ELSEVIER

MCN Molecular and Cellular Neuroscience

www.elsevier.com/locate/ymcne
Mol. Cell. Neurosci. 31 (2006) 161 – 177

Evolution of myelin proteolipid proteins: Gene duplication in teleosts and expression pattern divergence

Jörn Schweitzer,[a] Thomas Becker,[a] Melitta Schachner,[a] Klaus-Armin Nave,[b] and Hauke Werner[b,*]

[a]*Zentrum fuer Molekulare Neurobiologie, University of Hamburg, D-20251 Hamburg, Germany*
[b]*Department of Neurogenetics, Max-Planck-Institute of Experimental Medicine, Hermann-Rein-Str. 3, D-37075 Goettingen, Germany*

Received 21 July 2005; revised 30 September 2005; accepted 12 October 2005
Available online 11 November 2005

The coevolution of neurons and their supporting glia to the highly specialized axon-myelin unit included the recruitment of proteolipids as neuronal glycoproteins (DMβ, DMγ) or myelin proteins (DMα/PLP/DM20). Consistent with a genome duplication at the root of teleosts, we identified three proteolipid pairs in zebrafish, termed DMα1 and DMα2, DMβ1 and DMβ2, DMγ1 and DMγ2. The paralogous amino acid sequences diverged remarkably after gene duplication, indicating functional specialization. Each proteolipid has adopted a distinct spatio-temporal expression pattern in neural progenitors, neurons, and in glia. DMα2, the closest homolog to mammalian PLP/DM20, is coexpressed with P0 in oligodendrocytes and upregulated after optic nerve lesion. DMγ2 is expressed in multipotential stem cells, and the other four proteolipids are confined to subsets of CNS neurons. Comparing protein sequences and gene structures from birds, teleosts, one urochordate species, and four invertebrates, we have reconstructed major steps in the evolution of proteolipids.
© 2005 Elsevier Inc. All rights reserved.

Keywords: Myelin protein evolution; Pelizaeus–Merzbacher disease; PLP/DM20; Gpm6a; Gpm6b; Tetraspan transmembrane proteins; Alternative splicing; Teleostei; Genome duplication; Nerve regeneration; Ascidian *Ciona intestinalis*; *Drosophila melanogaster*; *Apis mellifera*; *Anopheles gambiae*; *Danio rerio*; CNS regeneration; Remyelination; Neurite outgrowth; Optic nerve lesion

Introduction

Rapid neuronal signaling is a prerequisite for avoiding predation or attacking prey. The evolution of early vertebrates included the insulation of axons with multilayered myelin sheaths for 'saltatory' impulse propagation, improving nerve conduction velocity. Myelin sheaths are assembled as highly specialized lipid-rich membrane by both, oligodendroglial cells in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS) (Hildebrand et al., 1993, 1994). The protein composition of myelin is less complex than that of other membranes and has undergone significant changes during vertebrate evolution (Franz et al., 1981; Waehneldt et al., 1985; Kirschner et al., 1989; Morris et al., 2004). The elaboration of the spirally compacted myelin membrane is mechanistically poorly understood, and significant interest has therefore focused on the role of membrane proteins in axon–glia interaction and the axonal ensheathment process.

The major myelin membrane component in the mammalian CNS is a highly hydrophobic tetraspan protein, termed proteolipid protein (PLP), which is coexpressed with its smaller splice variant DM20 (Milner et al., 1985; Nave et al., 1987; Kronquist et al., 1987; Simons et al., 1987). The high proteolipid content of CNS myelin is likely contributing to the membrane's unique biophysical properties. The myelin ultrastructure observed in PLP/DM20-deficient mice suggests that myelin proteolipids act as a molecular strut in the extracellular membrane apposition zone of the compact CNS myelin architecture (Rosenbluth et al., 1996; Klugmann et al., 1997; Stecca et al., 2000; Sporkel et al., 2002). Though the molecular mechanism is unclear, presence of PLP/DM20 in CNS myelin is a prerequisite for long-term oligodendrocyte-to-axon support (Griffiths et al., 1998; Stecca et al., 2000; Edgar et al., 2004). Together, the gene targeting experiments demonstrated that PLP enrichment per se is dispensable for myelin assembly. This view was confirmed by the finding that proteolipids are only minor components of PNS myelin (Anderson et al., 1997; Garbern et al., 1997). Nevertheless, PLP/DM20 deficiency in PNS myelin leads to peripheral neuropathy in humans (Garbern et al., 1997; Shy et al., 2003).

