Cellular and Molecular Aspects of Myelin Protein Gene Expression

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Contents

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Abstract

The cellular and molecular aspects of myelin protein metabolism have recently been among the most intensively studied in neurobiology. Myelination is a developmentally regulated process involving the coordination of expression of genes encoding both myelin proteins and the enzymes involved in myelin lipid metabolism. In the central nervous system, the oligodendrocyte plasma membrane elaborates prodigious amounts of myelin over a relatively short developmental period. During development, myelin undergoes characteristic biochemical changes, presumably correlated with the morphological changes during its maturation from loosely-whorled bilayers to the thick multilamellar structure typical of the adult membrane.

Genes encoding four myelin proteins have been isolated, and each of these specifies families of polypeptide isoforms synthesized from mRNAs derived through alternative splicing of the primary gene transcripts. In most cases, the production of the alternatively spliced transcripts is developmentally regulated, leading to the observed protein compositional changes in myelin. The chromosomal localizations of several of the myelin protein genes have been mapped in mice and humans, and abnormalities in two separate genes appear to be the genetic defects in the murine dysmyelinating mutants, *shiverer* and *jimpy*. Insertion of a normal myelin basic protein gene into the *shiverer* genome appears to correct many of the clinical and cell biological abnormalities associated with the defect. Most of the dysmyelinating mutants, including those in which the genetic defect is established, appear to exhibit pleiotropy with respect to the expression of other myelin genes.

Post-translational events also appear to be important in myelin assembly and metabolism. The major myelin proteins are synthesized at different subcellular locations and follow different routes of assembly into the membrane. Prevention of certain post-translational modifications of some myelin proteins can result in the disruption of myelin structure, reminiscent of naturally occurring myelin disorders. Studies on the expression of myelin genes in tissue culture have shown the importance of epigenetic factors (e.g., hormones, growth factors, and cell–cell interactions) in modulating myelin protein gene expression. Thus, myelinogenesis has proven to be very useful system in which to examine cellular and molecular mechanisms regulating the activity of a nervous system-specific process.

Index Entries: Myelin; protein; gene expression; post-transitional events; isoforms; in whole brain, developmental changes.

Nomenclature

bp	Base pair(s)	MBP	Myelin basic protein
cDNA	Complementary DNA	nt	Nucleotide(s)
CNP	2',3'-cyclic nucleotide 3'-phosphodiesterase	PAGE	Polyacrylamide gel electrophoresis
		PLP	(30 kdalton myelin) proteolipid protein
CNS	Central nervous system	PNS	Peripheral nervous system
DM20	25 kdalton myelin proteolipid protein	RIA	Radioimmunoassay
GC	Galactosyl Ceramide	SCD	Subacute combined degeneration
G_{M4}	Sialosylgalactosylceramide	SDS	Sodium dodecyl sulfate
MAG	Myelin associated glycoprotein	T_3	Triiodothyronine

Introduction

Myelin and its components have been the subject of extensive study for the past several decades; thus, the physical, chemical, and biological properties of the major myelin proteins are well known. It is not surprising, therefore, that the myelin protein genes and their expression have been among the first to be studied intensively by cellular and molecular neurobiologists. Since several excellent reviews on the structure and metabolism of myelin and its components have appeared recently (*see* Morell, 1984; Norton, 1984) this review will focus primarily on molecular and cellular aspects of the expression of myelin protein genes in vivo and in vitro in the central nervous system.

Myelination is a major event in the development of the nervous system. In the CNS of rats and mice it occurs postnatally and follows, in a concerted fashion, a period of rapid proliferation of oligodendroglial cells. Myelin and/or its components can be detected in the spinal cord of humans during the second trimester of gestation (Niebroj-Dobosz et al., 1980; Kronquist et al., 1987) and, in rat and mouse brain, within a few days after birth (Sternberger, 1984). Myelination generally proceeds from the top of the spinal cord and the base of the brain toward the frontal areas of the brain.

In the central nervous system, myelin appears to be a specialized extrusion of the oligodendroglial cell plasma membrane, which wraps around axonal segments (Peters et al., 1970). Unlike the peripheral nervous system where there is close proximity of the Schwann cell and the myelin sheath that it produces, the oligodendrocyte in the CNS may be some distance from the myelin sheath that it elaborates, connected only by a slender cytoplasmic process. A single oligodendrocyte may myelinate multiple axons at varying distances from the cell body, and these axons may be part of different fiber tracts (Sternberger et al., 1978a).

The major myelin proteins, representing 70–80% of the protein content of the membrane, fall into two classes—the basic proteins and the proteolipid proteins. Both of these classes consist of multiple polypeptide chains derived through altenative splicing of a single gene. No systematic method has been adopted yet for naming the isoforms of the two classes, in part because their actual structural and genetic relationships to each other have been determined only recently.

The myelin basic protein (MBP) is composed of at least six isoforms in mice (deFerra et al., 1985; Newman et al., 1987a,b) and four isoforms in humans (Kamholz et al., 1986; Roth et al., 1986, 1987). These have come to be referred to by their apparent molecular weights on SDS-polyacrylamide gels or by their masses obtained through protein or cDNA sequencing studies (e.g., 14, 17, 18.5 , 20 , and 21.5 kdalton MBP). Other names such as SBP or B_f (for the 14 kdalton MBP), LBP or B_s (for the 18.5 kdalton), prelarge (for the 21.5 kdalton MBP) and presmall (for the 17 kdalton MBP) also appear in the literature. In view of what is now known about the structure and metabolism of these proteins, these latter designations seem somewhat inappropriate.

The two isoforms of the proteolipid protein are generally referred to as the myelin proteolipid protein (PLP) [mol mass = 30 kdalton] and the DM20 protein [mol mass = 25 kdalton]. The structural relationship between these proteins has been established only recently through cDNA analysis, clearing up some confusion about the relationship of these proteolipids to each other. Other, lower molecular weight, proteolipid proteins, apparently related to the major myelin PLP, have been observed in proteolipid preparations isolated from brain (Chan and Lees, 1974; Macklin et al., 1983, 1984; Lepage et al., 1986) and synthesized in vitro with brain mRNAs (Sorg et al., 1986). Their structural relationship to the myelin

PLPs only now is beginning to emerge, but has not been exactly defined yet (Lepage et al., 1986).

No function other than a structural role has been attributed to these two major classes of myelin proteins. Both have unusual chemical and physical properties. The proteolipids are integral membrane proteins, constituting about 50% of the total protein content of myelin (Eng et al., 1968). They are extremely hydrophobic and aggregate relatively easily under a variety of experimental manipulations (Agrawal and Hartman, 1980; Lees, 1982). The PLP undergoes post-translational fatty acylation such that it contains approximately 2–4% by weight fatty acid covalently attached to the apoprotein, which further contributes to its hydrophobicity.

The myelin basic proteins are hydrophilic, extrinsic membrane proteins with isoelectric points greater than 10.6, making them even more basic than the histones. They have been localized, immunohistochemically, to the major dense line of myelin that is formed by apposition of the cytoplasmic surfaces of the extruded oligodendroglial plasma cell membrane during myelinogenesis. Unlike the proteolipids, which are believed to be transmembrane proteins traversing the unit bilayer, the myelin basic proteins appear to remain associated with the cytoplasmic side of the unit bilayer. The MBPs undergo a number of post-translational modifications, including *N*-terminal acetylation, phosphorylation, and methylation. The physiological significance of these post-translational modifications is not at all clear at the present time.

A number of other proteins and enzymes are found to be associated with myelin, making up the remainder of the 20–30% of its protein content (Lees and Sapirstein, 1983). All of these appear to be of higher molecular weight and lower concentration in myelin than the proteolipid and basic proteins. Establishing that these minor proteins were, in fact, components of myelin was a complex process; but two of these proteins that are of particular relevance to this review are the myelin-associated glycoprotein (MAG) and 2':3'-cyclic nucleotide 3'-phosphodiesterase (CNP).

MAG is the major glycoprotein associated with CNS myelin, constituting approximately 1% of the total myelin protein (Quarles, 1979). The fully glycosylated MAG has an apparent mol mass of about 100 kdalton on SDS gels, approximately 30% of which is carbohydrate (Quarles et al., 1983). The polypeptide portion of MAG consists of two isoforms with apparent mol masses of 72 and 67 kdalton (Frail and Braun, 1984), although their actual masses, determined recently from sequence analysis of the cDNAs encoding them were 69.3 and 64.2 kdalton (Salzer et al., 1987). For purposes of consistency with the literature, however, they will be referred to as the 72 and 67 kdalton MAG polypeptides in this review. A significant portion of the MAG in myelin is exposed to the extracellular surface of the unit bilayer (Poduslo et al., 1976) and it shares a carbohydrate determinant with a number of molecules proposed to mediate cell–cell interactions in the nervous system such as *N*-CAM, L1, J1, and ependymins (Salzer et al., 1987; Kruse et al., 1984, 1985; McGarry et al., 1983; Shashoua et al., 1986; Holley and Yu, 1987). The physiological role of MAG in myelin is not clear, but it has been proposed to be involved in the association of the myelin membrane with the axon (Quarles, 1983, 1984). Immunohistochemical localization data from several groups are consistent with this hypothesis (Martini and Schachner, 1986; Sternberger et al., 1979; Trapp and Quarles, 1982; Trapp et al., 1984), and recent sequence data show that MAG is related in structure to cell adhesion molecules (Salzer et al., 1987). This interpretation has been challenged, however, based on contradictory findings that suggest that MAG is localized primarily within the myelin lamallae rather than in the periaxonal regions (Webster et al., 1983).

CNP [E.C. 3.1.4.37] catalyzes the hydrolysis of several 2',3'-cyclic nucleoside monophosphates. The activity of the enzyme is very high in the central nervous system where it has been shown to be a myelin-associated enzyme (Kurihara and Tsukada, 1967, 1968; Olafson et al., 1969). The purified enzyme consists of two polypeptide chains (Drummond, 1979; Sprinkle et al., 1980a), which appear to comprise a significant portion of the "Wolfgram protein" fraction of myelin (Drummond and Dean, 1980; Sprinkle et al., 1980b), a loosely defined, heterogeneous group of high molecular weight proteins, originally obtained by fractionation of myelin (Wolfgram, 1966). These two Wolfgram polypeptides have been shown to share identical molecular, chemical, and immunological characteristics with the enzyme (*see* Lees and Sapirstein, 1983).

In summary, all the well-studied myelin proteins appear to exist in at least two molecular isoforms which, in turn, have been shown to be the translation products of separate mRNAs probably derived through alternative splicing mechanisms.

Myelin Protein Gene Expression in the Developing Brain

Summarizing the studies performed to date on the expression of the myelin protein genes in the developing CNS is difficult because rarely have more than two or three proteins been examined at a given time. This is, in part, because of the fact that the immunological and recombinant DNA probes for all the myelin proteins either had not been developed yet or they were not available to a laboratory at the time the study was performed. Also, fundamental information, such as the numbers and structures of the myelin protein isoforms, has become available only recently and might not have been known at the time the study was executed. This particularly true for the myelin basic proteins where the multiplicity and structures of the isoforms were only established through cDNA analysis; and very likely the same will be true for the proteolipid proteins. For example, in the early 1970s it was thought that humans contained only one MBP isoform and that species belonging to the suborders *Myomorpha* and *Sciuromorpha* (e.g., rats and mice) contained two myelin basic protein isoforms. We now know that humans contain at least four MBP isoforms and that mice contain at least six. One reason why it has taken so long to determine the numbers of MBP isoforms is that their numbers and proportions differ significantly from species to species, from one region of the CNS to another, and from age to age with development. Since most of the isoforms are of lower molecular weight than the 18.5 kdalton MBP it was difficult to rule out the possibility that a band on a polyacrylamide gel was not a degradation product. In addition, the quality of the separation of myelin proteins in the early SDS polyacrylamide gel systems was often not as good as in later studies and closely migrating bands were overlooked. In the case of the myelin proteolipid proteins, because of the intractability of these highly hydrophobic proteins, it was unclear for some time whether the DM20 protein was a conformational isomer of the PLP or whether PLP and DM20 were, possibly, different multimeric aggregates of a common subunit. Only very recently, with the determination of the amino acid sequences of the two proteins through cDNA analysis, has it been possible to determine unambiguously that the two forms are structurally related and the products of the same gene. Thus, many of the findings related to expression of myelin protein genes are incomplete in view of current knowledge. Nonetheless, general patterns have emerged with respect to the relative expression of the major classes of myelin proteins and of the various myelin protein isoforms.

Developmental Changes in Isolated Myelin

Prior to the studies of Smith and her coworkers in the late 1960s demonstrating that myelin components within the membrane turned over at measurable rates (Smith, 1968), myelin was considered to be an essentially inert membrane. About the same time, interest grew in developmental changes in myelin protein composition, and work from a number of laboratories proved that the composition of the myelin changed with age in a predictable fashion (for a review, *see* Norton and Cammer, 1984). With development, myelin isolated from the brains of rats and mice became increasingly enriched in the major myelin proteins relative to the higher molecular weight polypeptides. In addition, it has been consistently noted that the 14:18.5 kdalton MBP ratio increased considerably with age (Einstein et al., 1970; Morell et al., 1972; Adams and Osborne, 1973; Magno-Sumbilla and Campagnoni, 1977). No clear consensus has emerged with respect to changes in the ratios of proteolipids to basic proteins with development (Banik et al., 1974; Morell et al., 1972; Magno-Sumbilla and Campagnoni, 1977). Although the proportion of the high molecular weight proteins has been observed to decrease with age, the levels of MAG and Wolfgram proteins/mg total myelin protein have been reported to remain constant from 14–60 d (Druse et al., 1974) and 10–300 d, respectively (Morell et al., 1972).

Developmental Changes in Whole Brain

In addition to myelin protein compositional changes occurring within the membrane as a function of age, studies on the developmental expression of myelin proteins in whole brain have largely fallen into two categories: (a) those measuring changes in the proteins either through enzymatic activity, biochemical isolation of the protein, or immunoblot analysis; and (b) those measuring synthesis of the myelin proteins in vivo and in vitro (i.e., cell-free synthesis) or by titering the mRNAs with cDNA probes. Clearly, all these measurements yield important information generally related to expression of the myelin protein genes, but individual techniques measure different aspects of "expression."

