Neuroscience 316 (2016) 130–142

SELECTIVE AND COMPARTMENTALIZED MYELIN EXPRESSION OF HspB5

S. QURAISHE,[a]* A. WYTTENBACH,[a] N. MATINYARARE,[a] V. H. PERRY,[b] R. FERN[c] AND V. O'CONNOR[a]

[a] *Centre for Biological Sciences, Faculty of Natural and Environmental Sciences, Building 85, University of Southampton, Southampton SO17 1BJ, UK*

[b] *Centre for Biological Sciences, Mailpoint 840 (room LD80b), Level D Laboratories and Pathology Block, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK*

[c] *Peninsula School of Medicine and Dentistry, University of Plymouth, Devon, PL4 8AA, UK*

Abstract—In the present study, we reveal myelin-specific expression and targeting of mRNA and biochemical pools of HspB5 in the mouse CNS. Our observations are based on *in situ* hybridization, electron microscopy and co-localization with 2′,3′-Cyclic-Nucleotide 3′-Phosphodiesterase (CNPase), reinforcing this myelin-selective expression. HspB5 mRNA might be targeted to these structures based on its presence in discrete clusters resembling RNA granules and the presence of a putative RNA transport signal. Further, sub-cellular fractionation of myelin membranes reveals a distinct sub-compartment-specific association and detergent solubility of HspB5. This is akin to other abundant myelin proteins and is consistent with HspB5's association with cytoskeletal/membrane assemblies. Oligodendrocytes have a pivotal role in supporting axonal function via generating and segregating the ensheathing myelin. This specialization places extreme structural and metabolic demands on this glial cell type. Our observations place HspB5 in oligodendrocytes which may require selective and specific chaperone capabilities to maintain normal function and neuronal support.

Key words: myelin, small heat-shock protein, alphaB-crystallin, oligodendrocyte, CNS, chaperone.

INTRODUCTION

The small heat-shock proteins (sHsps) are evolutionarily conserved, ubiquitous proteins with diverse functions. They are grouped together based on their structural homology and low molecular weight (Taylor and Benjamin, 2005). They form large, dynamic, oligomeric complexes of variable size and are able to bind several non-native proteins per complex (Haslbeck et al., 2008). As their name suggests, the sHsps are classically defined as a class of protein that are induced by heat shock and other cellular stressors/insults. However, they are not exclusively induced by stress, but are established proteins that exert their function under physiological conditions as part of normal homeostatic cell regulation (Mymrikov et al., 2011; Bakthisaran et al., 2015). They regulate protein folding and (dis)aggregation, influence pathways that can modulate aging, inflammatory processes, the cytoskeleton and cell death (Beere, 2004; Haslbeck et al., 2005; Arrigo et al., 2007; Chen and Brown, 2007). The diverse and important homeostatic cellular roles of the sHsps are well illustrated by the finding that mutations in certain members of this protein family result in myopathies, neuropathies and cataract (Beere, 2004; Benndorf and Welsh, 2004; Haslbeck et al., 2005; Arrigo et al., 2007; Chen and Brown, 2007; Mymrikov et al., 2011; Simon et al., 2013; Benndorf et al., 2014).

We previously characterized the brain expression of ten mammalian sHsps. Four sHsps (HspB1/Hsp27, HspB5/αB-crystallin, HspB6/Hsp20 and HspB8/Hsp22) were unequivocally expressed at the protein level in the mouse CNS under physiological conditions (Quraishe et al., 2008). Interestingly, the expression pattern of HspB5 was distinct among the CNS-selective sHsps since it displayed selective white matter/myelin expression. Indeed, two systematic proteomics studies previously identified HspB5 as part of the CNS myelin proteome (Dumont et al., 2007; Jahn et al., 2009) and HspB5 expression has also been reported in Schwann cells and oligodendrocytes under physiological conditions (Iwaki et al., 1992; Klemenz et al., 1993).

Oligodendrocytes are highly specialized cells that send out many projections (~60) from a single cell body, each of which is able to wrap extensively around a single axon forming myelin internodes (Fanarraga et al., 1998; Chong et al., 2012). They synthesize a vast amount of myelin membrane and specific sub-sets of proteins and lipids that are associated with biochemically distinct compact and non-compact cellular sub-domains (Simons et al., 2000; Baron and Hoekstra, 2010). Myelin biogenesis requires the selective targeting, transport and integration of proteins and lipids into the emerging myelin sheath, which may lie a significant distance from

*Corresponding author. Address: Faculty of Medicine, Clinical and Experimental Sciences, Life Sciences Building 85, Highfield Campus, University of Southampton, Southampton SO17 1BJ, UK.
E-mail address: s.quraishe@soton.ac.uk (S. Quraishe).

Abbreviations: CNPase, 2′,3′-Cyclic-Nucleotide 3′-Phosphodiesterase; CNS, central nervous system; EDTA, ethylenediaminetetraacetic acid; MAP, microtubule-associated protein; MBP, myelin basic protein; MS, multiple sclerosis; MT, microtubule; NG2, neuron-glial antigen 2; OPCs, oligodendrocyte precursor cells; PBS, phosphate-buffered saline; PI, protease inhibitor; RTS, RNA transport signal; sHsps, small heat-shock proteins.

http://dx.doi.org/10.1016/j.neuroscience.2015.12.035
0306-4522/

the cell body. This is achieved by an orchestrated interplay of cytoskeletal elements that co-ordinate outgrowth during oligodendrocyte differentiation and regulate vesicular transport within the cytoplasmic channels of myelin. Microtubules (MTs) enable the polarized and directed transport of mRNA granules, vesicles and organelles within oligodendrocyte processes akin to axonal transport (Carson et al., 1997; Lunn et al., 1997; Richter-Landsberg, 2000). Multiple membrane-trafficking pathways, including lipid rafts, direct material to compact sub-domains and function as signaling platforms (Kramer et al., 2001; Poliak and Peles, 2003; Baron and Hoekstra, 2010; Simons et al., 2012).

