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Cell-Free Synthesis of Myelin Basic Proteins in Normal and Dysmyelinating Mutant Mice

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Abstract: Total polyribosomes were isolated from the brains of 16–20-day C57BL/6 mice, four neurological mutants (*qk/qk*, *shi/shi*, *mld/mld*, and *jp*/Y), and four heterozygote or littermate controls (*qk*/+, *shi*/+, *mld*, and *jp* littermates) and translated in a homologous, cell-free system. No differences were observed among the nine genotypes in either the yield of polysomes (32.2 ± 0.6 A_{260}/g brain) or in the incorporation of [^{35}S]methionine into trichloroacetic acid-precipitable protein. However, when the four myelin basic proteins (BPs) were isolated from the translation mixtures little incorporation of [^{35}S]methionine into the BPs was noted in those assays directed by polysomes from *mld/mld* or from *shi/shi* animals. Compared with C57BL/6 polysomes, *mld* littermate and *shi*/+ polysomes incorporated approximately half the levels of label into the four BPs while *qk*/+ and *qk/qk* incorporated normal and close-to-normal levels. Polysomes from *jp* littermates and *jp*/Y brains synthesized 66% and < 15% of the levels of the 14K BP compared with C57BL/6 polysomes. Incorporation of label into the other three BPs was normal with *jp* littermate polysomes and about half the control levels with *jp*/Y polysomes. The data indicate that *shi/shi* and *mld/mld* mutants either produce altered BPs not recognized by our antibody or synthesize very low levels of BP. The data provide additional support for the notion that the *qk/qk* mutant synthesizes much higher levels of MBP than are incorporated into myelin. They also indicate that in the jimpy mutant the synthesis of the four BPs is affected to differing extents; thus, the mutant cannot be easily characterized as either an "assembly" or "synthesis" defect.
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A number of mouse neurological mutants have been characterized that are abnormal in the structure, composition, and/or metabolism of myelin (Baumann, 1980). These include quaking (*qk*), myelin-deficient (*mld*), and shiverer (*shi*), which are autosomal recessive, and jimpy (*jp*), which is a sex-linked recessive mutation. The mutants *mld* and *shi* have been shown to be alleles (Lachapelle et al., 1980). All four of these dysmyelinating mutants are characterized by severe reductions in the levels of myelin in their brains, although the reasons for the myelin deficit are probably different in each case. Recent reviews are available that summarize in detail the morphological and biochemical defects in these animals (Hogan, 1977; Baumann, 1980). In brief, *qk* has been the most well-studied dysmyelinating mutant. In this mutation both CNS and PNS myelin are affected. Myelin composition is abnormal and most of the enzymatic activities associated with myelin lipid synthesis are low. Evidence has been presented that synthesis of myelin proteins is normal in quaking, but that myelin assembly is defective. The *jp* mutation differs from the *qk*, not only in its chromosomal location, but also in

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Abbreviations used: BP, Myelin basic protein; DTT, Dithiothreitol; HEPES, *N*-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid; diMePOPOP, *p-bis*-[2-(4-Methyl-5-phenyloxazolyl)]benzene; PPO, 2,5-Diphenyloxazole; SDS, Sodium dodecyl sulfate; TCA, Trichloroacetic acid.

the fact that the PNS does not appear to be affected. Furthermore, while *qk* mice have nearly normal life spans, *jp* mice die by 3–4 weeks of age. Jimpy CNS contains very little myelin of apparently abnormal composition, and many enzymatic activities associated with myelin lipid synthesis have been found to be reduced. It has been suggested that *jp* represents a specific defect of oligodendroglial cell metabolism (Norton, 1980) that is expressed as an arrest of myelinogenesis (Matthieu et al., 1973; Burkhart et al., 1981), possibly because of a defect in myelin assembly (Braun et al., 1980).