Enzyme activity measurements and estimates of the levels of individual myelin proteins by biochemical purification or from immunoblot analysis measure accumulation of these proteins, not rates of synthesis. Estimates of the levels of myelin proteins at different ages reflect, primarily, their accumulation during development. Whereas there is a tendency to consider mRNA levels as a measure of the capacity of the cell to synthesize the proteins, in fact, the specific mRNAs measured by dot blot and Northern blot analysis may not all be translatable. An additional complication can result if the cDNA probe hybridizes with a related, but not identical, transcript derived, for example, by alternative splicing mechanisms. Indirect estimates of mRNA levels by cell-free translation may be complicated by differences in translational efficiencies in the mRNAs. Thus, these caveats should be kept in mind in attempting to compare studies on the developmental "expression" of myelin proteins.

A number of workers have attempted to define the patterns of myelin protein gene expression in the developing brains from several species. There has been considerable interest in the temporal expression of the individual myelin proteins with respect to each other. The data from several laboratories indicate that maximal developmental expression of MBP occurs 16–20 d postnatally in mice as determined by in vivo synthesis measurements (Campagnoni et al., 1978), by translation of brain mRNAs in cell-free systems (Carson et al., 1983; Roth et al., 1985), and by dot blot and

Northern blot analysis (Miskimins and Yu, 1986; Roth et al., 1985; Sorg et al., 1987; Zeller et al., 1984; Gardinier et al., 1986).

In a study comparing CNP activity and levels of MBP as determined by radioimmunoassay, Sprinkle et al. (1978), reported a direct correspondence between the levels of the enzyme and MBP over development, suggesting identical developmental profiles in the rat. In the developing chick brain, the appearance of CNP enzyme activity appeared to lag behind MBP and PLP protein accumulation on immunoblots (Macklin and Weill, 1985). In the developing mouse brain, Monge et al., (1986) reported the immunohistochemical detection of Wolfgram proteins (including CNP) 4 d prior to the appearance of MBP. This group also has performed a study using cDNA probes for CNP and observed mRNA for the protein during embryonic development in the rat brain (Kanfer et al., submitted). Their studies show that there is variability in the temporal expression of mRNAs for MBP, PLP, and CNP among brain regions. In their studies, however, most regions had peaks of mRNA levels for CNP at about 15 d and for MBP at 18 d.

In vivo synthesis studies indicate that the developmental profile of PLP synthesis peaks a few days later than that of MBP synthesis in the mouse brain, i.e., 21 or 22 d for PLP vs 18 d for MBP (Campagnoni and Hunkeler, 1980). These findings were confirmed by Northern and slot blot analysis of *polysomal* mRNA with cDNA probes. However, *nuclear* PLP and MBP RNA levels were both maximal at 18 d, suggesting the possibility that there may not be a perfect correlation between the transcriptional activity of the PLP gene in the nucleus and the synthesis of PLP in the cytoplasm. The slight difference in timing of expression of PLP and MBP has been noted in the developing mouse brain by immunocytochemical methods (Monge et al., 1986), in the developing chick brain by immunoblot analysis (Macklin and Weill, 1985), in the developing human spinal cord by immunoblot and Northern blot analysis (Kronquist et al., 1987), and in many regions of the developing rat brain by Northern and dot blot analysis (Kanfer et al., submitted). In the developing rat optic nerve, Tennekoon et al. (1977) observed that the rapid developmental increase in RIA-detectable MBP preceded the increase in chloroform–methanol soluble proteolipids by several days. In a recent study of rat whole brain homogenates during development, MBP and PLP were found to appear concomitantly on immunoblots (Macklin et al., 1983, 1984).

Expression of MAG has been examined in number of ways. In early work, MAG was detected by in vivo fucose labeling, isolation of myelin, and separation of the proteins by SDS–PAGE. Using this approach, Matthieu et al., (1974) were able to detect a fucose labeled glycoprotein in isolated myelin as early as myelin could be isolated from the immature rat and mouse brain (7–9 d). The mouse MAG mRNA has been detected by in vitro translation at 10 d (Frail and Braun, 1984) and rat MAG mRNA has been detected on Northern blots at the same age (Sutcliffe et al., 1983) or a few days earlier (Lai et al., 1987), although Salzer et al., (1987) reported that they were only able to detect the rat MAG mRNA in significant concentrations at 14 d. The concentration of MAG mRNA appears to increase in the brains of both species until about 25–27 d after which it declines (Frail and Braun, 1984; Matthieu et al., 1986; Salzer et al., 1987; Sutcliffe et al., 1983). In the developing human fetal spinal cord, immunodetectable MAG of the appropriate molecular size was not detected until 31 wk after conception (Marton and Stefansson, 1984), at least 13 wk after PLP and 15–17 wk after MBP can be detected on immunoblots (Kronquist et al., 1987). Thus, in spite of the popular notion that MAG may play an important role in the early interaction between the growing myelin sheath and the axon, the developmental data suggest that the protein is

not expressed earlier than the major myelin proteins (MBP and PLP), and that the developmental peak of its synthesis is somewhat later than that of MBP.

In summary, the in vivo data suggest that in most species PLP is expressed slightly later than MBP by 1 or 2 d, MAG is expressed no earlier than MBP and probably later, and CNP mRNA is evident in rat brain before MBP mRNA whereas in the chick brain its enzymatic activity appears after MBP polypeptides. The lack of agreement among the various studies with respect to the temporal expression of these proteins may be resulting from a number of factors. Some of it may be because of differences in the species and/or brain regions examined. There is some evidence that differences may exist in the ratios of myelin protein polypeptides from one region of the nervous system to another (Morell et al., 1973; Newman et al., 1987b; Lees and Paxman, 1974) and it is possible that there may be regional variation in the timing of the expression of the myelin protein genes as suggested by the work of Kanfer et al., (submitted).

Investigators have used different criteria to define the order of expression of myelin proteins. Some workers have attempted to rely on the earliest age at which a protein could be detected to determine its order of expression. However, differences in the sensitivities of antibodies, or the specific activities of cDNA probes could lead to misleading results. Another complication is the recent finding that alternative splicing mechanisms may generate related but not identical mRNAs containing common sequences but encoding other proteins (Breibart et al., 1987). Thus, early detection of mRNAs hybridizing to myelin protein cDNAs may be detecting structurally-related, but not identical, mRNAs. The same problems exist with antibodies that might react with related but separate proteins.

In order to determine the temporal expression of myelin proteins, other workers have compared the developmental curves of the different myelin proteins. In such studies, if the overall developmental curve of a protein generally lagged behind that of another protein, then its expression was considered to be delayed even if the first protein appeared to be present in slightly higher levlels at the earliest age examined. Clearly, all methods of analysis have a degree of uncertainty and it may be difficult to establish with absolute certainty the order of expression of those myelin protein components expressed within a day or two of each other. It is important to bear this in mind when interpreting the results.

Developmental Expression of Myelin Protein Isoforms

Studies performed almost a decade ago on the in vivo synthesis of the MBPs indicated that the developmental increase in the 14/18.5 kdalton MBP ratio in myelin was reflected in the relative rates of synthesis of the two proteins (Campagnoni et al., 1978). Thus, these and the studies on developmental alterations in the protein patterns of isolated myelin established some time ago that there was a form of developmental regulation over the expression of the MBP gene, although its nature and complexity was far from appreciated. Defining the number and nature of the MBP isoforms has taken a fair amount of time to sort out, for reasons outlined above. Sequence analysis proved that the rat 14 kdalton MBP was structurally similar to the 18.5 kdalton bovine and human MBPs except for the deletion of a 40 amino acid sequence within the interior of the molecule (Dunkley and Carnegie, 1974), a structural relationship proposed somewhat earlier on the basis of peptide "fingerprinting" studies of the rat proteins (Martenson et al., 1972). In retrospect, there was evidence for the presence of other MBP variants (Berlet and Volk, 1980; Newcombe et al., 1982; Kerlero de Rosbo, 1984; Agrawal et al., 1986; Sorg et al.,

1986), but, at the time, it was difficult to rule out the possibility that these were breakdown products of bona fide MBPs.

The extent of the multiplicity of the MBP isoforms was not fully appreciateduntil immunochemical and recombinant DNA approaches were used to study the problem. In the late 1970s, immunochemical techniques revealed the presence of four mouse MBPs with apparent mol masses of 14, 17, 18.5, and 21.5 kdalton (Barbarese et al., 1977). After some initial confusion about whether the 21.5 and 17 kdalton MBPs were precursors to the 18.5 and 14 kdalton MBPs, respectively, several laboratories showed that each of the MBP isoforms was encoded by a separate mRNA (Colman et al., 1982; Yu and Campagnoni, 1982; Carson et al., 1983). Analysis of SDS-PAGE patterns of myelin polypeptides indicated that at 15 d mouse myelin is proportionately richer in the 21.5 and 17 kdalton MBPs relative to the other MBP isoforms than mouse myelin at 60 d (Barbarese et al., 1978). Subsequent work using immunoblots of whole brain to measure the different MBP isoforms, and cell-free synthesis studies of brain mRNA to measure the levels of individual MBP mRNAs, indicated that each isoform exhibited its own developmental pattern of expression and accumulation (Carson et al., 1983). The general pattern appears to be that the 21.5 kdalton protein is expressed to a greater extent during early brain development and that it is found in proportionately higher concentrations in myelin isolated from the brains of young rodents. With age, the proportion of the 21.5 and the 17 kdalton MBPs falls relative to the 18.5 and 14 kdalton isoforms.

There is now evidence for the existence of a number of other MBP isoforms. For example, a second mouse 17 kdalton MBP has recently been identified (Newman et al., 1987a), so our current knowledge about the metabolism of the mouse 17 kdalton MBP actually reflects the metabolism of a mixture of the two isoforms. This second 17 kdalton MBP is, apparently, the only form present in humans (Kamholz et al., 1986; Roth et al., 1986,1987; Deibler et al., 1986). A 20 kdalton MBP has been identified in fetal human spinal cord, and immunoblots suggest that it exists in other species as well (Kerlero de Rosbo et al., 1984), although it may be more prevalent in some regions of the CNS than others (Newman et al., 1987b). It appears to be expressed to a high degree in the fetal human spinal cord (Roth et al., 1987; Kronquist et al., 1987) and less so in newborn human brain (Kamholz et al., 1986). In rat brain, Agrawal et al. (1986) have identified a 23 kdalton phosphorylated protein that is immunologically related to MBP. Immunoblots of whole brain homogenate with both polyclonal (Carson et al., 1983) and monoclonal (Bansal et al., 1987) anti-MBP antibodies indicate the presence of higher molecular weight immunoreactive proteins. The metabolic relationship among all these proteins and the more well-characterized MBP isoforms is not clear at the present time.

The most well studied of the myelin proteolipids are PLP, the major form found in myelin, and DM20, a quantitatively less abundant form (Agrawal et al., 1972). These proteolipids have been identified in a number of species (Lees and Macklin, 1988) and a variety of recent data clearly indicate that the polypeptides are structurally related in the same way that the MBP isoforms are related to each other (Trifilieff et al., 1986; Nave et al., 1987a; Macklin et al., 1987). In vitro synthesis experiments have shown that these proteolipids are the products of separate mRNAs (Dautigny et al., 1983; Sorg et al., 1986) and that these mRNAs are derived by alternative splicing of the transcript of a single gene (Nave et al., 1987; Macklin et al., 1987). Very recently, immunoblots have shown that the appearance of the DM20 protein seems to precede the appearance of PLP in the developing human spinal cord (Kronquist et al., 1987) and in the developing mouse brain (Gardinier and Macklin, submitted). Quantitatively, the DM20 isoform is present in much lower abun-

dance than PLP in the adult brain (~ 10–20% of PLP) but at 18 wk after conception it is the principal, if not the only, isoform present in the human spinal cord. Several studies have reported the presence of multiple proteolipid polypeptides in mouse, rat, and bovine brain (Lerner et al. 1974; Nussbaum and Mandel, 1973; Campagnoni et al., 1976; Chan and Lees, 1974; Lepage et al., 1986). Very recently, Lepage et al. (1986) have used fast atom bombardment–mass spectrometry to characterize two proteolipid polypeptides with apparent mol masses of 14 and 16 kdalton. The 14 kdalton proteolipid contains six peptides (corresponding to a total of 35 amino acids) that are identical to peptides derived from the first 113 amino acids of the myelin PLP plus an extra unknown blocked *N*-terminal peptide. The 16 kdalton proteolipid contains eight peptides (corresponding to a total of 67 amino acids) belonging to a C-terminal fragment of the PLP. These data suggest the presence of additional proteolipids in the brain that share common structural features with the myelin proteolipids. Supporting this notion are immunoblot data that reveal the presence of lower molecular weight proteolipids in rat brain that crossreact with anti-PLP antisera (Macklin et al., 1983, 1984), and cell-free protein synthesis data in which proteins of similar molecular weights have been immunoprecipitated from reticulocyte lysates programmed with mouse brain mRNA (Sorg et al., 1986). These data suggest that the myelin proteolipid protein gene, like the basic protein gene, may encode a more extensive family of proteins than has been recognized thus far.

Both MAG and CNP exist as two polypeptide isoforms, each encoded by a separate mRNA (Lai et al., 1987; Salzer et al., 1987; Bernier et al., 1987). Early studies by Matthieu et al. (1974) indicated that there was developmental shift in the glycosylated form of MAG in rats. The protein was detected by in vivo fucose labeling and SDS-PAGE of myelin, and it has been consistently observed that, in rats, glycosylated MAG in immature myelin has a higher apparent molecular weight than in mature myelin. This shift in apparent molecular mass was evident in rats, gerbils, and hamsters, but not in mice. Cell-free translation studies have shown that there are two mouse MAG mRNAs (Frail and Braun, 1984; Matthieu et al., 1986), each encoding a separate MAG apoprotein with apparent molecular masses of 67 and 72 kdalton. The proportions of these mRNAs change with development such in that cell-free translations of 15–18 d brain mRNA, the 72 kdalton MAG apoprotein is the predominant immunoprecipitated product, whereas in translations programmed with brain mRNA from older ages (>50 d), the 67 kdalton MAG apoprotein is the predominant immunoprecipitated product (Frail and Braun, 1984; Matthieu et al., 1986). Thus, even though there is a clear developmental shift in the MAG apoprotein isoform expressed, the ultimate size of the glycosylated protein as determined by fucose labeling and SDS-PAGE, is very similar for both apoproteins. Very recently, it has been shown in rats that there also exist two MAG mRNAs and translated products (Salzer et al., 1987). Whether or not the developmental shift observed in glycosylated MAGs in the rat reflects differences in the glycosylation of the two apoproteins or two different levels of glycosylation for each of the apoproteins remains to be established.

