In situ Hybridization Analysis of Myelin Gene Transcripts in Developing Mouse Spinal Cord

Craig Jordan, Victor Friedrich, Jr., and Monique Dubois-Dalcq

Laboratory of Molecular Genetics, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892

We analyzed the location and abundance of transcripts for the 4 CNS myelin protein genes, myelin basic protein (MBP), proteolipid protein (PLP), myelin-associated glycoprotein (MAG), and 2',3'-cyclic nucleotide phosphohydrolase (CNP), in the mouse cervical spinal cord from the time of rapid myelination until adulthood (8-45 d). In the white matter, maximal levels of transcripts were found for each of the myelin genes at the peak of myelination (6 d). Total MBP and PLP mRNAs stayed high until 20 d and showed a minor decrease thereafter. In contrast, MAG and the MBP exon 2 containing transcripts (coding for the 21.5 and 17 kDa MBP isoforms) decreased sharply between 8 and 20 d, suggesting that high levels of these transcripts are needed primarily during the initiation of myelination. CNP transcripts were less abundant, maintained high expression until 20 d, and then decreased sharply. PLP, MAG, and CNP transcripts were clustered in the oligodendrocyte cell body, while MBP mRNAs were scattered throughout the cell body and processes. In contrast to the white matter, all these myelin specific transcripts in the gray matter showed a marked increase from 8 to 20 d, as did the number of oligodendrocytes identified by CNP immunostaining. MAG transcripts were found in white matter and in satellite and other oligodendrocytes of the gray matter but not in neurons identified by their expression of neurofilament transcripts. The results of our quantitative in situ hybridization study are in good agreement with those of previous molecular studies and provide new information on the cellular and topographic distribution of myelin-specific mRNAs during myelination.

Oligodendrocytes are multipolar glial cells of the CNS that synthesize, transport, and integrate myelin components in their processes before wrapping them around axons to form multilamellar myelin internodes (reviewed in Benjamins and Smith, 1984; Raine, 1984). The genetic program controlling the expression of myelin proteins in oligodendrocytes appears tightly coordinated and regulated (reviewed by Lemke, 1986; Sutcliffe, 1987). With the recent cloning and sequencing of myelin protein genes-MBP, PLP, CNP, and MAG-in the developing CNS and PNS myelin genes at the peak of myelination (6 d). Total MBP and PLP mRNAs stayed high until 20 d and showed a minor decrease thereafter. In contrast, MAG and the MBP exon 2 containing transcripts (coding for the 21.5 and 17 kDa MBP isoforms) decreased sharply between 8 and 20 d, suggesting that high levels of these transcripts are needed primarily during the initiation of myelination. CNP transcripts were less abundant, maintained high expression until 20 d, and then decreased sharply. PLP, MAG, and CNP transcripts were clustered in the oligodendrocyte cell body, while MBP mRNAs were scattered throughout the cell body and processes. In contrast to the white matter, all these myelin specific transcripts in the gray matter showed a marked increase from 8 to 20 d, as did the number of oligodendrocytes identified by CNP immunostaining. MAG transcripts were found in white matter and in satellite and other oligodendrocytes of the gray matter but not in neurons identified by their expression of neurofilament transcripts. The results of our quantitative in situ hybridization study are in good agreement with those of previous molecular studies and provide new information on the cellular and topographic distribution of myelin-specific mRNAs during myelination.

Received Feb. 19, 1988; revised May 31, 1988; accepted June 1, 1988.

We would like to thank Drs. L. D. Hudson, R. A. Lazzarini, and K. Koller for the gift of probes; Dr. W. S. Young III for advice and use of the Loats image-analysis system; Drs. F. de Ferra, N. K. Zeller, and I. H. Hudson for helpful discussions; Mr. R. Rusten for photographic printing; and Mrs. J. Carrillo for painstaking manuscript preparation.

Correspondence should be addressed to Dr. Craig Jordan, NIH/NINCDS, Building 36, Room 4A01; Bethesda, MD 20892.

0270-6474/89/0102-48-10$0.00/0
to hyperpolymer formation, which may influence quantitation, and anti-sense probes can be used for control of specificity (Haase et al., 1981; Cox et al., 1984). In addition, oligonucleotide probes can be designed to differentiate between different types of transcripts. In mouse, alternative splicing generates 4 MBP transcripts, each of which codes for 1 of the 4 MBP isoforms (Zeller et al., 1984; de Ferra et al., 1985). Exon 2-containing transcripts code for 2 of these MBP isoforms (21.5 and 17 kDa) whose expression appears developmentally regulated (Barbarese et al., 1978; Carson et al., 1983); in this study we have utilized exon 2-specific probes to study the expression of early developmental forms of MBP mRNA directly in situ.