The *shi* and *mld* mutations have been characterized to a lesser extent than either *jp* or *qk*. These mutants have extremely low levels of myelin and basic proteins (BPs) in the CNS. There is also a lack of compaction at the major dense line of CNS myelin in both mutations. Ganser and Kirschner (1980) have shown that even though PNS myelin appears normal in *shi*, it contains very low levels (< 0.4% of controls) of immunoreactive basic protein. In contrast with the low levels of BP and myelin-associated lipids (Bird et al., 1978), the levels of several enzyme activities associated with myelin lipid synthesis and 2′,3′-cyclic nucleotide phosphodiesterase have been shown to be normal in *shi* and *mld* mice (Bird et al., 1980; Matthieu et al., 1981).

The levels of BP in all four mutants have been measured by radioimmunoassay (Barbarese et al., 1979; Zimmerman and Cohen, 1979; Ganser and Kirschner, 1980; Matthieu et al, 1980; Barbarese and Carson, 1981; Delassalle et al., 1981). Using this technique the CNS of the mutants has been determined to contain the following levels of BP as a percentage of controls: *shi*, 0.1–0.4%; *mld*, 2–3%; *jp*, 3–12%; and *qk*, 5–20%. These presumably represent steady-state levels of BP wherein the protein is associated with myelin or some myelin-like structure. Little is known about the synthesis of BP in the mutants, although *in vivo* studies have suggested that BP synthesis is normal in *qk* (Greenfield et al, 1977; Brostoff et al., 1977) and *jp* (Carson et al., 1975). Incorporation of newly synthesized BP into the membrane may possibly be defective in these animals.

It is now clear that there are four immunologically and structurally related BPs in myelin (Barbarese et al., 1977) that appear to be metabolically unrelated and the products of four separate mRNAs (Yu and Campagnoni, 1982). Synthesis of these four BPs (designated 14K, 17K, 18.5K, and 21.5K on the basis of the apparent molecular weights originally estimated by Barbarese et al., 1977) can be detected on isolated polyribosomes translated in homologous brain systems (Yu and Campagnoni, 1982) or in mRNA-stimulated reticulocyte or wheat germ lysates (Yu and Campagnoni, 1982; Colman et al., 1982; Hall et al., 1982).

The overall purpose of the studies reported here was to examine the *in vitro* protein synthetic capacity of polyribosomes isolated from the brains of C57BL/6 mice and the dysmyelinating mutants *qk*, *jp*, *shi*, and *mld*. One aim was to determine if overall rates of protein synthesis were altered in any or all of the mutant mice. A second aim was to examine directly the *in vitro* rates of protein synthesis of the individual mouse BPs and determine if all four proteins were affected to the same extents in the mutations. We hoped the study might be used to help classify these dysmyelinating mutations into those that are capable of synthesizing BPs, but in which the newly synthesized BPs are not incorporated into myelin (i.e., "assembly" defects or defects subsequent to synthesis) and those mutants that cannot synthesize BPs (i.e., "synthesis" defects).

EXPERIMENTAL PROCEDURES

Materials

Phosphocreatine, creatine phosphokinase, heparin, GTP, ATP, amino acids, and kallikrein inactivator were purchased from Sigma Chemical, St. Louis, MO. L-[^{35}S]Methionine (translation grade, > 1,000 Ci/mmol) and En^{3}Hance were obtained from NEN Chemicals, GmbH, Dreieich, F.R.G. Pansorbin and Sansorbin were purchased from S.A.P.B. Hoechst-Behring, Paris, France.

Animals

All animals were bred in the Animal Colony of the Laboratoire de Neurochimie. The *qk* mutation was on the C57BL/6 strain and the *jp* mutation was maintained on the hybrid B6/CBA background. The *shi* mutation on the C3H/SWV background was obtained from Washington University (Bird et al., 1978); and the *mld* animals originated from Indiana (Doolittle and Sweikart, 1977). Shiverer heterozygotes were obtained by crossing homozygous shiverer males with normal females. Quaking heterozygotes and homozygotes were obtained from crosses of the double heterozygote *qk*+/+*T* in which the progeny +*T*/+*T* are lethal, *qk*+/+*T* have short tails, and *qk*+/*qk*+ have normal tails.