The two isoforms of CNP appear to have mol masses of 44–48 kdalton, depending upon the species (Karin and Waehneldt, 1985) and they are apparently the products of separate, alternatively spliced mRNAs (Bernier et al., 1987). Two CNP bands have been observed in immunoblots of myelin from mouse, rat, human, and pig and their sizes appear to differ slightly among species (Karin and Waehneldt, 1985). No major difference has been reported

in their developmental expression, relative to each other, although this has not been examined extensively (Waehneldt, 1975).

Myelin Protein Gene Structure and mRNAs

Very recently, the genes coding for several of the myelin proteins have been isolated and their structures determined (deFerra et al., 1985; Diehl et al., 1986; Sutcliffe et al., 1987; Takahashi et al., 1985; Macklin et al., 1987). The system now represents one of relatively few neurobiological systems that can be studies at the molecular genetic level. Ironically, one of the reasons many investigators found myelinogenesis an attractive neurobiological process to study from the molecular biological standpoint was its relative simplicity with respect to its protein components (Campagnoni, 1985). However, the molecular biological studies have shown that the numbers of major myelin protein isoforms, and the regulation of their expression, is far more complex than originally expected. Nonetheless, in the past several years a number of important advances have been made in our understanding of the myelin protein genes, their structures, and their expression in several organisms and in dysmyelinating mutant mice.

Myelin Basic Protein

Cell-free translations of mouse and rat brain mRNA indicated that the four major isoforms of the myelin basic proteins were encoded by separate MBP mRNAs (Yu and Campagnoni, 1982; Colman et al., 1982) and this logically led to investigations into whether or not more than one gene coded for the multiple MBP isoforms. An approach taken by several laboratories (Roach et al., 1983; Zeller et al., 1984; Mentaberry et al., 1986; Roth et al., 1986) was to isolate cDNAs for the mouse MBP mRNAs in order to determine the structural relationship among the MBP isoforms through an analysis of their cDNAs and to use the cDNAs to determine the number, location, and structure of the MBP gene(s).

The gene has been mapped by both *in situ* hybridization and somatic cell hybridization techniques to the distal end of chromosome 18 in both the mouse (Roach et al., 1985) and human (Saxe et al., 1985; Sparkes et al., 1987). The mouse MBP gene is very large, extending over 30 kb. It consists of seven exons, some of which are as small as 33 bp in length, and which are interrupted by introns that can be quite large (Takahashi et al., 1985; deFerra et al., 1985). Southern blot analysis indicates the presence of only a single gene in the mouse (Takahashi et al., 1985; deFerra et al., 1985) and the human (Kamholz et al., 1986).

Several laboratories have isolated cDNAs of mRNAs encoding a wider variety of MBP isoforms than had been anticipated (Roach et al., 1983; deFerra et al., 1985; Kamholz et al., 1986; Roth et al., 1986, 1987; Newman et al., 1987a). For example, cDNAs of mRNAs encoding five forms of the mouse MBP now have been isolated. These cDNAs are derived from mRNAs that arise through the alternative splicing of three exons (the second, third, and sixth) from the MBP gene primary transcript. These cDNAs encode MBP isoforms with mol masses of approximately 21.5, 18.5, 17, and 14 kdalton. Complementary DNAs have been isolated that encode two different 17 kdalton MBP isoforms that are so close in mass that the proteins would be indistinguishable by polyacrylamide gel electrophoresis. From the abundance of the two 17 kdalton MBP cDNA clones in a cDNA library, it appears that one of the forms is present in greater abundance than the other at 18 d. Of five cDNA clones encoding a 17 kdalton mouse MBP isolated in one study (Newman et al., 1987a), one was missing exons 2 and 5 and four were missing exon 6. A sixth MBP isoform

with a mass of ~20 kdalton (missing peptide sequence encoded by exon 5) also has been detected by immunoblotting in mouse CNS, but the cDNA encoding this isoform only has been isolated from humans (Roth et al., 1987). Unpublished data suggest that there may be even more alternatively spliced forms of MBP cDNAs than have been reported to date, although they seem to represent relatively minor forms in mouse 18–20 d cDNA libraries (Kitamura, Newman, Kerlero de Rosbo, and Campagnoni, unpublished results).

Four MBP cDNAs encoding human MBP isoforms with masses of approximately 21.5, 20, 18.5, and 17 kdalton have been isolated from fetal spinal cord and newborn brain cDNA libraries (Kamholz et al., 1986; Roth et al. 1986, 1987). The mRNAs represented by these cDNAs are apparently derived by alternative splicing of exons 2 and 5 of the MBP gene primary transcript. The 20 kdalton MBP mRNA is missing exon 5; the 18.5 kdalton MBP mRNA is missing exon 2, and the 17 kdalton MBP mRNA is missing exons 2 and 5. The human 17 kdalton MBP mRNA is analogous to that present in 18 d old mouse brain in only minor amounts. In their screens of human cDNA libraries, neither Kamholz et al. (1986) nor Roth et al. (1987) found a human cDNA that was missing exon 6. Thus, exon 6 is probably not spliced out in the processing of human MBP premRNA, in contrast to mouse where it appears that at least three exons (i.e., exons 2, 5, and 6) can be alternatively spliced. This putative difference in the MBP mRNA splicing pathways of mouse and man is noteworthy since it provides an example of a difference in the alternative splicing pathway of the same gene between species.

All of the MBP mRNA structures published thus far contain a relatively short 5' untranslated region (<48 nt) and a very long 3' untranslated region (>1 kb). On Northern blots, the MBP mRNAs migrate as a broad band around 2.0–2.4 kb, presumably reflecting the heterogeneity of the MBP mRNA population. There are two polyadenylation signals near the 3' end of both mouse and human MBP mRNAs, and in humans both appear to be used, resulting in mRNAs that have slightly different 3' ends (Roth et al., 1987). In addition, there is a second AUG codon in the 5' untranslated region, 5 bases upstream of the initiator codon, which is immediately followed by a termination codon. This structural feature has been postulated to explain the poorer translation initiation efficiencies exhibited by MBP mRNAs relative to brain mRNAs as a whole (Campagnoni et al., 1987a).

Proteolipid Protein

Proteolipid protein cDNAs have been isolated from rat (Dautigny et al., 1985; Milner et al., 1985; Gardinier et al., 1986), mouse (Hudson et al., 1987; Nave et al., 1986; Sorg et al., 1987), bovine (Naismith et al., 1985), and human (Fahim and Riordan, 1986; Kronquist et al., 1987) species. The mRNA for the proteolipid protein is heterogeneous and consists of a family of mRNAs with the most common members being approximately 1.5–1.6, 2.4–2.6, and 3.0–3.4 kb in length. In the rat, 1.6 and 3.2 kb PLP cDNAs have been cloned and they are identical except for the length of the 3' untranslated region (Milner et al., 1985). The origin of the 1.6 kb PLP mRNA is presumably related to the presence of an alternate polyadenylation signal in the 3' untranslated region. However, such a signal is also present in the human PLP mRNA (Kronquist et al., 1987) and very little 1.6 kb PLP mRNA is detectable in this species (Campagnoni et al., 1987b; Kronquist et al., 1987). Furthermore, in the mouse, the major forms of the PLP mRNA are 2.4–2.6 and 3.0–3.4 kb in length (Gardinier et al., 1986; Sorg et al., 1987; Nafe et al., 1986) and the 1.6 kb PLP mRNA is present in lesser amounts. Thus, the relative proportion of the members of the PLP family of

mRNAs appears to differ from species to species, suggesting that some factor in addition to the presence of a polyadenylation signal is important in determining a polyadenylation site.

Nave et al. (1987a) have recently isolated a near full-length cDNA of the 2.4kb mouse mRNA which encodes the DM20 protein. The sequence of this cDNA is identical to the PLP mRNA except for the deletion of 105 bp within the coding region of the molecule corresponding to amino acid residues 116–150 of the PLP. Independently, Macklin et al. (1987) have arrived at essentially the same conclusion through S1 nuclease protection experiments. These findings complement and prove the cell-free protein synthesis work indicating that the two proteolipids were the products of separate mRNAs (Dautigny et al., 1983; Sorg et al., 1986) and the peptide mapping and immunological data (Trifilieff et al., 1985,1986) predicting that the DM20 was identical to the PLP except for a deletion of approximately 40 amino acid residues in this region of the molecule. At the moment, it is unclear whether DM20 mRNAs are represented in all the members of the family of PLP mRNAs or whether they are confined to the 2.4–2.6 kb class.

The myelin proteolipid protein gene has been assigned to the human and mouse X chromosomes using somatic cell hybridization (Willard and Riordan, 1985) and chromosomal *in situ* hybridization techniques (Mattei et al., 1986). The gene now has been isolated and characterized in both species (Diehl et al., 1986; Macklin et al., 1987), and it contains seven exons distributed over approximately 17 kb. There is a remarkable degree of homology in the protein coding regions of the mouse, human, and rat genes. The 5' untranslated regions of the mouse and human genes are 92% and 93% homologous, respectively, to the same region of a rat cDNA (Macklin et al., 1987; Kronquist et al., 1987). Although there is significant homology between the 3' untranslated regions of the mouse and rat genes (~90%) (Macklin et al., 1987), the human and rat genes exhibit only 73% homology in this region (Kronquist et al., 1987). Furthermore, there is considerable variability in the homology within the 3' untranslated region, with the middle of the 3' noncoding region (i.e., nucleotides 2000–2500) exhibiting only 55% homology between the rat and human genes.

The DM20 mRNA is derived by alternative splicing of the PLP gene primary transcript (Nave et al., 1987a; Macklin et al., 1987). There appears to be an alternative splice site located within exon 3 of the gene which may be utilized to produce the mRNA for the DM20 protein.

A number of other PLP-related mRNAs are evident on Northern blots of mouse RNA (Gardinier et al., 1986; Sorg et al., 1987). One of these is rather large, approximately 4.5–5 kb, and appears to be present primarily in the nucleus, suggesting that it may be a stable nuclear precursor mRNA (Sorg et al., 1987). Most of the other PLP-related mRNAs are smaller than 2kb. Whether any of these other PLP-related RNAs represent mRNAs for some of the other PLP-related polypeptides identified in other types of studies (Lepage et al., 1986) remains to be determined. Also whether the PLP-related RNAs are additional alternative splice products of the PLP gene is an interesting question that remains to be investigated.

Myelin-Associated Glycoprotein

The complete amino acid sequences of the two isoforms of rat MAG now have been determined through analyses of cDNAs isolated independently in several laboratories (Sutcliffe et al., 1983; Arquint et al., 1987; Salzer et al., 1987; Lai et al., 1987). The two isoforms, 67 and 72 kdalton MAG, differ in their C-termini as a result of alternative splicing patterns that produce their mRNAs. A rat MAG cDNA was first isolated by Sutcliffe et al. (1983) as an unidentified, brain specific cDNA with the designation

1B236 and subsequently it has been shown to encode MAG (Salzer et al., 1987; Lai et al., 1987; Arquint et al., 1987). The MAG gene is approximately 16 kb in length containing 13 exons, and it has been mapped to mouse chromosome 7 by RFLP analysis (Sutcliffe, 1987). The mRNA encoding the 72 kdalton MAG contains all the exons of the gene except exon 12; and the mRNA encoding the 67 kdalton MAG contains all the exons of the gene except exon 2 (Lai et al., 1987). Exon 12 contains a termination signal which creates a shorter coding region in the MAG mRNA than would be present in its absence (Salzer et al., 1987; Lai et al., 1987); thus the protein for which it codes is smaller (the 67 kdalton MAG). Exon 2 is part of the 5' untranslated region of the gene, and its absence results in a mRNA with a shorter 5' noncoding region (Lai et al., 1987); thus, its presence or absence has no effect on the size of the protein encoded. S1 nuclease protection studies, conducted to detect the proportion of these two MAG transcripts in mRNA preparations isolated from rat hindbrain at different ages, indicate that the two mRNAs have different developmental patterns of expression (Lai et al., 1987). The 72 kdalton MAG mRNA appeared more abundant in the hindbrain at earlier ages, increased to its highest level at 17–29 d and decreased by d 50. The 67 kdalton MAG mRNA was present at low levels in the younger ages and increased continuously to 50 d when it became the major MAG mRNA in the hindbrain. Transcription of the MAG gene appears to result in the production of a number of distinct MAG mRNAs of approximately 2500 nucleotides in length, apparently produced by alternative splicing and the use of at least two polyadenylation sites (Sutcliffe, 1987). A minor band of about 3000 nucleotides has also been observed on Northern blots (Sutcliffe et al., 1983; Salzer et al., 1987). Thus, the MAG gene, like the MBP and PLP genes expresses a multiplicity of mRNAs through alternative splicing mechanisms which encode more than one isoform of each protein.

2',3'-Cyclic Nucleotide-3'-Phosphodiesterase

Less is known about the molecular biology of CNP than the other myelin proteins. Very recently, two groups have reported the isolation of cDNAs encoding the bovine (Kurihara et al., 1987) and the rat enzymes (Bernier et al., 1987). The clones from the two species have some homology but they appear to encode enzymes with significantly different amino acid sequences. Kurihara et al. (1987) isolated two partial length cDNAs comprising a full-length bovine cDNA of 2305 bp. Subclones of the bovine cDNAs, including the coding region, were found to hybridize to a bovine cerebellar mRNA of about 2600 nucleotides but not to any mRNA present in poly A(+) RNA from rat brain (Kurihara et al., 1987). The bovine cDNAs encode a protein of 400 amino acids, including the initiator methionine, with a mol mass of approximately 45 kdalton. Within the coding region there is a stretch of nucleotides corresponding to 236 amino acids of the bovine *elastase* 2',3'-cyclic nucleotide-3'-phosphodiesterase. If the bovine CNP clone corresponds to a mRNA that encodes this enzyme, it would probably have to arise by post-translational processing of the larger CNP polypeptide since the segment encoding the elastase enzyme is not defined by a second open reading frame.

Bernier et al. (1987) isolated a 2.6 kb rat CNP cDNA clone encoding a 46 kdalton polypeptide. Translation of the mRNA transcription product of the insert subcloned into a riboprobe vector indicated that the clone encodes the smaller of the two CNP polypeptides found in rat brain. Analysis of the deduced primary structure of the polypeptide indicates that it contains a polypeptide segment with homology to cAMP binding sites found in several other proteins. The rat probe hybridized to mRNAs of approximately 2400 and 2800 nucleotides in brain and sciatic nerve; and to a mRNA of about 2600 nucleotides in thymus. A 373 bp segment from the 5' end of the clone was found

to hybridize only with the 2800 nt mRNA, suggesting that not all CNP mRNAs share the same 5' ends. Bernier et al. (1987) have suggested that there is a single CNP gene that can be alternatively spliced to produce the various mRNA transcripts.