Here we describe the distribution and relative abundance of transcripts for 4 myelin protein genes in the mouse spinal cord from 8 to 45 d after birth. In the white matter, the peak expression of each type of transcript coincides with the peak of myelination. However, subsequent expression of the different transcripts varies: high levels of MAG and MBP exon 2-containing transcripts are observed only during the most active myelination period while mature MBP and PLP transcripts are still steadily expressed in the adult white matter. CNP expression shows a more complex pattern. The expression of all 4 myelin specific genes is delayed in gray matter as compared to white matter. Our results indicate that in situ hybridization is a most sensitive and precise method to study regional distribution and relative abundance of myelin gene transcripts during development.

Figure 1. Oligonucleotide probe specificities and Northern analysis of both DNA and RNA probes. A. The myelin genes for MBP, PLP, and MAG are schematically represented (genes and exons are not to scale) in order to show the position of the oligonucleotide probes along the genome (shaded boxes). Oligonucleotide probes for CNP 3' and 5' regions (shaded boxes) are positioned along a cDNA clone (see details in Materials and Methods) (Bernier et al., 1987). B, Northern blot demonstrates specific transcripts detected by the following probes: MAG exon 7-8 (lanes a, b), PLP exon 2 (lanes c, d), PLP exon 3 (lanes e, f), MBP exon 2 (lanes g, h), and MBP exon 1 (lanes i, j) were hybridized to 5 μg/lanes of total brain RNA (lanes a, c, e, g, i) or total liver RNA (lanes b, d, f, h, j) from 20- to 40-d-old mice. Note that MBP exon 1 probe (lane i) detected mRNA trapped in ribosomal RNA markers, and this accounts for the additional bands (which were not seen when poly A-selected RNA was used). C, Northern blot of poly(A)+ RNA from 20-d-old mouse brain (lane a, 10 μg; lane b, 5 μg) hybridized with a mixture of CNP 3' and 5' probes (lane a) and with cRNA PLP probe (lane b).

Materials and Methods

Tissue preparation. C57BL/6N mice 8, 20, and 45 d old were anesthetized with methoxyflurane followed by chloral hydrate administered intraperitoneally (0.35-0.5 mg/gm body weight). Mice were perfused with 4% formaldehyde freshly prepared from paraformaldehyde in 0.1 M phosphate buffer (pH 7.2), and spinal cords were dissected out, cut into transverse pieces, cryoprotected overnight in 15% sucrose (RNAse buffer), and then stored up to 3 weeks at -70°C before use.

Probes. Single-stranded RNA probes were prepared in the following way: A 930 base pair fragment corresponding to the mouse PLP-coding region and some 5'-untranslated sequence was subcloned into pGEM 3 (kindly supplied by L. D. Hudson; Hudson et al., 1987), which contains SP6 and T7 promoters. The PLP plasmid was linearized with Eco-RI (kindly supplied by L. D. Hudson; Hudson et al., 1987) and transcribed with SP-6 polymerase, following the conditions recommended by Promega Biotec (Madison, WI) in the presence of 30 μCi of (α-32P) UTP (1250 Ci/mmol, Amersham Corp., Arlington Heights, IL). The RNA probes were purified on G-50 Sephadex columns, alkali-hydrolyzed to 200-400 base fragments (Cox et al., 1984), and stored with 100 mM dithiotreitol (DTT) and 1200 U/ml RNAse-inhibitor (Boehringer Mannheim Biochemicals, Indianapolis, IN).

