Biosynthesis of the major integral protein of rat peripheral nerve myelin

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Schwann cells produce myelin as an extension of their plasma membrane and ensheath nerve fibres in the peripheral nervous system (PNS). Several myelin proteins are common to both central nervous system (CNS) and PNS myelin but the major integral protein (P0) of PNS myelin is peculiar to this membrane (Greenfield et al., 1973). P0 comprises around 60% of the total protein of PNS myelin. SDS/polyacrylamide-gel analysis indicates an apparent M, of 28 000–30 000 and the protein is known to have a single N-linked oligosaccharide chain (Ishaque et al., 1980). Other post-translational modifications include acetylation (Agrawal et al., 1982), phosphorylation (Wiggins & Morell, 1980) and sulphation (Matthieu et al., 1975).

The purpose of this work was to identify the subcellular site of synthesis of P0 in Schwann cells and determine the mechanisms by which this protein is inserted into membranes before transport to the Schwann cell plasma membrane. mRNA isolated from sciatic nerves of 15-day-old rats was translated in a wheatgerm system and P0, was immune-precipitated and analysed on SDS-gels (Colman et al., 1982). The primary translation product had a M, of 30 700 compared with an M, of 28 500 for the mature protein. This result indicated a cleavable signal sequence in the primary translation product of P0 mRNA. Removal of the single oligosaccharide from mature P0, with endoglycosidase F showed the M, of the mature deglycosylated protein to be 24 800. The apparent M, of the cleaved signal was therefore 5900, which is relatively large. Interestingly, a significant fraction of the mature oligosaccharide was susceptible to endoglycosidase H-catalysed hydrolysis, indicating a large amount of high mannose oligosaccharide in the mature protein. Translation of PNS mRNA in the presence of dog pancreatic microsomes enriched with signal recognition particle (Walter & Blobel, 1983) confirmed the presence of a cleavable signal sequence. Recently, Lemke & Axel (1985) have sequenced a cDNA that encodes P0. The presence of a single methionine codon at nucleotide 32 followed by codons for 28 uncharged amino acids up to the N-terminal isoleucine for the mature protein was taken to indicate the existence of a cleavable sequence. This we have confirmed. This discrepancy in the relative sizes of the signal deduced from our work and that from the cDNA sequence is the subject of current investigation.

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Biosynthesis of the 2',3'-cyclic nucleotide phosphohydrolases of rat central nervous system myelin

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Colman et al., (1982) have shown that the major proteins of rat central nervous system (CNS) myelin, the proteolipid protein (PLP) and myelin basic proteins (MBPs), are synthesized on bound and free polysomes respectively. Of particular interest was their observation that MBP mRNA is enriched in the RNA associated with myelin. These authors speculated that the close proximity of MBP mRNAs to their target membrane provided a mechanism for the rapid incorporation of these proteins into the growing myelin membrane. The purpose of the experiments reported here was to determine whether another major group of CNS myelin proteins were also synthesized on polysomes close to the myelin process.

Although myelin contains relatively few enzymes, there is a 2',3'-cyclic nucleotide phosphohydrolase (CNPase; EC 3.1.4.37) which has received considerable attention. The activity has been shown to reside in two polypeptides previously termed the Wolfram proteins (Wolfram & Kotori, 1968; Drummond & Dean, 1980; Sprinkle et al., 1980). Together the two polypeptides of M, 48 000 and M, 50 000 comprise about 4% of total CNS myelin protein. Free polysomes and bound polysomes were isolated from myelinating rat brain and their RNA was extracted and translated in a wheatgerm system in vitro. The CNPases were immune-precipitated from the translations and analysed on SDS-gels (Colman et al., 1982). The CNPases were clearly synthesized on free polysomes, therefore we investigated whether these polysomes might be enriched in the myelin-associated polysomal fraction, as is the case for MBP mRNA. Myelin was isolated and RNA extracted as described by Colman et al., (1982). There was no enrichment of CNPase mRNA in the myelin RNA fraction. It appears that there are distinct mechanisms for the incorporation of peripheral membrane proteins into rat CNS myelin. It will now be of great interest to determine whether other myelin proteins that are synthesized on free polysomes share the type of distribution in the myelin-forming cell exemplified by the MBPs or the CNPases.

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Abbreviations used: CNS, central nervous system; PLP, proteolipid protein; MBP, myelin basic protein; CNPase, 2',3'-cyclic nucleotide phosphohydrolase.

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