Translational and Post-translational Events

Little attention has been given to potential translational regulation of myelin protein synthesis. Recently, cell-free synthesis studies have shown that the mRNAs for the myelin basic proteins are poorly initiated during translation relative to brain mRNAs as a whole, and this appears to be related to the presence of an additional translational initiation signal within the 5' untranslated region of MBP mRNAs which is immediately followed by a termination signal (Campagnoni et al., 1987a). In most eukaryotic mRNAs, the first AUG encountered at the 5' end of the molecule serves as the initiator codon but, in poorly initiated mRNAs, additional AUGs frequently are found upstream of the true initiator codon in the untranslated region of the mRNA. These are often followed relatively closely by an in-phase termination codon (Kozak, 1981). In the case of the MBP, this structural arrangement can cause a pause in the translation of the mRNA (resulting from false starts at the upstream initiation site) so that the message is translated less efficiently. It also has been observed that the 14 kdalton MBP mRNA appears to be translated less efficiently than the other MBP mRNAs, a somewhat curious finding since presumably all MBP mRNAs have the same 5' untranslated regions (Campagnoni et al., 1987a). Recently, however, several mouse cDNAs encoding the 14 kdalton MBP have been isolated that have an unusual 5' untranslated region. The 5' noncoding region of these cDNAs contains many upstream AUGs followed by downstream, in frame, termination signals which might make these mRNAs even less efficiently initiated than the "normal" MBP mRNAs (Kitamura, Newman, and Campagnoni, unpublished data). These results suggest that at least the 14 kdalton MBP mRNA population contains two classes of messages with differing efficiencies of initiation and that this could, in part, account for the translation findings.

Sites of Myelin Protein Synthesis and Transport to Membrane

Since the major myelin proteins differ so considerably in their biochemical properties and in their topological localization in the membrane, it should not be surprising that they have different subcellular sites of synthesis and appear to follow different paths of assembly into the membrane. Several laboratories have observed, both in vivo and in vitro, that the myelin basic proteins and Wolfgram proteins (CNP) are rapidly incorporated into myelin following synthesis and that there is a lag before PLP is incorporated into the membrane (Benjamins and Morell, 1978; Benjamins et al., 1975, 1976; Colman et al., 1982; Karin and Waehneldt, 1985; Konat, 1981). The majority of evidence favors the view that the myelin basic proteins are synthesized on free ribosomes (Campagnoni et al., 1980; Colman et al., 1982; Campagnoni, 1985) and that the proteolipid proteins are synthesized on membrane bound ribosomes (Colman et al., 1982). It is likely that MAG is synthesized, like all other glycoproteins, on membrane-bound ribosomes and that CNP is synthesized on free ribosomes (Karin and Waehneldt, 1985). Neither PLP nor MBP are synthesized with "leader" or "signal" sequences. A putative "signal" peptide and its cleavage site have been proposed for the MAG polypeptides based upon the cDNA sequence (Arquint et al., 1987; Salzer et al., 1987); however, the *N*-termini of the MAG apoproteins are not yet known with certainty.

A number of studies strongly indicate that PLP and MBP follow different routes of assembly into the membrane. For example, monen-

sin has been shown to interrupt the appearance of newly synthesized PLP into myelin and to have no effect on the appearance of newly synthesized myelin basic proteins into the membrane (Townsend and Benjamins, 1983). These data and other results on the sites of synthesis of the proteins, the entry times for the two classes of proteins into the membrane, and immunocytochemical staining differences of the two protein classes during the early stages of myelination (Agrawal and Hartman, 1980; Sternberger et al., 1978a,b) all indicate separate intracellular processing of the MBPs and the proteolipids. The intracellular route followed by the PLPs probably involves passage through the Golgi apparatus and other membranes, which may result in its delayed incorporation into myelin relative to the basic proteins. Colman et al. (1982) have suggested that polyribosomes involved in synthesizing the myelin basic protein (and, therefore, carrying the MBP mRNA) enter the oligodenrocyte processes and translate protein close to the sites at which MBP is assembled into the membrane. Synthesis of MBP at this subcellular location might then explain the rapid appearance of newly synthesized MBP into myelin. This hypothesis should be testable by *in situ* hybridization techniques now that cDNA probes for these mRNAs are readily available. In the case of CNP, newly synthesized enzyme appears to become associated rather quickly not only with myelin, but also with membranes that sediment with the microsomal fraction (Karin and Waehneldt, 1985). No precursor-product relationship was observed, however, between the CNP polypeptides found in the microsomal fraction and in myelin.

Post-translational Modifications of Myelin Proteins

Many of the myelin proteins undergo post-translational modifications, such as acetylation, phosphorylation, methylation, glycosylation, or acylation. The biological relevance of most of these post-translational modifications is unclear, although in the case of the MBP methylation, prevention of the modification has resulted in myelin structural abnormalities (Gandy et al., 1973; Small et al., 1981).

Methylation

It has been known for some time, from structural studies on the basic protein, that the *N*-terminus is acetylated (Eylar, 1970; Carnegie, 1971; Dunkley and Carnegie, 1974). An additional modification, also noted from early protein sequencing studies, was the presence of a single methylated arginine residue at position 107 of the human 18.5 kdalton MBP and at the analogous position of the protein in several other species (Baldwin and Carnegie, 1971; Brostoff and Eylar, 1971; Small and Carnegie, 1982). The enzyme responsible for the methylation is *S*-adenosyl-L-methionine: protein-L-arginine *N*-methyl-transferase, E.C.2.1.1.23 (Paik and Kim, 1975; Lee et al., 1977). There are apparently at least two distinct enzymes in the brain, one of which methylates histones and the other of which methylates the myelin basic protein (Lee et al., 1977; Miyake, 1975; Kim et al., 1984). The two enzymes exhibit different developmental patterns of activity; with the histone-specific methylase being most active in newborn mouse brain and declining with age, and the MBP-specific methylase activity peaking at 17 d in the developing mouse brain (Crang and Jacobson, 1982; Amur et al., 1984; Kim et al., 1984; Chanderkar et al., 1986). In the spinal cord, the MBP-specific methylase activity continues to increase postnatally up to 100 d (Crang and Jacobson, 1982). The myelin basic protein has been reported to be a specific uncompetitive inhibitor of the histone-specific methylase (Park et al., 1986). Chanderkar et al. (1986) have performed in vivo double-labeling studies and observed that newly synthesized MBPs are more highly methylated at younger

ages than at older ages, correlating to some extent with the pattern of enzyme activity in the developing mouse brain. The point in the translation/assembly process at which the protein becomes methylated is not known at the present time. The activity of the MBP-specific methylase has been observed to be abnormal in both the *jimpy* and *shiverer* mouse mutants (Kim et al., 1984, 1986).

Several studies with inhibitors of protein methylation indirectly suggest that methylation of MBP is important for myelin integrity. Sinefungin and cycloleucine are protein methylation inhibitors, each acting at different steps in the transmethylation pathway. Cycloleucine acts indirectly on protein methylation by inhibiting the cellular production of S-adenosylmethionine, a substrate of the methyl transferase reaction. Sinefungin is a structural analog of S-adenosylmethionine and causes the inhibition of protein methylation, presumably by acting as a competitive inhibitor in the methyltransferase reaction (Amur et al., 1986). Small et al. (1981) found in chickens injected in vivo with cycloleucine that there was decreased incorporation of methyl groups into methylarginine in myelin basic protein. They found also that chickens injected with the inhibitor developed vacuolation of myelin similar to the subacute combined degeneration (SCD) of myelin seen in humans with vitamin B_{12} deficiency (Agamanolis et al., 1978) and in lesions induced in animals by nitrous oxide administration (Scott et al., 1981).Nitrous oxide intoxication may block transmethylation, since it oxidizes vitamin B_{12} to a form that can no longer function in the methylation of homocysteine to methionine, a metabolic precursor of S-adenosylmethionine. Amur et al. (1986) found that sinefungin inhibited the MBP-specific methylase activity of cultured cerebral cells from embryonic mice. Electron microscopic examination of the cultures revealed significant alterations in the ring-like multilamellar membranous structures, which bear characteristics of myelin in the untreated cultures. In the treated cultures, the myelin-like figures were devoid of multilamellar periodicity and compactness and were reminiscent of the vacuolated myelin observed in SCD and in nitrous oxide, or cycloleucine-treated animals. Thus, there is some evidence that the methylation of myelin basic proteins is important in the maintenance of the multilamellar structure of myelin. It is of interest that the only methylated amino acid residue in MBP identified to date lies within the region of the protein encoded by exon 5. Thus, presumably the 20 and the 17 kdalton MBP (missing exons 2 and 5), both of which are present very early in the developing human spinal cord, are incapable of being methylated at this necessarily missing residue. If these isoforms represented a greater proportion of the MBPs produced in the earliest stages of myelin, then perhaps there is a relationship between the fact that very early myelin is uncompacted and the fact that these two MBP isoforms should be unable to be methylated.

Phosphorylation

The myelin basic protein has been shown to be phosphorylated by a number of different kinases, both in vitro and in vivo (Carnegie et al., 1974; Steck and Appel, 1974; Miyamoto et al., 1974; Miyamoto and Kakiuchi, 1974; Stecket et al.,1976; Endo and Hidaka, 1980; Suhlakhe et al., 1980; Wu and Ahmad, 1984; Turner et al., 1984; Kobayashi et al., 1984). The rabbit protein is phosphorylated in vivo at five residues: ser^{7}, ser^{56}, thr^{96}, ser^{113}, and ser^{163} (Martenson et al., 1983). However, the amount of phosphorylation is only 0.2 mol phosphorus/mol MBP, somewhat less than stoichiometric (Miyamoto and Kakiuchi, 1974), and only a small fraction of the potential sites are phosphorylated in the total basic protein, i.e., from about 2–6% for each site (Martenson et al., 1983).

All of the characterized isoforms of the MBP appear to be phosphorylated in vivo in the rat (Agrawal et al., 1982a, 1986), rabbit (Agrawal et

al., 1981), and the mouse (Ulmer and Braun, 1986a,b). After in vivo labeling, the specific activities of the phosphorylated isoforms have been found to be similar within a given age in 15 d rabbit brain (Agrawal et al., 1981) and in 12, 30, and 50 d mouse brain (Ulmer and Braun, 1986a). During development, the incorporation of radioactive phosphate into MBPs and the resultant specific activities of the MBPs appear to be related to the specific activities of the acid soluble phosphate pool of myelin (Ulmer and Braun, 1986a). No major developmental differences appear to occur in the timing of the phosphorylation of the MBP isoforms relative to one another. Phosphorylation of the basic proteins has been reported to occur within one min after the intracranial injection of isotope (Ulmer and Braun, 1986a), consistent with other results indicating a very rapid turnover rate for the phosphate groups in the myelin basic proteins (DesJardins and Morell, 1983).

The physiological role of MBP phosphorylation is not clear. Murray and Steck (1984) reported that action potential conduction, but not electrical stimulation, increased the state of MBP phosphorylation in rat optic nerve incubated in vitro. In studies on myelinogenesis with cultured adult oligodendrocytes, Vartanian et al. (1986) observed that oligodendrocyte adherence to substratum activated a protein kinase C-dependent phosphorylation of MBP in addition to promoting the synthesis of MBP. Although the results of several of the studies suggest that MBP may be phosphorylated *in situ* in myelin, Ulmer and Braun (1984, 1986b) have presented evidence that MBP phosphorylation also can occur prior to its incorporation into compact myelin. This would be a cell biological prerequisite for the proposal by Stoner (1984) that phosphorylation of MBP may be important in the folding of the protein and its insertion into the membrane. If MBP dimer formation plays a role in the myelin compaction process at the major dense line, then phosphorylation of MBP would lower the positive charge on each MBP monomer and reduce intermolecular charge repulsion. This charge repulsion needs to be overcome for dimer formation to occur. Thus, whereas there is growing evidence that phosphorylation of MBP may be altered by biologically relevant phenomena, and theoretical considerations consistent with its being important, it is still unclear if phosphorylation of MBP has a role in the assembly, metabolism, or structural integrity of myelin.

Acylation

From their first isolation from brain tissue, proteolipid apoprotein preparations were thought, and subsequently shown, to contain covalently-bound fatty acids (Folch and Lees, 1951; Braun and Radin, 1969; Stoffyn and Folch-Pi, 1971; Gagnon et al., 1971). The bovine myelin proteolipid apoprotein contains approximately 2% by weight fatty acid, principally palmitic [55%], oleic [26%], and stearic acids [19%] (Lees and Macklin, 1988). Although the analytical data indicate the presence of 2 mol fatty acid/mol protein, evidence for only one amino acid residue, thr^{198}, as a site of fatty acyl estification has been obtained to date (Stoffel et al., 1982). The other site(s) has not been identified yet.

In metabolic studies, several groups have shown that both the myelin PLP and DM20 proteolipids can be acylated in vivo (Agrawal et al., 1982b; Bizzozero et al., 1984; Garwood et al., 1983; Bizzozero et al., 1986) and in vitro (Townsend et al., 1982; Bizzozero et al., 1983; Konat et al., 1986). None of the other major myelin proteins were found to be acylated in these studies. Several lines of evidence suggest that fatty acylation of the proteolipids occurs at or near myelin. In marked contrast to the delay in the incorporation of newly synthesized PLP into myelin, incorporation of fatty acids into PLP and DM20 in the membrane occurs very

rapidly and in a linear fashion in tissue slices (Townsend et al., 1982). In the same system, acylation of the proteolipids continues for at least 2 h after pre-incubation of the tissue slices with the protein synthesis inhibitor, cycloheximide. Acylation also continues after addition of monensin, which has been shown to block PLP transport to the membrane (Townsend and Benjamins, 1983). In pulse-chase experiments, increased incorporation of [^{3}H]palmitate stopped immediately after the addition of unlabeled palmitate to the slices (Bizzozero et al., 1983). Finally, Bizzozero et al., (1987b) have demonstrated the presence of an endogenous fatty acyltransferase activity in myelin capable of catalyzing the transfer of fatty acyl groups from fatty acyl CoA to PLP and DM20 within the membrane. Recently, Bizzozero et al. (1987a) have reported that deacylated PLP is capable of "autocatalyzing" incorporation of fatty acyl groups from palmitoyl CoA into deacylated PLP without the addition of an exogenous enzyme. The substrate concentration curve followed Michaelis kinetics and denaturation of the protein inhibited the reaction. Thus, there is growing evidence that acylation of the PLP and DM20 proteins occurs at the myelin membrane, possibly through an "autocatalytic" process involving the deacylated PLP.