Oligonucleotide probes (48-54 nucleotides) were synthesized on an Applied Biosystems DNA synthesizer following the manufacturer's recommendations and published sequences for PLP (exon 2, nucleotides no. 220-267; exon 5, nucleotides no. 514-561, following the numbering of Hudson et al., 1987), MBP (exon 1, nucleotides no. 38-85; exon 2, nucleotides no. 238-285, following the numbering of de Ferra et al.,
resed on formaldehyde/agarose gels, and transferred to nitrocellulose
old C57B1/6N mice with guanidinium thiocyanate (Maniatis et al.,
poly A+ mRNA was selected with oligo (dt)-cellulose, electropho-
but were usually purified on 8 M urea/10% acrylamide gels.
ated with 100 mmr+r DTT for up to 6 weeks.
of K. Koller) was made following the published sequence (nucleotides
responding to the mouse small neurofilament (NF) gene (the kind gift
of developmental patterns among the different probes (Fig. 8), values
of 100% for the most intense binding.
immunocytchemistry. Sections were immunolabeled for CNP by the
immunochemistry method (Stemberger et al., 1970). Sections were
immersed in acetone at -20°C for 10 min, rinsed with 0.5 M Tris buffer,
the numbering of Arquint et al., 1987), and CNP (5'-end probe, nu-
cleotides no. 46-93; 3'-end probe, nucleotides no. 1087-1134, following
the numbering of Bernier et al., 1987). A synthetic oligonucleotide cor-
responding to the mouse small neurofilament (NF) gene (the kind gift
of K. Koller) was made following the published sequence (nucleotides
no. 117-170, from the cDNA clone of Lewis and Cowan, 1985). The
oligonucleotides were usually purified on 8 M urea/10% acrylamide gels.
Labeling was accomplished with terminal deoxynucleotidyl-transferase
and (35S) dATP (1200 Ci/mmol, Amersham Corp.) (Zeller et al., 1985).
Probes had tail lengths in the range of 15-25 residues and were stored
at 4°C with 100 mM DTT for up to 6 weeks.
Northern analysis. RNA was extracted from brains and livers of 20-
day-old C57Bl/6N mice with guanidinium thiocyanate (Maniatis et al.,
1982). Poly A+ mRNA was selected with oligo (dt)-cellulose, electropho-
resed on formaldehyde/agarose gels, and transferred to nitrocellulose
membranes. Filters were hybridized overnight at 40°C with the RNA
or DNA probes, washed, and visualized by autoradiography.
In situ hybridization. The technique is basically that of Young et al.
(1986). The cryostat sections were removed from -20°C storage and
placed at room temperature for 10 min. Sections were then fixed again
in 4% formaldehyde in PBS, rinsed, acetylated, dehydrated, delipidated,
and air-dried. Sections were then hybridized with a mixture containing
50% denatured formamide (Fukui Chemical Corp., Rochester, NY),
4x saline sodium citrate or SSC (1 x SSC consists of 150 mM NaCl,
and 15 mm sodium citrate), 500 &µg/ml sheared single-stranded salmon
sperm DNA, 250 &µg/ml yeast t-RNA (both from Sigma Chemical Com-
pny), 1x Denhardt's solution (0.02% ficoll, 0.02% polyvinyl pyrroli-
done, 0.02% BSA fraction V), 10% dextran sulfate (MW = 500,000),
100 mm DTT, and 1-2 x 10^6 cpm of probe per 30 µl. Usually, groups
of 2-4 spinal cord sections were hybridized with 20 µl of the mixture,
covered with a piece of paraffin, and incubated overnight in a
humid chamber at 37°C.
After hybridization the paraffin coverslips were removed in 1 x SSC
followed by 3 quick rinses in 1 x SSC. At this point, specimens hybrid-
bred with cRNA probes were treated differently than those with
DNA oligonucleotide probes. Sections hybridized with cRNA probes
were treated with (1) RNase A (100 &µg/ml) and RNase T1 (10 &µl/ml)
(but from Boehringer Mannheim, Indianapolis, IN) in 1 mm EDTA,
0.5 M NaCl, 10 mm Tris, pH 8.0, for 30 min at 37°C; (2) 1 x SSC; (3)
2 x SSC with 50% formamide at 40°C for two 15 min changes; and (4)
0.1 x SSC at 50°C for two 15 min changes. Sections hybridized with
DNA oligonucleotide probes were washed in (1) 2 x SSC with 50%
formamide at 40°C for four 15 min changes; (2) 1 x SSC at room
temperature for two 30 min changes. All slides were rinsed in H2O followed
by ethanol and air-dried. Dried slides were dipped into Kodak NTB2
emulsion diluted 1:1 with H2O at 45°C and allowed to expose at 4°C
for 14-40 d. The emulsion was developed with D-19 (Kodak, ½ strength)
and Kodak fixer before being washed and counterstained with cresyl
violet (0.5% in 0.1 M sodium acetate, pH 4). Sections were partially
destained with 5 mm of the sodium acetate buffer in 95% ethanol.
Densitometric analysis. Autoradiograms were analyzed with a Loats
image-analysis system (Loats, Westminster, MD) using dark-field illu-
mination and measurement windows from 2-40 x 10^3 µm^2. Two to
four optical density determinations were made from each of 6 standard
locations in 2 sections for each age/probe combination. For comparison
of developmental patterns among the different probes (Fig. 8), values
were corrected at each age for background (binding to substantia gelu-
sos and central canal) and data for each probe scaled to give a value
of 100% for the most intense binding.