It is of evolutionary and cell biological interest to compare PLP with its counterpart in the PNS. The major myelin membrane protein in the mammalian PNS is a cell adhesion molecule of the immunoglobulin superfamily, protein zero (P0), which is structurally unrelated to proteolipids (Lemke and Axel, 1985). Lack of P0

* Corresponding author. Fax: +49 551 3899 758.
E-mail address: hauke@em.mpg.de (H. Werner).
Available online on ScienceDirect (www.sciencedirect.com).

1044-7431/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.mcn.2005.10.007

causes dysmyelination in the PNS of mice and humans (Giese et al., 1992; Hayasaka et al., 1993; Kulkens et al., 1993). In the most ancient myelinated species, cartilaginous fish (sharks and rays), P0 is the major myelin protein in both PNS and CNS (Kirschner et al., 1989; Saavedra et al., 1989; Yoshida and Colman, 1996). Adhesive properties have been demonstrated both for mammalian and fish P0 (Filbin et al., 1990; D'Urso et al., 1990; Schneider-Schaulies et al., 1990; Lanwert and Jeserich, 2001). In zebrafish, P0 mRNA expression correlates with CNS myelination (Brosamle and Halpern, 2002; Schweitzer et al., 2003) and remyelination after injury (Schweitzer et al., 2003).

In evolutionary terms, proteolipids were suggested to be younger myelin constituents, compared to P0. Proteolipid mRNA (the DM20 ortholog in fish has been termed DMα) is expressed in shark and ray myelinating glia (Kitagawa et al., 1993), but with the available antibodies incorporation into myelin could not be demonstrated (Yoshida and Colman, 1996). In both CNS and PNS myelin of bony fish and amphibia, P0 is coexpressed with DMα or PLP, respectively (Takei and Uyemura, 1993; Yoshida and Colman, 1996). The lack of P0 in oligodendrocytes and CNS myelin is confined to mammals.

At the root of tetrapode evolution, i.e., after diversion from lungfish, the glial DM20/DMα gene must have acquired an alternative splice donor site for the inclusion of exon 3B. Exon 3B encodes a 35–36 residue cytoplasmic loop that is specific for PLP and absent from DM20 (Nave et al., 1987; Schliess and Stoffel, 1991; Yoshida and Colman, 1996; Venkatesh et al., 2001). Although DM20 is incorporated into myelin in the absence of PLP (Stecca et al., 2000; Sporkel et al., 2002), the PLP-specific domain appears to increase the efficiency of targeting proteolipids to myelin (Trapp et al., 1997). The evolution of the PLP-specific domain has been suggested to be a prerequisite to displace P0 from mammalian CNS myelin (Yoshida and Colman, 1996). In humans, mutations in the PLP-specific domain cause spastic paraplegia (type 2), which is a milder neuropathy when compared to Pelizaeus–Merzbacher disease caused by mutations in DM20 (Werner et al., 1998; Cailloux et al., 2000; Duncan, 2005).