Myelin Protein Gene Expression in Cultured Cells

Myelin protein gene expression has been studied in a variety of cultured cell systems. Most myelin proteins and their mRNAs have not been detected in cell lines, with the exception of CNP, which is expressed in some glioma cell lines (Pfeiffer et al., 1981a). The discussion in this section will focus on primary cells, since investigations on myelin gene expression in these cells might be expected to more closely reflect the in vivo expression of these genes. Furthermore, the studies on the expression of such myelin genes as MBP and PLP have only been conducted on primary cultures, since these proteins or mRNAs have not been found in cell lines. This review will focus primarily on myelin protein gene expression, rather than oligodendrocyte cell biology. This will limit significantly the scope of the discussion on cultured oligodendrocytes, but several recent reviews have discussed in more detail the extensive area of investigation on oligodendrocyte cell biology (Pfeiffer, 1984; Wood and Bunge, 1984; Bologa, 1985).

Whereas primary cultured oligodendrocytes differentiate to produce galactosyl ceramide (GC), CNP, MBP, PLP, and other myelin markers in the apparent absence of any neural signals, their differentiation process in vitro is somewhat more plastic than in vivo, and differences among studies may result from this plasticity. One important early element of this plasticity is whether the oligodendrocyte will differentiate from its progenitor cell. Raff et al. (1983) reported the existence of a cell type in rat optic nerve primary cultures, which can differentiate into a type 2 astrocyte in the presence of fetal calf serum or into an oligodendrocyte in defined media with no fetal calf serum. This oligodendroctye progenitor cell appears to stop dividing and to begin differentiation into oligodendrocytes or type 2 astrocytes within 2–3 d in culture (Raff et al., 1983; Temple and Raff, 1985), whereas in vivo, these progenitor cells continue to divide for weeks after birth (Skoff et al., 1976a,b). Interactions between these oligodendroctye progenitor cells and type 1 astrocytes apparently affect the time course of oligodendrocyte proliferation and differentiation in vitro (Noble and Murray, 1984; Raff et al., 1985; Temple and Raff, 1986). Thus, it appears that oligodendrocyte differentiation can be manipulated in culture by media or by cell–cell interactions, and the studies to be discussed

here will analyze myelin gene expression in these cells under several such culture conditions. For example, investigations in cultured oligodendrocytes can provide infomation on: (a) specific factors that modulate myelin protein gene expression; (b) the effects of growth conditions on the time course of myelin gene expression; (c) the intracellular localization of certain myelin proteins; and (d) cell–cell interactions that influence myelin gene expression.

Oligodendrocytes cultured in the presence or absence of other CNS cells express every myelin marker investigated and they can clearly make a myelin-like membrane (Matthieu et al., 1979; Sarlieve et al., 1983; Szuchet et al., 1986; Rome et al., 1986). Nevertheless, in the absence of an appropriate axonal target, this membrane has distinct differences from myelin produced in vivo (Sarlieve et al., 1983; Szuchet et al., 1986). Myelin assembly in vitro may be altered relative to normal, and such differences might feed back and alter the synthesis of myelin components in these cells, affecting the interpretation of the results.

Primary glial cultures contain both oligodendrocytes and astrocytes and generally very few neurons. McCarthy and deVellis (1980) developed a protocol to isolate pure rat oligodendrocyte cultures, by shaking oligodendrocytes off the mixed glial cultures. By various adaptations of this protocol, cultures containing a mixture of oligodendrocytes and astrocytes (mixed glial cultures) or cultures containing primarily oligodendrocytes (enriched oligodendrocytes) have been prepared and maintained for many weeks.

From studies on mixed glial cultures, it has been possible to determine the rat oligodendrocyte progenitor cells are committed by the late fetal period to express myelin markers two or three wk later in culture. Neurons are not required to induce the synthesis of MBP, PLP, CNP, GC, or cerebroside sulfotransferase (McCarthy and deVellis, 1980; Pfeiffer et al., 1981b; Dubios-Dalcq et al., 1986a). However, although they are clearly programmed to differentiate and produce myelin proteins, the behavior of the cultured cells differs from cells in vivo in certain respects. For example, during early stages of myelination in vivo, the 14 kdalton MBP is expressed at lower levels than the 18.5 kdalton MBP. With development, the ratio of the 14:18.5 kdalton MBP increases significantly until the 14 kdalton isoform predominates (Banik and Smith, 1977; Matthieu et al., 1973; Morell et al., 1972; Magno-Sumbilla and Campagnoni, 1977). In contrast, in culture, the 14 kdalton MBP is expressed earliest, and it remains the predominant isoform up to 39 d in culture, although the other isoforms accumulate from 27–39 in culture (Barbarese and Pfeiffer, 1981).

The temporal expression of myelin markers has been studied in primary glial cultures, and for many markers, it appears that the normal temporal developmental pattern of expression observed in vivo is reproduced in these cells. For example, galactolipid synthesis, MAG, CNP, and PLP become detectable approximately 10 d after plating, and their levels increase significantly for the next two wk, just as would occur in cells remaining in the brain (Pfeiffer et al., 1981b; Nussbaum et al., 1983; Bansal and Pfeiffer, 1985; Singh and Pfeiffer, 1985; Dubois-Dalcq et al., 1986a; Wernicke and Volpe, 1986). Interestingly, in one study, the first myelin protein to be detected in oligodendrocyte processes in cultures was MAG (Dubois-Dalcq et al., 1986a), which contrasts somewhat with the developmental studies on MAG expression in vivo (Frail and Braun, 1984; Matthieu et al., 1986; Lai et al., 1987; Salzer et al., 1987). On the other hand, expression of cholesterol ester hydrolase activity is delayed. Two cholesterol ester hydrolase isoenzymes exist in oligodendrocytes, one localized in the microsomes and one in myelin. Both enzyme activities are quite low until 20 d in culture, at which point they increase significantly to a peak at 30 d in culture. This time course

lags approximately 10 d behind the normal in vivo developmental expression of these enzymes (Bhat and Pfeiffer, 1985).

Conflicting results have been reported on the timing of MBP expression in cultured rat oligodendrocytes (Pfeiffer et al., 1981b; Dubois-Dalcq et al., 1986a), but a possible explanation for the differences may provide insight into the regulation of MBP expression. In one study, cells were cultured in 10% fetal calf serum (Pfeiffer et al., 1981b), and in the other study, in 1% fetal calf serum (Dubois-Dalcq et al., 1986a). In cells grown in the higher levels of fetal calf serum, MBP expression was delayed, relative to normal development, yet no differences in the timing of expression of CNP, sulfatide, or PLP were observed (Macklin and Pfeiffer, 1983; Bansal and Pfeiffer, 1986; Singh and Pfeiffer, 1986). These results suggest that MBP gene expression might be, for a presently unknown reason, more responsive to epigenetic factors than that of the other myelin markers. This hypothesis is consistent with other in vitro studies in which alterations in the growth conditions were found to modulate MBP expression significantly, without altering the number of GC+ cells (Bologa et al., 1986).

Two types of studies, performed with cultured cells, indicate that CNP is localized on the intracellular surface of the oligodendrocyte plasma membrane (McMorris et al., 1984). No enzyme activity could be measured in intact viable cells, and it was possible only to assay CNP in cells permeabilized by freezing, suggesting that the catalytic site of the enzyme was located on the intracellular surface of the oligodendrocyte. Also, it was not possible to stain viable cells for CNP by immunofluorescence: only fixed, permeabilized cells stained for CNP, indicating that the major antigenic sites of the enzyme were also intracellular. Similar types of studies have also been performed to localize domains of the proteolipid protein within the membrane of cultured cells (Dubois-Dalcq et al., 1986b).

Oligodendrocytes from adult sheep have been cultured and utilized to study the reinitiation of myelin expression in cells that have ended active myelination *in situ* (Szuchet et al., 1980; Mack and Szuchet, 1981; Szuchet et al., 1983; Szuchet and Yim, 1984; Yim et al., 1986). These cells could be maintained in two states, floating and attached; and overall protein synthesis was comparable in the two sets of cells. Floating cells synthesized low levels of MBP, which did not change over time; and they accumulated significant amounts of CNP and low levels of MAG (Yim et al., 1986). In contrast, attached cells synthesized high levels of MBP, which increased with prolonged time of attachment, along with high levels of CNP, MAG, and PLP. Thus, the physical attachment of these cells to a substratum appeared to enhance the expression of certain myelin genes, in particular those of the "structural" elements of myelin. In addition, attachment of oligodendrocytes to a solid surface also enhanced phosphorylation of MBP via protein kinase C (Vartanian et al., 1986). When floating cells were treated with phorbol esters or diacylglycerol, this same phosphorylation enhancement was observed, suggesting that protein kinase C can be activated in floating cells and that it can phosphorylate MBP.

The effects of thyroid hormone on myelin gene expression in cultured mouse oligodendrocytes have been examined extensively (Bhat et al., 1979; Bhat et al., 1981a,b; Shanker and Pieringer, 1983; Shanker et al., 1984; Amur et al., 1984; Shanker et al., 1985; Shanker et al., 1987). Removing thyroid hormone from the cell culture medium reduced the expression of all myelin markers examined. For example, sulfatide synthesis was reduced in cells grown without thyroid hormone, and it returned to normal levels when thyroid hormone was returned to the medium (Bhat et al., 1981b). Similarly, the expression of CNP and 5'-nucleotidase was reduced in the absence of thyroid hormone and returned to normal levels by me-

dium supplementation with thyroid hormone (Bhat et al., 1981a; Shanker et al., 1984). The cellular response to thyroid hormone is relatively rapid with respect to myelin protein expression. For example, recovery of CNP and 5'-nucleotidase activity was observed within 24–48 h after introduction of thyroid hormone to the cultures (Bhat et al., 1981a; Amur et al., 1984).

Several aspects of MBP gene expression are affected by the presence or absence of thyroid hormone. Almazen et al. (1985) demonstrated that MBP synthesis in suspension cultures is reduced in hypothyroid culture media. This reduced synthesis most likely results from a reduction in MBP mRNA levels, as Shanker et al. (1987) found that hypothyroid cultures have significantly reduced levels of MBP mRNAs. The activity of the MBP-specific methylase also has been found to increase in the presence of thyroid hormone and decrease in its absence (Amur et al., 1984). Thyroid hormone had no effect on the histone-specific methylase in this study, and it appears to selectively modulate myelin protein gene expression in these cultures. Thyroid hormone appears to act at several levels: RNA metabolism, protein synthesis, and post-translational modification.

There appears to be a critical period in early oligodendrocyte differentiation during which the cells are responsive to thyroid hormone. After cells have been in culture more than 20 d, they have little responsiveness to either the absence or presence of thyroid hormone, in terms of CNP activity or sulfatide synthesis (Shanker et al., 1985). Interestingly, one of the few myelin markers that was shown to have little response to the absence of thyroid hormone was the synthesis of G_{M4} ganglioside. This synthetic reaction occurs relatively late in culture, peaking after 48 d, and it would appear to occur after the early critical period of thyroid hormone sensitivity. If the cell cultures were made hypothyroid during that early critical period, they did not express G_{M4} at the later stages of development (Shanker and Pieringer, 1983).

This series of studies is important because it had been known for some time that the presence or absence of systemic thyroid hormone could affect the normal myelination process (Balasz et al., 1969; Balazs et al., 1971; Walravens and Chase, 1969; Flynn et al., 1977), but it was not known whether this was a specific effect on the myelination process or a general effect on brain cell metabolism. Furthermore, it was not possible to assess whether this was an effect of thyroid hormone directly on the oligodendrocyte or on another cell type (either within or without the CNS) that had a secondary effect on the oligodendrocyte. These studies with cultured cells suggest that thyroid hormone has a selective effect on myelin protein gene expression in mouse oligodendrocytes in vivo.

Rat oligodendrocytes in mixed glial cultures express approximately 20-fold more MBP and CNP per oligodendrocyte than do isolated oligodendrocytes purified away from astrocytes and other cells in the mixed cultures (Bhat et al., 1981c). When the isolated oligodendrocytes are added back to the astrocyte-containing cultures, they again express the higher MBP and CNP levels. This suggests that the presence of cell(s) and/or factor(s) in mixed primary glial cultures plays some role in regulating the expression of these two myelin components. Bhat and Pfeiffer (1986) established that a soluble astrocyte factor(s), which is nondialyzable, heat-labile, and trypsin-sensitive, enhances MBP and CNP expression in purified oligodendrocytes. In these experiments, it appeared that the enhancement resulted from an increase in the number of MBP+ or CNP+ cells in the cultures, rather than from an increase in MBP or CNP content per oligodendrocyte. This astrocyte factor(s) may be comparable to that identified by Noble and Murray (1984) in supernatants of cultured type 1 astrocytes, which stimulates proliferation of oligodendrocyte progenitor cells.

In addition to interactions with astrocytes, oligodendrocytes clearly interact with neurons in culture. Soluble neuronal factor have been shown to affect oligodendrocyte differentiation, possible shifting the differentiation of oligodendrocyte progenitor cells towards oligodendrocytes, rather than astrocytes (Sakellaridis et al., 1986). Soluble rat and chick neuronal factors enhanced MBP expression in mixed rat glial cultues (Bologa et al., 1986). After three d exposure to neuronal factors, the number of MBP+ cells in the cultures increased dramatically (1800-fold), without increasing the number of GC+ cells. Without neuronal factors, only a small number of GC+ cells were MBP+. The soluble neuronal factors may regulate the timing of MBP expression in these mixed glial cells, without affecting GC expression.

Experiments co-culturing purified rat oligodendrocytes with chick neurons enhanced MBP and PLP mRNA and protein levels (Macklin et al., 1986). The mRNA levels were enhanced 4–8-fold over a period of 1–3 wk of co-culture with neurons. Since these were purified oligodendrocytes, these responses would appear to be the direct effect of neuronal interaction with the oligodendrocytes, and it is not yet known whether these interactions were mediated by soluble factors or by cell–cell contact. The increase in MBP content in these cells was significantly below the increase in the number of MBP+ cells observed by Bologa et al. (1986). This may result from neuronal interactions with astrocytes in the mixed cultures, which have additional effects on the oligodendrocytes, or it may result from experimental design differences between the two studies. Bologa et al. (1986) studied neuronal effects within three d; possibly these early effects were advancing a "clock," which would eventually raise the number of MBP+ cells, even in the absence of neurons. Since Macklin et al. (1986) were analyzing cells at a later stage, the dramatic differential between the two sets of cells might have been missed. Clearly the effects of astrocytes and neurons on the time course and magnitude of myelin gene expression will be an important area of future investigation.