Results
Characterization of the probes
Figure 1A represents the position of the MBP exon 1- and exon
2-specific oligonucleotide probes along the MBP genome. Both
oligonucleotide MBP probes hybridized on Northern blots of
mouse total brain RNA to a wide band of approximately 2.2
kilobase (kb), as expected for rodent MBP (Fig. 1B, g and i)
(Zeller et al., 1984; Mentaberry et al., 1986; Popko et al., 1987).
Signal from the exon 2 probe (Fig. 1B, g) is less intense than
that from the exon 1 probe (Fig. 1B, i) since the exon 2 probe
binds only to the 2 early forms of MBP mRNAs (coding for the

Figure 2. Comparison of cRNA and oligonucleotide DNA probes by
in situ hybridization. Twenty-day-old mouse spinal cord cross sections
were hybridized with the cRNA PLP probe (a), oligonucleotide probes
for PLP exon 2 and exon 3 (b), and an oligonucleotide complementary
to the PLP exon 3 probe as a control (c). Note the typical clustering of
grains in both white and gray matter (see also Fig. 4). The cRNA probes
showed some background binding over the gray matter between the
clusters of grains. All sections were treated identically, exposed for 14
d and photographed in dark-field illumination as are Figures 3-5. Scale
bar, 500 µm.
Figure 3. Differential expression of MBP exon 1- and exon 2-containing transcripts. Cervical spinal cord sections from 8-, 20-, and 45-d-old mice were hybridized with either a MBP exon 1-specific (left side) or a MBP exon 2-specific probe (right side). While binding sites in the white matter are very high with exon 1 probe at 8 and 20 d, exon 2 binding sites dramatically decrease from 8 to 20 d. In contrast, gray matter signal increased substantially with both probes from 8 to 20 d (see also Figs. 7 and 8 for quantitation data). With exon 1 probe at day 20, grains are seen aligned along myelinating fibers crossing from the dorsal funiculi to the dorsal horns (arrows). Although MBP signal is predominantly detected as a diffuse grain pattern in the white matter, clusters of grains are seen at day 8 in the gray matter. For all procedures, sections were treated identically and exposed for 14 d. Different age cross sections are scaled to equivalent sizes. Scale bar, 1 mm.

21.5 and 17 kDa proteins), while the exon 1 probe binds to all 4 major MBP mRNA forms.

We used 3 PLP probes (1 cRNA, 2 exon-specific oligonucleotide probes) (see Materials and Methods). The diagram in Figure 1A shows the position of the oligonucleotide probes for exon 2 and exon 3 along the PLP genome. The exon 3 probe corresponds to a region deleted in one type of PLP transcript (referred to as DM20). The 2 oligonucleotide probes were mixed together for in situ hybridization to increase the hybridization signal. When the cRNA probe or the oligonucleotide probes were hybridized to mouse brain mRNA on Northern blots (Fig. 1B, c and e; and 1C, b), they revealed 2 strong bands and 1 weak band corresponding to the expected sizes (4.8, 3.2, and 2.4 kb) for mouse PLP mRNAs (Hudson et al., 1987). These transcripts result from the use of alternative polyadenylation sites but cover the same coding region (Milner et al., 1985; Hudson et al., 1987).