The analysis of proteolipid function in the nervous system, as well as their evolutionary origin, was boosted by the identification of two PLP/DM20-related proteins in fish and tetrapode neurons, termed M6A and M6B. The names reflect that they were identified in an expression screen with a monoclonal antibody termed "M6" (Yan et al., 1993). M6A (or Gpm6a/EMA, Edge Membrane Antigen) is expressed in a subset of epithelial cell types and in most CNS neurons (Lund et al., 1986; Yan et al., 1993, 1996; Mi et al., 1998), promoting neurite outgrowth and contact stabilization of cellular processes in vitro (Sheetz et al., 1990; Baumrind et al., 1992; Lagenaur et al., 1992; Mukobata et al., 2002). M6B (or Gpm6b) is abundant in both neurons and oligodendrocytes (Yan et al., 1996; Werner et al., 2001) and is present in a myelin-enriched membrane fraction (Klugmann et al., 1997). By sequence analysis, all proteolipids share a common overall structure with four hydrophobic transmembrane domains, intersected by three hydrophilic loop regions. The fish and amphibian M6A and M6B orthologs have been termed DMβ and DMγ, respectively (Kitagawa et al., 1993; Geltner et al., 1998; Yoshida and Colman, 2000; Yoshida et al., 1999). Thus, we will use in the following the term DMα for all PLP/DM20 orthologs, DMβ for M6A, and DMγ for M6B.

Proteolipid genes have been identified as pairs in Xenopus (Yoshida et al., 1999), best explained by tetraploidization of the amphibian genome (Hughes and Hughes, 1993). However, the divergence of amino acid sequences and expression patterns between xenPLP1 and xenPLP2 or between xenDMγ1 and xenDMγ2 is low. Only a single DMβ gene was found in Xenopus.

A protein related to vertebrate proteolipids has been predicted from *Drosophila* genomic and mRNA sequences (Stecca et al., 2000; Werner et al., 2001) that shares 34% and 31% amino acid similarity with mouse M6A and M6B, respectively. Thus, proteolipids are phylogenetically much "older" than vertebrates and thus have been involved in myelination at a later step in evolution. The M6-protein of arthropods is likely a functional homolog to mammalian proteolipids.

The emergence of proteolipids as myelin proteins must have occurred in early vertebrate evolution (Yoshida and Colman, 1996). Expression data at the cellular level is an essential prerequisite to understand the evolution of genes and the cellular function of their products. However, knowledge on the expression of proteolipids has been very limited in teleost fish, the most species-rich and diverse vertebrate group. It was thus tempting to fill this gap utilizing the teleost zebrafish (*Danio rerio*), a widely used model system of genetics with well-defined developmental stages (Kimmel et al., 1995). We have identified six PLP/DM20-related gene products in zebrafish, termed DMα1, DMα2, DMβ1, DMβ2, DMγ1, and DMγ2. In contrast to the amphibian homologues, zebrafish proteolipids have diverged from each other remarkably in sequence and spatio-temporal expression.

Results

Identification of six proteolipid genes in zebrafish

To identify proteolipids in the teleost species *D. rerio*, we have screened the EST databases at the NCBI using rodent sequences for PLP (Milner et al., 1985), M6A and M6B (Yan et al., 1993), and a *Drosophila* M6-ortholog (Werner et al., 2001) as queries. 19 zebrafish EST clones with significant homology were obtained from the IMAGE consortium, and cDNA sequences of both strands were determined. Large stretches of nucleotide sequence identity allowed us to group all cDNA clones as products of six distinct genes. The 5′-end of one partial cDNA (DMγ2) was completed by 5′-RACE in four independent experiments. All other cDNA clones contained putative complete open reading frames. The encoded proteins were predicted by conceptual translation (Table 1). We identified two zebrafish orthologs for each of the known vertebrate proteolipids DMα, DMβ, and DMγ. These were named DMα1, DMα2, DMβ1, DMβ2, DMγ1, and DMγ2, following the nomenclature for fish and amphibian proteolipids. Nucleotide and amino acid sequences have been deposited at the NCBI database (accession numbers AY070259-AY070267).

Structural conservation

The primary sequence of the six zebrafish proteolipids displayed four regions of high hydrophobicity. Using SMART software (http://smart.embl-heidelberg.de), the same amino acid stretches were identified as likely transmembrane domains (TMD), corresponding to the topological model of mammalian PLP/DM20, thought to be tetraspan membrane proteins (Popot et al., 1991; Weimbs and Stoffel, 1992; Inouye and Kirschner, 1994). The percentage of hydrophobic amino acids (39–46%) and the

Download English Version:

https://daneshyari.com/en/article/2199537

Download Persian Version:

https://daneshyari.com/article/2199537

Daneshyari.com