Gene Expression in Dysmyelinating Mutants

The dysmyelination mutants, primarily mouse mutants, are useful models for investigating the normal process of myelination (Hogan and Greenfield, 1984; Baumann, 1980). These animal models provide a uniform system in which to study a specific defect in myelination, and the secondary effects resulting from that defect. Several of the mouse mutants have been extensively characterized biochemically. The *shiverer* mutation and its allele, myelin deficient (*shi*^{mld}), the *jimpy* mutation and its allele, myelin synthesis deficient (*jp*^{msd}), and the *quaking* mutation all produce primary dysmyelination, i.e., an oligodendrocyte-specific defect. Some of these mutants were identified over 30 y ago, but it is only within the past few years that significant progress has been made on identifying the genes that are altered in these animals. For two of the mutants, *shiverer* and *jimpy*, the mutation has been localized to the structural genes for the myelin basic protein and the myelin proteolipid protein, respectively (Roach et al., 1985; Mattei et al., 1986).

Shiverer (shi) *and Myelin Deficient* (shi^{mld})

The *shiverer* mutation and its allele *shi*^{mld} (Doolittle and Schweikert, 1977; Doolittle et al., 1981) are autosomal recessive, and they map to mouse chromosome 18 (Roach et al., 1985; Sidman et al., 1985). These mice exhibit tremors beginning approximately at postnatal d 12, which become progressively worse. Convulsions appear in older animals and these ani-

mals do not survive past 90–150 d (Chernoff, 1981). Morphological analyses indicate that many axons have no myelin, and those that are myelinated have only low amounts of myelin. A striking feature of *shiverer* CNS myelin is the aabsence of the major dense line but the presence of the intraperiod line (Jacque et al., 1978; Dupouey et al., 1979; Privat et al., 1979; Inoue et al., 1981). This suggests that there is a greater loss of myelin basic protein in these animals than of proteolipid protein or of other proteins expressed on the extracellular surface of myelin.

With the dysmyelinating mouse mutants, it has been important to establish that the mutation is generating dysmyelination because of a specific alteration of an oligodendrocyte/myelin gene. To assess whether the *shiverer* mutation affected oligodendrocyte metabolism directly, or through a secondary regulatory mechanism, Mikoshiba et al. (1982) and Inoue et al. (1986) produced chimaeras of normal C57BL/6N and *shiverer* Balb/C mice. In brains of animals having predominantly normal oligodendrocytes, oligodendrocytes of a *shiverer* morphology were seen. These cells made contact with axons to form nodes of Ranvier, but they formed abnormal myelin. Thus, the *shiverer* oligodendrocytes apparently maintained their altered phenotype in a "normal" environment. Interestingly, in a predominantly *shiverer* brain environment, as defined morphologically, the presence of *shiverer* oligodendendrocytes apparently reduced the ability of normal oligodendrocytes to initiate the appropriate connections with axons. Nevertheless, normal oligodendrocytes were present and they were morphologically unaffected by the *shiverer* brain environment. These data suggested that the *shiverer* defect was intrinsic to the mutant oligodendrocytes, and could not affect normal oligodendrocytes in that environment.

Biochemical analysis of *shiverer* tissue indicates essentially the total absence of myelin basic protein in *shiverer* brain, (Bourre et al., 1980; Barbarese et al., 1983). Myelin basic protein RNA levels in these animals have been measured directly by dot blot or Northern blot analysis and indirectly by in vitro translation. Although in one study using in vitro translation of *shiverer* brain mRNA, normal levels of the 21.5 and 17 kdalton MBPs and low levels of the 18.5 and 14 kdalton MBPs were observed (Barbarese et al., 1983), in several other studies, using both RNA blot analysis and in vitro translation, less than 1% of the normal level of MBP mRNA was observed (Roach et al., 1983; Campagnoni et al., 1984).

As with *shiverer* animals, *shi^{mld}* mice appear to have a major deficit in MBP. Myelin is poorly compacted, and the major dense line of myelin is essentially absent (Matthieu et al., 1980). However, more MBP (3% of normal levels) was observed in *shi^{mld}* brains than in *shiverer* brains. The level of MBP mRNA in *shi^{mld}* mice was quite low, approximately 2–5% of normal in young animals (Ginalski-Winkelmann et al., 1983; Campagnoni et al., 1984; Roch et al., 1986; Okano et al., 1987; Popko et al., 1987), but the size of the *shi^{mld}* MBP mRNA was normal, 2.2–2.4 Kb (Okano et al; 1987; Popko et al; 1987). The developmental expression of MBP mRNA in *shi^{mld}* mice was unusual (Okano et al., 1987; Popko et al., 1987), in that low levels were seen at 18 d (2% of normal) and these levels increased up to 90 d, at which point they were approximately 10% of normal levels (Popko et al., 1987). This contrasts with normal animals, which have a peak of MBP mRNA expression of 16–20 d and then a reduction in the level of MBP mRNA.

Recently it has been established that the *shiverer* and *shi^{mld}* mutations are within the structural gene for the myelin basic protein. The alleles map to the distal end of mouse chromosome 18, which contains the MBP gene (Doolittle and Schweikart, 1977; Roach et al., 1985; Sidman et al., 1985). When the MBP gene in these mutants was analyzed, clear alterations in the MBP gene were identified. The

shiverer mutation results in a deletion of exons 3–7 of the MBP gene (Roach et al., 1983; Roach et al., 1985; Kimura et al., 1985, Molineaux et al., 1986), and the production of low levels of MBP-related RNA. This MBP-related mRNA is aberrantly spliced and poorly polyadenylated. In contrast, in *shi*^{mld} mice, there appear to be multiple MBP genes. There is one complete MBP gene, and at least one extra partial or complete gene that is closely juxtaposed to the complete MBP gene (Popko et al., 1987). Thus, it would appear that the partial or complete duplication of the MBP gene, near a normal MBP gene, can reduce the expression of the normal gene to 2% of normal and alter the normal developmental expression of the gene.

Alterations of the MBP structural gene in *shiverer* and *shi*^{mld} mice have pleiotropic effects on the expression of other myelin genes. CNP activity in many regions of *shiverer* brain does not differ significantly from control brains (Mikoshiba et al., 1980a; Matthieu et al., 1981; Cammer and Zimmerman, 1983), although when the tissue is homogenized and fractionated, the membranes containing the highest levels of CNP activity sediment differ from normal myelin (Mikoshiba et al., 1980b; Cammer and Zimmerman, 1983). Whereas CNP activity for whole brain is approximately the same between controls and *shi*^{mld} mice, the isolated myelin from *shi*^{mld} mice is four-fold enriched in CNP activity relative to controls (Matthieu et al., 1981).

When *shiverer* oligodendrocytes are immunostained for carbonic anhydrase, an early oligodendrocyte marker, there is in fact elevated staining relative to normal animals (Cammer et al., 1985). When *shiverer* tissue was assayed, carbonic anhydrase levels were approximately 1.5-fold higher than normal up to 30 d of age, and even at 60 d of age, this enzyme level was above normal in *shiverer* mice.

In contrast to the relatively normal amount of CNP and the elevated level of carbonic anhydrase in *shiverer* mice, levels of both PLP and MAG are reduced. Myelin isolated from *shiverer* brains contained low levels of PLP, although they were significantly above the MBP levels (Mikoshiba et al., 1980a). PLP mRNA levels in 21 d old *shiverer* brain were measured at approximately 50% of normal, although the amount of PLP that accumulated in these brains was less than that (Sorg et al., 1986). When PLP RNA levels were measured in *shiverer* polysomes and nuclei, some distinct differences were noted relative to normal animals. The developmental pattern of PLP mRNA in polysomes mirrored the increase observed in normal animals from 12–27 d, although quantitatively, it was only 40–50% of normal (Sorg et al., 1987). In contrast, nuclear PLP RNA as a percentage of normal was much lower than polysomal RNA, and the developmental pattern of expression of PLP RNA in the nucleus did not at all mirror the developmental pattern of normal animals. In contrast to *shiverer* mice, close to normal levels of PLP mRNA were reported in *shi*^{mld} mice when analyzed by in vitro translation (Matthieu et al., 1983, 1984), and dot blot analysis indicates the PLP mRNA levels are approximately 60–70% of normal throughout development (Okano et al., 1987).

In young *shiverer* animals, mRNAs for both forms of MAG (p72 and p67) are present, although at slightly lower levels than normal, whereas in adult animals, the mRNA levels are close to normal (Frail and Braun, 1985). These data contrast somewhat with the observation that MAG protein levels in adult *shiverer* brains are reduced relative to controls (Sheedlo and Siegel, 1986). When analyzed by immunocytochemistry, oligodendrocyte-like cells were observed that contained MAG in the cytoplasm and the periphery of their perikarya; such cells were not observed in normal animals (Sheedlo and Siegel, 1986).

In *shi*^{mld} mice, the expression of the MAG gene is quite different from normal (Matthieu et al., 1986). At 18 d of age, the level of MAG mRNA in *shi*^{mld} brain is significantly higher

than in normal brain. In young *shi^{mld}* mice, it appears that there is a high synthetic rate for MAG protein, but also a rapid turnover, with production of dMAG, which in normal animals is often seen during demyelination. These data suggest instability of the myelin membrane in young *shi^{mld}* mice. The normal shift in expression of the two MAG mRNAs from the 72 to the 67 kdalton MAG mRNA, is delayed in *shi^{mld}* mice, not occurring until after 50 d of age. In older *shi^{mld}* mice, dMAG is not observed and the total amount of MAG protein is less than in young *shi^{mld}* mice, with a concomitant reduction in the level of MAG mRNA. A possible explanation for these changes in MAG gene expression in older *shi^{mld}* mice may come from the observation that, as MBP accumulates in these mice, myelin compaction increases. Thus, in older *shi^{mld}* animals, the presence of compact myelin may stabilize MAG (Matthieu et al., 1986).

Myelin gene expression has been studied in *shiverer* or *shi^{mld}* heterozygotes. In one study, both MBP and PLP were significantly reduced in heterozygous *shiverer* mice (Cammer, 1982). In another study, the reduction in PLP levels in heterozygotes was not apparent, but the MBP concentration measured by RIA in brain and sciatic nerve was approximately 50% of normal from 15–90 d of age (Barbarese et al., 1983). This protein reduction in whole tissue was also observed in isolated myelin, which contained approximately 50% of the normal level of MBP, although the yield of myelin was normal. When analyzed by in vitro translation, the MBP mRNA level in the heterozygotes was intermediate between normal and *shiverer* animals. In *shi^{mld}* heterozygous mice, MBP mRNA levels were measured by both in vitro translation and dot blot analysis, and the level of MBP mRNA appeared to be 39–57% of normal; MBP protein levels were approximately 52% of normal (Roch et al., 1986). The data from these studies would suggest that a single normal MBP gene in either *shiverer* or *shi^{mld}* animals cannot increase its expression to compensate for the presence of the *shiverer/shi^{mld}* MBP gene.

Attempts have been made to enhance myelination in *shiverer* brains utilizing either transplantation of normal tissue or production of transgenic mice carrying a normal MBP gene. These studies have provided interesting and important new information. Gumpel and coworkers transplanted newborn normal tissue into newborn *shiverer* brain, and studied the distribution of myelin-producing cells by immunohistochemistry and electron microscopy (Gumpel et al., 1983; Lachapelle et al., 1983, 1984; Gumpel et al., 1985; Gansmuller et al., 1986). Normal oligodendrocytes were able to survive, migrate, and myelinate in the *shiverer* brain. A single axon could be myelinated by both normal and *shiverer* oligodendrocytes, suggesting that the early behavior of both types of oligodendrocytes in contacting axons is comparable. Normal oligodendrocytes were found well outside the site of the graft, suggesting that these cells can migrate long distances and can survive for an extended period of time in the *shiverer* brain environment, at least until 130 d after transplantation. Similar studies have been conducted by others with comparable results (Friedman et al., 1986).

Hood and coworkers produced transgenic *shiverer* mice carrying one or two copies of the MBP transgene (Readhead et al., 1987). The transgene is expressed in a tissue-specific manner with the correct developmental pattern. *Shiverer* mice carrying the MBP transgene produce more MBP mRNA and protein than the original *shiverer* mice, and they live longer, with a less severe disorder. *Shiverer* mice carrying a single transgene produced approximately 12.5% of normal MBP mRNA and 8.5% of normal protein, whereas mice homozygous for the transgene produced roughly twice as much mRNA and protein. The heterozygous transgenic mice survived somewhat longer than original *shiverer* mice, but they still had convulsions and died at approximately 6 mo. Homo-

zygous transgenic mice appeared to be phenotypically closer to normal, for example, they had no tremors or convulsions, but some subtle behavioral abnormalities were observed. Despite the presence of only 25% of normal levels of MBP mRNA in the homozygous transgenic mice, the myelination of the optic nerve, spinal cord, and cerebellar white matter was significantly increased relative to the heterozygous transgenic mice. It was possible to produce *shiverer/shi*^{mld} heterozygotes and *shi*^{mld} homozygotes that carried one or no copy of the transgene (Popko et al., 1987). These mice all had abnormal myelin, but those carrying the transgene produced far less of this abnormal membrane. These mice all had fewer seizures than those carrying no transgene.

These studies with transgenic mice suggest that insertion of a normal MBP gene into the genome of mice carrying either a deletion or a duplication of the normal gene can produce a tissue-specific and developmentally accurate expression of the transgene. However, the level of expression was not normal, since even with two copies of the transgene, only 25% of normal MBP mRNA was observed. The fact that the transgene was expressed at comparable levels in *shiverer* and *shi*^{mld} mice suggests that the deleterious effect of the MBP gene duplication on MBP gene expression in *shi*^{mld} mice is *cis*-acting, not *trans*-acting. Clearly the production of these transgenic mice, and the future use of these mice will provide new insight into myelin gene expression.

Jimpy (jp) and Myelin Synthesis Deficient (*jp*^{msd})

The *jimpy* mutation and its allele *jp*^{msd} (Meier and MacPike, 1970; Eicher and Hoppe, 1973) are recessive X-linked mutations. These mice exhibit tremors, which become progressively worse, beginning approximately at postnatal d 11. Convulsions appear in older animals and these animals do not survive past 25–30 d. This is exclusively a central nervous system disorder with no peripheral nervous system involvement (Billings-Gagliardi and Adcock, 1981). Histological examination indicates virtually the total lack of myelin, and there is a significant loss of oligodendrocytes, resulting apparently from a reduced oligodendrocyte lifespan (Sidman et al., 1964; Knapp et al., 1986). both *jimpy* and *jp*^{msd} animals have a similar clinical course, but it appears that *jimpy* animals have approximately one half the myelin of *jp*^{msd} mice and that the *jimpy* mutation causes a more severe disease (Billings-Gagliardi et al., 1980, Wolf et al., 1983).