The single probe used for MAG transcript detection is an oligonucleotide that spans the junctions of exons 7 and 8 (Fig. 1A). This region is common to both the early and late forms of MAG (Lai et al., 1987), corresponds to an extracellular portion of the MAG molecule located between 2 immunoglobulin-like domains, and has less than 35% nucleotide homology to N-CAM, another member of the Ig superfamily expressed in the CNS (reviewed by Williams, 1987). When hybridized on Northern of mouse brain RNA, a single species of approximately 3.0 kb was recognized (Fig. 1B, a). This message is of the appropriate size, although we were not able to resolve the 2 MAG messages described as a doublet in the 2.5-3.5 kb range (Lenoir et al., 1986; Arquint et al., 1987; Salzer et al., 1987).
The 2 oligonucleotide probes for CNP correspond to 3' and 5' regions of a known cDNA clone (Fig. 1A). For hybridization, the 2 probes were mixed to maximize signal and increase the amount of genetic information detected since splicing is likely to occur with CNP (Bernier et al., 1987). As can be seen from the Northern blot in Figure 1C, a, these oligonucleotide probes detected a broad band probably corresponding to the 2.4 and 2.8 kb transcripts described by Bernier et al. (1987). [The bands are not distinct because we used more poly A selected RNA (10 μg) in this lane to enhance its detection.]

Comparison between RNA and oligonucleotide probes

cRNA probes corresponding to full-length coding sequences for PLP and MBP were compared with 48 nucleotide DNA oligonucleotide probes in order to evaluate relative levels of background and sensitivity during in situ hybridization. Results obtained with both single-stranded RNA and DNA probes were consistent and reproducible (Fig. 2).

With the same short autoradiographic exposure time, cRNA probes displayed higher sensitivity than the oligonucleotide probes. This is most likely due to the much shorter sequence represented in the oligonucleotide probes. After longer exposure times (Fig. 2), the difference in sensitivity was no longer striking, but cRNA probes produced a higher background, especially noticeable in the gray matter between oligodendrocytes. This increased background was also seen with the MBP cRNA probe (data not shown) and is probably due to the non-specific binding of very small partial transcripts resulting from the alkaline hydrolysis performed after cRNA labeling (see Materials and Methods). Since cRNA probes cause increased background, require a cDNA clone as a template, and are susceptible to endogenous RNAse digestion, we have conducted our developmental experiments with oligonucleotide cDNA probes. In some cases, we mixed 2 of these probes to increase the number of binding sites on the transcripts to increase the hybridization signal.

Figure 4. Expression of PLP and MAG transcripts. As in Figure 3, cervical spinal cord sections from 8-, 20-, and 45-d-old mice were hybridized with either a combination of PLP exon 2 and exon 3 probes (left side) or the MAG exon 7-8 probe (right side) in the same conditions used for MBP probes. Transcripts are organized in discrete clusters both in gray and white matter at all ages. White matter signal decreased earlier with MAG (8–20 d) than with PLP (20–45 d). Gray matter signal increased dramatically from 8 to 20 d for both transcripts with a peak at 20 d. Scale bar, 1 mm.
Figure 5. Expression of CNP transcripts. Cervical spinal cord sections from 8-, 20-, and 45-d-old mice were hybridized with a combination of CNP 3' and 5' probes. As with PLP and MAG transcripts (Fig. 4), the hybridization signal is mostly arranged in clusters, although not as tightly organized (see also Fig. 6). White matter signal decreases with age in general, with the exception of the dorsal funiculus signal, which is still high at 20 d. As with the other genes, gray matter signal increases from day 8 to day 20 with a peak at day 20. All procedures were the same as for Figures 3 and 4 except for the exposure time, which was increased to 40 d. Scale bar, 1 mm.

In order to minimize variability and allow comparative analysis of data, all in situ hybridizations depicted in Figures 2-5 were performed simultaneously on proximate sections from one block of tissue for each age; these sections were treated with the same reagents, exposed for the same time (except where noted), and developed with the same chemicals.

MBP transcripts
As mentioned above, exon 2 is present only in the 2 mRNA forms coding for the 21.5 and 17 kDa MBPs while exon 1 is present in all 4 forms of MBP transcripts. Cryostat sections from 8-, 20-, and 45-d-old mouse cervical spinal cords were hybridized separately with MBP probes specific for exon 1 and exon 2 (Fig. 3). Both probes bound to similar regions of the spinal cord during development and were more abundant in white matter than in gray matter. In white matter, the amount of probe binding decreased with age with both exon 1 and exon 2 oligonucleotides; however, the exon 2-containing transcripts decreased earlier, more rapidly, and to a significantly lower level than exon 1-containing MBP transcripts. In contrast, MBP transcripts increased in the gray matter, peaking at 20 d. This increase was especially noticeable in areas where myelinated fibers pass from the dorsal columns to the dorsal horn.