The importance of functional oligodendrocytes and appropriate myelination becomes clear when considering diseases such as multiple sclerosis (MS). MS is an inflammatory demyelinating disease, where abnormalities in development, maintenance and damage to the myelin sheath results in a devastating and incapacitating disease with severe neurological symptoms (Lassmann and Lucchinetti, 2008). HspB5 is the most abundant transcript that is unique to early active MS lesions (Chabas et al., 2001). Despite being a negative regulator of inflammation, it has been reported to produce one of the strongest T cell responses in MS patients (van Noort et al., 1995), thus exacerbating inflammation and demyelination in MS (Ousman et al., 2007).

Furthermore, it is increasingly apparent that the neuronal cell population is not the only cell type adversely affected during various age-related neurodegenerative diseases. Associated structures, such as myelin sheaths and glial cells are also affected (Ciarmiello et al., 2006; Mitew et al., 2010). Given the emerging importance of white matter dysfunction during neurodegenerative diseases (Lu et al., 2014; Phillips et al., 2014), the early changes to white matter structural integrity in normal aging (Barrick et al., 2010; Sullivan et al., 2010; Bennett and Madden, 2014) and in view of the rather selective expression of HspB5, we wanted to better define and characterize the physiological expression profile of HspB5 within the CNS. Expression and localization of HspB5 mRNA and protein were assessed histologically by *in situ* hybridization (emulsion radiography), immunohistochemistry and electron microscopy in rodent brain, spinal cord and optic nerve tissue. Biochemical analysis of HspB5 in myelin-enriched samples was conducted by sub-cellular fractionation, followed by detergent, pH and salt extraction. Such biochemical analysis enables the separation and identification of sub-membranous, membrane-bound and scaffold-proteins within myelin sub-domains (Kim and Pfeiffer, 1999). This has been used to gain a better understanding of the function/role of abundant myelin proteins such as myelin basic protein (MBP) and 2′,3′-Cyclic-Nucleotide 3′-Phosphodiesterase (CNPase) in myelin biogenesis and has revealed distinct biochemical characteristics of these proteins (Kim and Pfeiffer, 1999; Debruin and Harauz, 2007). Our analysis suggests that HspB5, with its intrinsic chaperone capabilities is well placed to modulate oligodendroglial function in physiological and pathological conditions.

EXPERIMENTAL PROCEDURES

In situ hybridization

Adult, p60, C57BL/6J mice were sacrificed by cervical dislocation. Their brains and spinal cords were removed and immersed in Tissue Tek OCT (Bayer diagnostics, Newbury, UK), on 2-methylbutane over dry ice. 12-μM sections were cut on a cryostat, mounted onto Superfrost slides (Menzel-Glaser, Thermo Scientific, Loughborough, UK) and stored at −80 °C prior to use. *In situ* hybridization was performed as previously described (French et al., 2001), with an exposure time of 2 weeks. For emulsion autoradiography, slides were dipped in Ilford K-5 emulsion (exposure was extended to threefold the standard exposure time), developed and counterstained with Cresyl Violet. 45mer oligonucleotides specific to HspB5 were evaluated using the BLAST sequence database comparison software http://blast.ncbi.nlm.nih.gov/Blast.cgi. Antisense and sense oligonucleotides with perfectly matched nucleic acid sequence to HspB5 (100% homology) and low levels of homology to any other known mRNA were identified and supplied by Eurogentec:

HspB5 antisense: AAA CTC AAT GAG GAA AGG GGA TCT ACT TCT TAG GGG CTG CGG CGA
HspB5 sense: TCG CCG CAG CCC CTA AGA AGT AGA TCC CCT TTC CTC ATT GAG TTT

Immunohistochemistry

Coronal sections (10 μm) were cut from formalin-fixed, paraffin wax-embedded brains. Antigen retrieval was performed by boiling in citrate buffer, sections were then incubated in a humid chamber overnight at 4 °C with one of the following primary antibodies: anti-HspB5 polyclonal antibody (1:400; SPA223, Stressgen, Exeter, UK), anti-CNPase monoclonal antibody (1:400; AB6319, Abcam, Cambridge, UK) and anti-MBP monoclonal antibody (1:1000; Upstate Signalling). Negative control (primary antibody omission) sections were incubated in blocking serum. Sections were incubated in appropriate biotinylated secondary antibody (Vector Laboratories, Peterborough, UK), diluted 1:200 in 0.25% BSA/phosphate-buffered saline (PBS), and followed by incubation in the avidin–biotin complex (ABC) Kit (Vector Laboratories). Location of antibody binding was determined by incubation of sections in a 3,3′-diaminobenzidine (DAB) solution for 1–3 min. Sections were counterstained with Harris Haematoxylin (BDH). Alexa-conjugated secondary antibodies (Molecular Probes, Fisher Scientific, Leicestershire, UK) were used in place of biotinylated secondary antibodies for fluorescent labeling and sections were counterstained with DAPI (Vector Shield, Vector Laboratories LTD, Peterborough, UK). Images were taken using a Leica CM500 microscope. LAS-AF software was used for fluorescence microscopy and Q-Win image analysis for brightfield images.

Enrichment of crude myelin

Myelin membrane preparation and differential extraction was carried out using modified protocols (Kim and Pfeiffer, 1999; Phillips et al., 2001). All steps were carried out at 4 °C unless otherwise stated. Two adult mice brains