It was possible to use tissue culture to establish that the *jimpy* mutation was a specific alteration of oligodendrocyte function. Wolf, Billings-Gagliardi, and coworkers demonstrated that cerebella from *jimpy* and *jp*^{msd} animals could be maintained in culture and these explants exhibited the same myelin deficit seen *in situ* (Wolf and Holden, 1970; Billings-Gagliardi et al., 1980), providing strong evidence that the deficit was caused by the CNS tissue itself, not a systemic influence. When *jimpy* or *jp*^{msd} cerebellum was cultured in contact with normal optic nerve, significant myelination of the mutant cerebellar neurons was observed in the area closely juxtaposed to the normal optic nerve (Wolf et al., 1981; Billings-Gagliardi et al., 1983), suggesting that normal oligodendrocytes were migrating into the mutant tissue and myelinating axons within that tissue. These data suggest that the defect in these animals is not in the neurons, for example producing axons that cannot be myelinated, nor is it in cells producing inhibitory factors that prevent myelination. Rather, the defect is localized to the *jimpy* and *jp*^{msd} oligodendrocytes themselves, and they can be replaced by migrating normal oligodendrocytes.

The most extensive myelin deficit in *jimpy* mice appears to be the virtual absence of PLP (Nussbaum and Mandel, 1973; Lerner et al.,

1974; Sorg et al., 1986; Yanigisawa and Quarles, 1986). Quite early in the study of *jimpy* mice, alterations in PLP were noted. Whereas a number of brain proteolipid proteins are still found in *jimpy* mice, the myelin proteolipid proteins identified by electrophoretic mobility were shown to be reduced in *jimpy* mice (Nussbaum and Mandel, 1973; Lerner et al., 1974). When more extensive studies were conducted using immunoblotting to identify PLP and DM20, neither was observed, indicating that PLP was present at less then 0.5% of normal, if at all (Sorg et al., 1986; Yanigisawa and Quarles, 1986).

Through a series of experiments, the genetic defect in *jimpy* animals has now been established as a point mutation within the splice acceptor signal of exon 5 of the mouse PLP gene (Macklin et al., 1987; Nave et al., 1987b; Milner, personal communication). The PLP gene has been mapped to the *jimpy* locus by several groups (Willard and Riordan, 1985; Dautigny et al., 1986; Diehl et al., 1986; Hudson et al., 1987). It was established that there was an altered PLP mRNA in *jimpy* animals, which had an internal deletion of 74 nucleotides (Gardinier et al., 1986; Morello et al., 1986; Nave et al., 1986; Hudson et al., 1987). The deletion, which was initially indicated from PLP mRNA sizing studies (Gardinier et al., 1986) and from S1 nuclease mapping of PLP mRNA in *jimpy* mice (Morello et al., 1986; Hudson et al., 1987), was definitively established by sequencing a *jimpy* PLP cDNA (Nave et al., 1986), identifying the site of the missing 74 nucleotides. Nave et al. (1986) proposed from this sequence information and from mapping PLP genomic DNA in *jimpy* mice, that this deletion resulted from a splicing defect in the processing of the PLP mRNA. In addition, this alteration in the RNA results in a frameshift of the amino acid reading frame for the last segment of the PLP mRNA. Thus, the protein translated from this mRNA is identical to PLP for the first 206 amino acids, and the last 70 amino acids of the normal protein are changed to a sequence of 36 different amino acids.

The specific alteration of the PLP gene in jp^{msd} mice is not established yet. Hudson et al. (1987) found reduced levels of PLP mRNA in jp^{msd} brains, but S1 nuclease mapping of the PLP mRNA in jp^{msd} brain indicated no deletions or major structural alterations. Gardinier and Macklin (submitted) also saw no alteration in the mRNA when analyzed by S1 nuclease mapping and demonstrated that the smaller PLP mRNAs in jp^{msd} brain were reduced to a greater extent than the 3200 nucleotide mRNA. In this study, no structural alteration of the PLP gene was identified by Southern analysis. Thus, it would appear that there is no major structural alteration in the PLP gene in jp^{msd} mice, but these experiments would not identify point mutations or alterations in the upstream regulatory elements of the gene. The proteins generated from the PLP gene in jp^{msd} brain appeared by immunoblot to be PLP and DM20. The most interesting aspect of PLP gene expression in jp^{msd} mice was the observation that at all ages up to 23 d, DM20 was present at equivalent or greater levels than PLP (Gardinier and Macklin, submitted). Thus, some aspect of the regulation of the alternate splicing of these two mRNAs is affected in this mutant. The interpretation at present is that some control element of the PLP gene is altered in jp^{msd} mice, but the specific genetic alteration is not yet known.

An unusual feature of the genetic defect in *jimpy* mice, which is within the PLP gene, is its effects on other brain cells and on the lifespan of *jimpy* oligodendrocytes. It has been known for some time that prior to the onset of myelination, astrocytic changes can be observed in *jimpy* central nervous system (Skoff, 1976; Omlin and Anders, 1983). Data on early astrocyte abnormalities in *jimpy* mice suggest intrinsic alterations in astrocytes, independent of the

alterations in oligodendrocyte function. For example, *jimpy* astrocyte processes begin to branch abnormally, and most axons become surrounded by astrocytic processes before oligodendrocyte processes contact axons. Astrocytic cytoplasm hypertrophies, and at two d after birth, astrocytes in *jimpy* optic nerve appear to have a two- to four-fold redution in microtubules and an increase in filaments. At this same age, the oligodendrocytes appear normal, but less differentiated than controls, and there are approximately 50% of the normal number of oligodendrocytes (Skof, 1976; Omlin and Anders, 1983). A significant alteration of a unique astrocyte function has been observed in *jimpy* mice. Potassium-induced stimulation of oxygen uptake by astrocytes is absent, even in isolated *jimpy* astrocytes cultured from tissue taken prior to the onset of myelination (Keen et al., 1976; Hertz et al., 1980). Other data suggesting specific astrocytic changes in *jimpy* mice come from tissue culture studies. Bartlett et al., (1987) demonstrated that when *jimpy* glial cells were cultured in the presence of conditioned media from normal glial cell cultures, oligodendrocytes survived longer and differentiated to a greater extent than without the conditioned media. The conditioned media were from young cultures that contained primarily astrocytes with only small numbers of oligodendrocytes. Thus, it is possible that normal glial cells, most likely astrocytes, produce soluble factors that enhance *jimpy* oligodendrocyte survival in culture, and that these factors are not available from cultured *jimpy* astrocytes. These are still preliminary studies, and it is too early to establish the exact nature of the alteration in *jimpy* astrocytes, but this is clearly an important area of investigation.

In addition to astrocyte alterations, the *jimpy* mutation appears to dramatically alter oligodendrocyte metabolism and lifespan. Thymidine autoradiographic studies indicated increased incorporation of radioactive thymidine in the mutant neuroglial cels after 4 d of age, and the labeling index disparity between *jimpy* and control animals became greater in older animals (Privat et al., 1982; Skoff, 1982). With this high proliferative rate and the observation of mitotic figures in *jimpy* tissue, the number of oligodendrocytes should be quite high, but in fact it is approximately 50% of normal (Meier and Bischof, 1975; Skoff, 1976). Thus, a significant number of these rapidly proliferating cells must die, whereas others continue to divide. Clusters of dying cells, presumably oligodendrocytes, are often seen in older *jimpy* mice; at some ages, over 10% of the glial cells are dying (Knapp et al., 1986). This is a 10-fold greater rate of glial cell death than in normal animals.

Studies that address the issue of *jimpy* environmental elements affecting oligodendrocyte survival and differentiation come from transplantation investigations. *Jimpy* brain tissue, either from neonatal or affected 11–35 d animals, was transplanted into *shiverer* brains (Gumpel et al., 1985). Interestingly, significant areas of myelination were subsequently found within the *shiverer* host brain. In most of the animals implanted with affected *jimpy* tissue, large, well myelinated MBP-positive areas were found both close to the site of implantation and at a distance. These MBP-positive myelinated areas were morphologically quite similar to regions myelinated by normal transplanted tissue, rather than to regions myelinated *in situ* in *jimpy* tissue. Thus, some elements within the *shiverer* brain would appear to be enhancing the ability of *jimpy* oligodendrocytes to survive and differentiate better than they would in their own environment.

Since PLP gene expression has been considered a late differentiated function of oligodendrocytes, with the major PLP gene expression occurring from 14–28 d of age, these data on early astrocyte changes and environmental elements enhancing oligodendrocyte cell death

appear somewhat inconsistent with the known genetic defect in these animals, namely altered splicing of PLP mRNA leading to production of aberrant PLP protein. Two possible explanations exist, but no clear information is available yet. Macklin and coworkers demonstrated that as early as three d after birth, PLP mRNA can be detected at low levels in whole mouse brain (Gardinier et al., 1986), and that both PLP and DM20 protein can be extracted from whole brain and immunoblotted as early as four d after birth (Gardinier and Macklin, submitted). Thus, it is possible that in the early differentiation of oligodendrocytes, a low level of expression of the PLP gene occurs, and the aberrant protein expression in oligodendrocytes affects astrocyte function and the ability of the oligodendrocytes to survive. A second explanation of altered astrocyte function would invoke the expression of the PLP gene in astrocytes at a very early stage. Since PLP protein has not been identified in astrocytes, it is possible that another alternatively spliced PLP gene product exists that is expressed in astrocytes, and that the *jimpy* splicing defect removes exon 5 from this mRNA.

As with *shiverer* mice, the alteration of a major myelin protein gene, the PLP gene, in *jimpy* mice has pleiotropic effects on other myelin genes. The MBP proteins are significantly reduced in *jimpy* mice, relative to normal animals (Campagnoni et al., 1972; Barbarese et al., 1978; Bourre et al., 1980; Delassale et al., 1981; Kerner and Carson, 1984). Delassale et al. (1981) found that the deficit in MBP in *jimpy* mice ranged from 94–98% in different regions of the *jimpy* brain at 25 d of age. When the distribution of the four MBPs was studied, it appeared that in young *jimpy* mice, the deficit in the 21.5 kdalton MBP was less than the deficit in the other three MBPs; the overall deficit in MBP accumulation in animals in this study was 92% at all ages (Kerner and Carson, 1984). When MBP accumulation was compared in *jimpy* optic nerve and sciatic nerve, a significant reduction in MBP content was observed throughout development in optic nerve, whereas no reduction was apparent in sciatic nerve (Jacque et al., 1983). Thus, there are differences in the regulation of the MBP gene in *jimpy* oligodendrocytes and Schwann cells.

The level of MBP mRNAs was also measured in *jimpy* mice, with somewhat contradictory results. Carnow et al. (1984) observed normal levels of total MBP mRNA in both *jimpy* and jp^{msd} mice when measured by in vitro translation of polysomal RNA. They found a reduction in the level of mRNA for the 14kdalton protein, with a concomitant increase in the level of mRNA for the other three major MBP proteins over normal. In contrast, in vitro translation studies by Campagnoni et al. (1984) indicated a reduction in the level of all MBP mRNAs. The mRNAs for the 17, 18.5, and 21.5 kdalton proteins were approximately 43–48% of normal, and the 14 kdalton MBP mRNA was approximately 7% of normal. When the level of total MBP mRNA was analyzed by dot blots, it ranged from 19–42% of normal age-matched controls during development (Roth et al., 1985), and these reductions were observed for both polysomal and nuclear RNA (Sorg et al., 1987). Despite differences in MBP mRNA levels measured in these studies, in all cases, the level of detectable protein was significantly below the level of detectable mRNA, suggesting that whereas the MBPs may be synthesized to some extent, the amount that accumulates is quite low. These data would suggest that MBP that is not inserted into an appropriate membrane is turned over. This would be consistent with the conclusions drawn from experiments (discussed) below on the *quaking* mutant where the synthesis of MBP is significantly above the level of accumulation (Brostoff et al., 1977).

The expression of other myelin protein genes is also altered in *jimpy* mice. By immunoblot analysis, the MAG protein level in *jimpy* brains was 5.3% of normal at 20 d of age, and the mature protein was a comparable size to

normal MAG, indicating that MAG glycosylation in *jimpy* mice is apparently normal (Yanigasawa and Quarles, 1986). The MAG mRNA levels in *jimpy* mice were measured by in vitro translation, and they were reduced to less than 10% of normal (Frail and Braun, 1985). The ratio of the two mRNAs encoding the p67 and p72 initial translation products were comparable to the normal ratio at that age, i.e., mRNA for p72 was more abundant in *jimpy* mice at 15 and 20 d of age.

A reduction in CNP activity was one of the earliest observed myelin deficits in *jimpy* mice (Kurihara et al., 1969; Sarlieve et al., 1976). The level of CNP was approximately 10% of normal, and both MAG and CNP were present at a proportionately higher level than the MBP (Yanigasawa and Quarles, 1986). CNP enzyme levels have been measured in many regions of the CNS, and they ranged from 7–25% of normal (Mikoshiba et al., 1985).

A number of changes in myelination occur in heterozygous *jimpy* females, indicating a partial expression of the *jimpy* defect in heterozygotes. Morphological studies of optic nerve from heterozygous *jimpy* females indicated a mosaic pattern of myelination in both young and adult animals with patches of dysmyelination (Skoff and Nowicki-Montgomery, 1981). This is compatible with the Lyon hypothesis (Lyon, 1972), which states that on a random basis, one of the X chromosomes from each cell will be inactivated. Morphological mosaicism was not observed in the rest of the brain, but myelin proteins were reduced in heterozygotes. PLP levels were approximately 60% of normal (Kerner and Carson, 1984), and dot blot analysis of PLP RNA indicated approximately 60–70% of normal levels (Gardinier et al., 1986). In other studies, MBP levels are also reduced in heterozygotes; both young and old heterozygous *jimpy* females measured by RIA had approximately 50–60% of normal MBP (Kerner and Carson, 1984; Benjamins et al., 1984).

Benjamins et al. (1986) demonstrated that in heterozygous brains, the different MBP isoforms all recovered to essentially normal levels by 100 d of age, and that galactolipid synthesis, which was reduced in young heterozygous animals, also recovered in older animals. A detailed morphological analysis of myelin structure during develoment in heterozygotes also indicated a recovery from the early myelin deficit and suggested one mechanism of this recovery. Bartlett and Skoff (1986) observed a major myelin deficit in young heterozygous *jimpy* spinal cord, but this deficit was essentially gone by 150 d of age. One possible mechanism for this recovery could be the production of more myelin/oligodendrocyte, and it was observed that in a two wk period, the size of the myelin sheath increased 90% in heterozygotes and only 43% in normals. Thus, oligodendrocytes in the heterozygote were producing twice as much myelin during this period as the normal oligodendrocytes. Another mechanism of compensation could be production of a higher number of normal oligodendrocytes within the heterozygote. Rosenfeld and Friedrich (1986) demonstrated that there is an increased incorporation of ^{3}H-thymidine into oligodendrocyte DNA in *jimpy* heterozygotes compared to controls and that the total number of oligodendrocytes that are labeled in heterozygotes is greater than that in normals. Thus, several mechanisms may be involved in this apparent recovery from early hypomyelination in heterozygous *jimpy* animals.