Figure 6. Differential localization of myelin gene transcripts as seen in bright-field microscopy. Hybridization patterns for the different probes in spinal cord ventral horn which contains large neuron cell bodies stained by cresyl violet (arrowheads). In a, MBP exon 2 probe, 20 d tissue. The diffuse silver grains are seen around the neurons and in higher number in the lower-left portion of the picture, which shows part of the white matter. In b, CNP 3' and 5' probes, 20 d tissue. Grain clusters are associated with the stained nuclei of putative oligodendrocytes, some of them closely associated with neurons. These grains are not as densely packed as those seen with PLP or MAG (compare with c and d). In c, PLP exon 2 and 3 probes, 45 d tissue. Grains are clustered around nuclei sometimes closely associated with neurons and are tightly organized. In d, MAG exon 7-8 probe (45 d tissue) also shows transcripts in tight clusters around nuclei (arrows), although the signal is less intense than with PLP. In e, NF probe, 8 d tissue. Only the neurons are labeled, a pattern never seen with any of the myelin-specific probes tested here. Scale bars, 20 μm.
CNP transcripts are lower than that of the other myelin genes. Therefore, the emulsion exposure was prolonged to 40 d and gave the results shown in Figure 5. The general pattern of CNP transcript distribution was similar to that of the other myelin gene transcripts. In white matter the transcripts varied in abundance from region to region but as a whole were maximally expressed at 8 and 20 d and then decreased. As with the other myelin gene transcripts, CNP transcripts in the gray matter region increased from 8 to 20 d and then decreased. CNP transcripts were detected as perinuclear clusters, which were more loosely organized than the clusters seen with MAG and PLP (Fig. 6b). The low abundance of CNP transcripts as compared with PLP and MBP transcripts might be expected since CNP constitutes only 5% of myelin protein. A discrepancy between levels of message and protein could exist due to differences in turnover rates.

Quantitative and comparative analysis of in situ hybridization data

To confirm and extend the qualitative observations presented above, we measured grain densities in several regions of spinal cord using an image analyzer (as described in the Materials and Methods). The results for MBP exon 1 are presented in detail in Figure 7, which shows the highest binding in the 3 white matter areas measured (dorsal, lateral, and ventral funiculi) and the lowest binding over the substantia gelatinosa and central gray matter. Myelin and oligodendrocytes are almost completely absent from the latter 2 regions, and grains over those regions represent background; consequently, optical densities over other areas were corrected for this background. The 3 white matter regions showed similar grain densities (Fig. 7); in order to simplify comparison between different probes, we combined data from the 3 white matter regions.

Analyzed in this way, the densitometric data revealed different developmental patterns of transcript expression (Fig. 8). As expected, white matter always showed greater binding than gray matter. In white matter, MBP exon 1, PLP and CNP probe binding showed relatively little change from 8 to 20 d; by contrast, MBP exon 2 and MAG probe binding decreased dramatically during this period. Binding of all probes decreased in white matter between 20 and 45 d; this decrease was most pronounced for MBP exon 2 and CNP transcripts. The pattern exhibited in gray matter was substantially different. Binding increased dramatically between 8 and 20 d for all probes, including those whose binding to white matter decreased during the same interval (MBP exon 2 and MAG).

Densitometric analysis determines overall binding intensity but gives no information about the clustering of transcripts observed with certain genes. These clusters, which are found around the nuclei of small cells, most likely localized in the perikaryon of individual oligodendrocytes. Grain clusters were most clearly seen in the gray matter, where oligodendrocytes are scattered and can thus be counted easily. We counted oligodendrocytes in the gray matter after immunostaining for CNP and observed an increase of about 2.5-fold in number per spinal cord cross section between days 8 and 20 (data not shown). We then counted the number of grain clusters in gray matter after in situ hybridization with the CNP probe and observed a similar increase. Finally, we compared the number of grain clusters in spinal cord cross sections seen with CNP, PLP, and MAG (Fig. 9). All 3 probes yielded similar developmental curves: the number of clusters visualized was lowest at 8 d, increased several
fold at 20 d, and remained unchanged thereafter (Fig. 9). However, the absolute number of grain clusters detected at each age differed markedly among the 3 probes. This variation may reflect differences between the myelin genes in absolute number of transcript copies per cell and in the degree to which transcripts are confined to the cell body and excluded from processes. As shown in Figure 6, grain clusters in the PLP preparations were intense and more defined than those in the CNP sections, and many clusters in the CNP sections may not have been detected.