A provocative series of experiments have been conducted with double mutants carrying both the *shiverer* mutation and the *jimpy* mutation. Heterozygous *jimpy* females were backcrossed for three generations with homozygous *shiverer* male mice. The F_3 generation contained mice that were putative homozygous *shiverer*, hemizygous *jimpy* males. When these double mutants were analyzed biochemically or morphologically, they appeared to have a phenotype that was intermediate between that

of either single mutant (Kerner and Carson, 1986; Billings-Gagliardi et al., 1986). CNS tissue from the double mutants was immunoblotted for MBP, PLP, and CNP. In each case, levels of the proteins were found to be intermediate between the two mutants. Morphologically, these double mutants appeared unique, relative to the parent mutant strains (Billings-Gagliardi et al., 1986). The numbers of oligodendrocytes were not reduced, as they are in *jimpy* animals, and myelin was more abundant. Although myelin sheaths in certain brain regions lacked the major dense line, the number of myelin sheaths with more than four lamellae was significantly greater than in *shiverer* brains, and the number with major dense lines was 2–3%, in contrast to 0% of *shiverer* brains.

It is not immediately clear how these two particular mutations can be interacting in this manner, since the *shiverer* mutation causes a major deletion of the MBP gene, and the *jimpy* mutation causes a splicing defect within the PLP pre-mRNA that prevents normal PLP mRNA from being produced. Thus, none of the four MBPs, the PLP, or DM20 proteins could be produced from RNA transcribed from these two genes. The authors suggest that the ability of these double mutants to produce all four MBPs indicates the presence of a second MBP gene providing a "backup" program of MBP gene expression, although this backup program apparently does not function in the original homozygous *shiverer* animals (Kerner and Carson, 1986). The presence of a second PLP gene would presumably also be a necessary interpretation for the new ability of these double mutants to produce PLP, since the PLP gene in *jimpy* mice cannot produce normal PLP protein. Further investigation of these double mutants will be needed to ascertain the interactions of these two genes in the double mutants.

Quaking (qk)

The quaking mutation is autosomal recessive, and it maps to mouse chromosome 17 (Hammerberg and Klein, 1975). This mutation causes a disorder of both the central nervous system and the peripheral nervous system (for a review, *see* Hogan and Greenfield, 1980). These mice exhibit tremors, which continue throughout their lifetime, beginning approximately at postnatal d 12. Tonic seizures occur in these animals, although they have a normal lifespan. Affected females are fertile, but males are sterile, Histological examination indicates gray, translucent areas of the optic nerve and the myelinated tracts of the spinal cord and brainstem. There is a significant loss of CNS myelin, although PNS myelination is not severely defective at the light microscopic level. The density of oligodendrocyte cell bodies is increased along fiber tracts, often with vacuoles in the cytoplasm suggesting lysosomal degradation of myelin.

As with the other dysmyelination mouse mutants, it was important to establish whether the mutation in the quaking mouse results in a specific alteration of an oligodendrocyte/myelin gene. Comparable tissue culture studies to those with the jimpy mouse demonstrated that cerebella from quaking animals produced a myelin deficit, although it was unusual in that the myelin produced in culture was invisible by light microscopy (Billings-Gagliardi et al., 1980). When mutant cerebella were cultured in contact with normal optic nerve, significant myelination of the mutant neurons occurred (Wolf et al., 1981; Billings-Gagliardi et al., 1983). Thus, as with jimpy mice, these studies demonstrated that the quaking defect is localized to the oligodendrocytes themselves, and they can be replaced by migrating normal oligodendrocytes.

Unlike *shiverer* or *jimpy* mice, no oligodendrocyte/myelin gene product has been clearly implicated as the primary site of the genetic lesion in *quaking* mice. It cannot be a defect in MAG since the MAG gene has been mapped to mouse chromosome 7 (Sutcliffe, 1987); at present no myelin-specific genes have been mapped to chromosome 17. One unique feature to

the dysmyelination in *quaking* mice is the existence of a caudal-rostral gradient in the severity of the CNS dysmyelination, e.g., the spinal cord is less affected than the anterior commissure (Wisniewski and Morell, 1971; Friedrich, 1974), and the PNS deficit is even less. It has been suggested that the defect in *quaking* mice is a maturation defect that results in an inability of the myelin membranes to assemble correctly, rather than in an inability of the cell to synthesize the proteins (Brostoff et al., 1977; Greenfield et al., 1977).

There is a major reduction in the amount of myelin in both the central and peripheral nervous systems, and biochemical analysis indicates a reduction in the amount of most myelin lipids and proteins. The yield of myelin from adult *quaking* mice was only 5–10% of the amount from normal adult mice (Greenfield et al., 1971). One of the earliest myelin protein deficits noted was the loss of PLP (Nussbaum and Mandel, 1973; Lerner et al., 1974). When myelin proteins were electrophoresed, most of the proteins from *quaking* myelin were high molecular weight proteins, as compared with normal myelin, which contained predominantly PLP and the MBPs (Greenfield et al., 1971). When CNP activity was measured in quaking mice, it was quite low (Kurihara et al., 1970; Mikoshiba et al., 1979).

Because of the caudal-rostral gradient in the severity of the dysmyelination in the CNS, it was important to investigate differences in the myelin composition in different brain regions. The greatest protein deficit in quaking myelin was in PLP and 14 kdalton MBP, and this deficit was comparable for all regions examined (Fagg, 1979). Throughout the CNS regions studied, there was little change in the amount of the 17/18.5 kdalton MBP, and there was an increase in the amount of the higher molecular weight myelin proteins, relative to normal myelin. There was no clear gradient in the expression of specific proteins, and it would appear the gradient in severity of the dysmyelination results from the loss of the whole myelin membrane, rather than from a gradient in the loss of specific proteins.

An early study using a double isotope labeling protocol to study myelin protein synthesis in *quaking* mice indicated that overall incorporation of amino acids into *quaking* myelin was approximately 40% below that of normal myelin (Greenfield et al., 1977). This study utilized a double label protocol to assess the relative rate of protein synthesis in *quaking* and normal mouse brain, and the incorporation of proteins into different brain membranes. In *quaking* myelin, there was a preferential depression in amino acid incorporation into PLP and 14 kdalton MBP, relative to other myelin proteins. It was not clear from these experiments whether this selective reduction in amino acid incorporation was the result of depressed synthesis of the proteins or depressed incorporation of the newly synthesized proteins into the myelin membrane. These investigators subsequently studied this issue of PLP synthesis, relative to assembly into myelin, using chloroform-methanol extraction and electrophoresis to identify PLP. They concluded that the synthesis of PLP was essentially normal in both 18 and 34 d *quaking* mice, but that PLP incorporation into the myelin membrane was low (Greenfield et al., 1979).

These studies by Greenfield and coworkers suggested that PLP mRNA levels might be normal in *quaking* brain, but in another series of studies, measurement of PLP mRNA levels in *quaking* mice by both probe hybridization and in vitro translation indicated that PLP mRNA levels were quite low (Sorg et al., 1986; Sorg et al., 1987). By in vitro translation, the levels were approximately 15–22% of normal. Polysomal PLP mRNA levels measured by slot blot analysis were reduced to approximately 20–30% of the age-matched control. Nuclear PLP RNA levels were initially quite low and they steadily increased in quaking brain during development until, at 27 d of age, they were almost equivalent to the normal nuclear level

(Sorg et al., 1987). This suggested a possible defect in transport of PLP mRNA from the nucleus to the polysomes, which could lead to a build up of PLP mRNA in the nucleus.

Studies on the accumulation of MBP in different regions of the quaking nervous system indicated that the developmental curve was somewhat different between quaking and normal mice and that there was a delay in the appearance of MBP in every CNS region tested. Furthermore, the level of MBP was severely depressed, ranging from 5–20% of normal (Delassale et al., 1981; Jacque et al., 1983). Interestingly, the accumulation of MBP in spinal cord myelin did not stop at any discernable time during development, as it normally does (Delassale et al., 1981). Thus, at 80 d MBP content of spinal cord was approximately 25% of normal, whereas at 1 yr, it had risen to 35% of normal.

An early study on MBP biosynthesis in quaking mice suggested that the synthesis of the MPBs occurred at the normal rate, but that incorporation into the myelin membrane was deficient (Brostoff et al., 1977). This study utilized the double label protocol employed by Greenfield et al., (1977) to assess the relative rate of protein synthesis in quaking and normal mouse brain, and the incorporation of proteins into different brain membranes. The $^{3}H/^{14}C$ isotope ratio for total microsomal protein synthesis was measured, and the ratio for the synthesis of the 14 and 18.5 kdalton MBPs in the total brain homogenates was essentially equivalent to that. However, their double isotope ratio within isolated myelin was quite low, suggesting that a significant amount of newly synthesized MBP within quaking brain was not being assembled appropriately into myelin, and was presumably being turned over.

Other studies since have confirmed the presence of close to normal levels of MBP mRNA in quaking mice (Carnow et al., 1984; Campagnoni et al., 1984; Roth et al., 1985; Hudson et al., 1987). Quantitation of 27 d MBP mRNA by probe hybridization indicated 91% of normal levels of MBP mRNA (Roth et al., 1985), and Carnow et al. (1984) found no difference in the level of total MBP-specific mRNA in 15–25 d quaking brain relative to normal brain, when measured by in vitro translation.

Other studies quantitating the level of the different MBP mRNAs in 16–20 d quaking brain by in vitro translation indicated close to normal or elevated levels of the mRNAs for the 17, 18.5, and 21.5 kdalton MBP and approximately 70% of normal levels of the 14 kdalton mRNA (Campagnoni et al., 1984). These studies were pursued to analyze MBP mRNA levels developmentally by both dot blot analysis and in vitro translation (Roth et al., 1985). Dot blot analysis of young quaking brain samples indicated the presence of approximately 40–60% of normal MBP mRNA levels. In contrast, at 27 d, approximately 91% of normal mRNA was detected by dot blot, suggesting that the presence of normal levels of MBP mRNA in quaking mice is seen only in older quaking animals, and that in young animals, clear deficits are observed. Interestingly, in vitro translation of the same 27 d RNA samples indicated only 63% of normal MBP levels were translated, suggesting that the mRNA might be translated less efficiently than in normal animals or in younger quaking animals.

The expression of MAG has been studied, and it appears that MAG glycosylation is uniquely altered in quaking mice. Protein synthesis studies indicated that 30 d old quaking mice synthesize a fucosylated glycoprotein that migrates on gels as a slightly larger protein than MAG from control mice, but this protein has much less fucose on it than normal MAG (Matthieu et al., 1974). Since in mice no size shift in MAG is normally observed during development, it would appear that the *quaking* form of MAG is not related to any developmental stage of MAG biosynthesis in the mouse. This form of MAG and another minor form slightly lower in molecular weight than

normal MAG (Inuzuki et al., 1987) may be unique to *quaking* mice. Although normal amounts of MAG were seen in PNS, these unique forms of MAG apparently were the mature form of MAG in *quaking* PNS (Inuzuki et al., 1987).

The mRNAs encoding the two initial MAG translation products also have an unusual expression in *quaking* mice. In contrast to normal animals, the predominant MAG mRNA at both 15 and 25 d of age in *quaking* mice is the p67 MAG mRNA, which is normally seen only in older normal animals (Frail and Braun, 1984). Thus, in contrast to the current concept that the dysmyelination in *quaking* mice results from an arrest in the normal process of myelination, these data would argue that something quite different is occurring with respect to MAG gene expression, since the adult form of MAG mRNA is enriched in young *quaking* mice, and the protein produced from this mRNA has apparently altered carbohydrate processing to produce a protein of a larger size than normal.

Myelin Deficient Rat

A myelin deficient mutation has been identified in the Wistar rat, and it maps to the X chromosome. This mutation is quite similar to the *jimpy* mouse mutation. For example, it causes a disorder exclusively of the central nervous system. The first recognizable symptom is a head tremor beginning approximately at postnatal d 12–15, which becomes generalized within a few days. Seizures occur in these animals, and as with jimpy mice, these animals die before 30 d of age (Csiza and de Lahunta, 1979). Histological examination indicates gray tracts of the spinal cord. There is essentially a total loss of CNS myelin, whereas PNS myelination is not affected. Because of the extensive new information of the PLP gene and the jimpy mouse, a great deal of interest is currently focused on the myelin deficient rat, as a possible new example of a defective PLP gene.

Certain immunocytochemical and biochemical similarities to the *jimpy* mouse are apparent in myelin deficient rats. For example, interfascicular oligodendrocytes are not observed in 21 d myelin deficient rat corpus collosum, using carbonic anhydrase and 5'-nucleotidase antisera to identify cells (Kahn et al., 1986), and CNP activity in myelin deficient rats is extremely reduced relative to normal rats. Young rats have approximately 20–50% of normal CNP activity in brain and spinal cord, and the CNP level drops as the animals age (Kahn et al., 1986; Yanigasawa et al., 1986).

Other myelin proteins also have been quantitated in the myelin deficient rat, and striking deficits were noted. MBP levels in brain, as measured by RIA, were consistently 1–1.5% of normal levels from 8–25 d of age in brain, although the level of MBP in spinal cord was somewhat higher. MAG levels as measured by RIA were reduced in CNS, although spinal cord had higher levels than brain (Yanigasawa et al., 1986). In both CNS regions, MAG levels dropped with age. In no sample could PLP be detected by immunoblotting, which would have detected 0.2% of normal levels of PLP, adding further support to the possibility that the genetic lesion in these animals is within the PLP gene.

It is clear from the numerous studies on the dysmyelinating mutants that important information can be obtained with respect to the normal process of myelination. The pleiotropic effects of mutations in myelin genes on the expression of other myelin genes are distinctly different, depending on the specific mutation. Furthermore, introduction of a transgene into the mutant environment can apparently produce "normal" expression of the transgene and of the other genes affected by the pleiotrophy. Both production of transgenic *shiverer* mice and the utilization of tissue transplantation to enhance myelin production in mutants provides encouragement for the possibility of recovery from genetically-induced neurological deficits.

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