Discussion

In this in situ hybridization study of mouse spinal cord, we compared the location and abundance of transcripts coding for 4 CNS myelin protein genes from the myelination period to adulthood. Similar conditions of fixation, cryocutting, and hybridization with single-stranded probes of matched specific activity were rigorously applied to CNS tissues taken at 3 different time points. The quantitative observations made here by in situ hybridization parallel changes in transcript abundance observed by others using Northern blot analysis. In such a molecular analysis, PLP transcripts in adult were approximately 50% less than in 18-d-old mouse brain (Hudson et al., 1987; L. Hudson, personal communication); we found the same decrease in spinal cord with in situ hybridization. Total MBP mRNAs also remain high in adult CNS (Zeller et al., 1984), a pattern consistent with the substantial binding of exon 1 probe to adult spinal cord (Fig. 8). The sharp decrease of exon 2-containing MBP transcripts in the white matter observed here by in situ hybridization was also demonstrated by RNase protection experiments (de Ferra, in preparation).

In situ hybridization revealed a progressive decrease of MAG transcripts in spinal cord white matter with age, although less dramatic than that of MBP exon 2. According to Lai and coworkers (1987), MAG transcripts lacking exon 12 information predominate in the postnatal period while transcripts containing exon 12 predominate in adults. Our probes did not distinguish between these 2 transcripts; it seems likely that the substantial levels observed at 45 d correspond to the “late” MAG mRNAs.

Figure 8. Densitometric measurement of probe binding for different myelin gene transcripts. Grain density was measured for 5 different probes as shown for MBP exon 1 probe in Figure 7. Data for each probe was corrected and normalized independently. The central canal and substantia gelatinosa values were averaged and subtracted from all other values as a correction for background binding (see Fig. 7). All 3 white matter regions were averaged to yield the plotted white matter values ± SE (vertical bars). Ventral horn data is the average of multiple measurements ± SE. All values for a particular probe were normalized as a percentage of the maximal binding (100%). A, MBP exon 1 probe. B, MBP exon 2 probe. C, PLP exon 2 and exon 3 probes. D, MAG exon 7–8 probe. E, CNP 3′ and 5′ probes.

The 35S-labeled probes used in our experiments yielded sufficient resolution to reveal information about the cellular lo-
The increase in gray matter myelin-specific mRNAs parallels an increase in the number of oligodendrocytes (revealed by CNP immunostaining or in situ grain clusters), it seems likely that oligodendrocytes are generated or mature later in gray than in white matter. There is mounting evidence that the different myelination schedules of each tract of the CNS depend on differences in the timing of migration and/or mitosis of the progenitor cell of oligodendrocytes in these specific regions (Small et al., 1987)

In comparing transcript levels in white matter, one can distinguish 2 types of transcripts. MAG and MBP exon 2-containing transcripts are maximal at 8 d, a time when most tracts are starting to myelinate in the spinal cord, and are decreased substantially at 20 d. In contrast, PLP and MBP transcripts devoid of exon 2 information remain at a high level or increase between 8 and 20 d, and decrease moderately thereafter. CNP transcripts exhibit an intermediate pattern; at 20 d the average white matter binding is equal to that at 8 d (Fig. 8), but individual regions show substantial variation (Fig. 5). This difference in developmental pattern suggests that the level of expression of different transcripts is regulated independently. MAG has been implicated in initial recognition and adhesion between axons and oligodendrocyte processes (reviewed by Trapp and Quarles, 1984; Quarles, 1985). Exon 2 of MBP codes for a sequence of amino acids whose folding might be critical for interaction with myelin lipids and/or PLP in the early events of myelination (discussed in Kamholz et al., 1986). Thus, both MAG and MBP exon 2 transcripts might be critically required in the early events of myelination. On the other hand, PLP and mature forms of MBP make up the bulk of proteins within the myelin sheath (reviewed by Lees and Brostoff, 1984); they undergo some turnover in the adult and may therefore require sustained synthesis of their transcripts for both myelin compaction and maintenance.

Thus, in situ hybridization for myelin genes can be used as a reliable and convenient way to analyze gene expression at the level of single cells and fiber tracts in vivo. It provides information on in vivo events of differentiation and cellular and topographic organization not available from Northern blot analysis. Our approach also appears well suited to the analysis of myelin gene transcripts in focal lesions during the course of a demyelinating disease (Jordan et al., unpublished observations).

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