Preparation of the components of the *in vitro* system

Initiation factors and pH 5 enzymes were prepared as previously described by Yu and Campagnoni (1982). Total polysomes were isolated by a modification of the Ramsey and Steele (1977) procedure for obtaining bound polysomes from rat brain. Five or six brains of 15–21-day old mice were homogenized in nine volumes of 20 m*M* *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), pH 7.4; 250 m*M* KCl; 5 m*M* magnesium acetate; 100 μg/ml heparin (H buffer) containing 0.25 *M* sucrose, using 12 up-and-down strokes of a Potter-Elvehjem homogenizer. Then 1/9 volume of 10% Triton X-100 was added and the suspension was mixed with two or three strokes of the pestle. Once the nuclei had been removed by centrifugation for 5 min at 1,469 × *g* in a Sorvall SS-34 rotor, the sample was mixed with 1/9 volume of 13%

(wt/wt) DOC and centrifuged for 12 min at 18,800 × *g*. The supernatant was layered onto a step gradient consisting of 3 ml of 0.5 *M* sucrose in H buffer and 1 ml of 2 *M* sucrose in H buffer. The gradients were centrifuged for 22–24 h at 112,500 × *g* in a Spinco SW27 rotor. Polysomal pellets were resuspended in 20 m*M* HEPES, pH 7.6, 5 m*M* MgOAc, 100 m*M* potassium acetate, 0.1 m*M* EDTA, 1 m*M* dithiothreitol (DTT) (A buffer).

Incubation conditions

Each 100-μl incubation mixture contained 1 A_{260} polysomes; 375 μg pH 5 enzymes; 60 μg initiation factors; 2 μl kallikrein inactivator (20 KIU/μl, 2.8 μg/μl protein); 1 m*M* ATP; 0.4 m*M* GTP; 20 m*M* phosphocreatine; 0.1 mg/ml creatine phosphokinase; 20 m*M* HEPES, pH 7.6; 1 m*M* DTT; 75 m*M* potassium acetate; 5.05 m*M* magnesium acetate; 0.05 m*M* EDTA; 40 μ*M* each of 19 amino acids (except methionine); and 100 μCi of [^{35}S]methionine (1,000–1,200 Ci/nmol). Each sample was incubated 1 h at 37°C in a 1.5-ml microcentrifuge tube.

Isolation of newly synthesized BP

The incorporation of [^{35}S]methionine into trichloroacetic acid (TCA)-insoluble material was determined by treating aliquots of the incubation mixture spotted on glass filters as described previously (Campagnoni and Harris, 1977). The incorporation of [^{35}S]methionine into BP was determined by an abbreviation of the method of Yu and Campagnoni (1982). Following incubation, each sample was mixed with 1/9 volume of 10% sodium dodecyl sulfate (SDS) and boiled for 2 min. Once the SDS had been trapped by the dilution of the mixture with nine volumes of immunoprecipitation buffer containing 1% Triton X-100 (Dimitriadis, 1979), each was stirred overnight at 4°C after the addition of 5 μl (100 KIU) kallikrein inactivator and 47 μg purified rabbit anti-BP (Reidl et al., 1981). The next morning each sample was stirred for 1.5 h at room temperature with 40 μl of a 12% (wt/vol) suspension of Sansorbin in 50 m*M* Tris·HCl, pH 7.8; 150 m*M* NaCl, 10 m*M* EDTA, 1% Triton X-100, 2 mg/ml DL-methionine (immunoprecipitation buffer) and then centrifuged for 10 min at 4,400 × *g*. The supernatants were then stirred for 1.5 h at room temperature with 40 μl of a 12% (wt/vol) suspension of Pansorbin and centrifuged 10 min at 4,400 × *g*. The Pansorbin pellets were washed four times with 500 μl immunoprecipitation buffer, resuspended in 80 m*M* DTT, 2.8% SDS, 6.4% sucrose, and boiled for 1 min, and then vortexed and boiled again for 2 min to release the BP from the Pansorbin-antibody complex. The Pansorbin was precipitated by centrifugation for 10 min at 4,400 × *g*. The BP-containing supernatant was loaded on a 16 × 14 × 1 mm 10% Weber and Osborn (1969) gel and electrophoresed until a cytochrome *c* standard had moved 10 cm from the origin. The gel was then fixed for 45 min in 50% methanol-10% acetic acid-40% water and then soaked in two changes of 40% methanol-5% acetic acid-55% water. The gel was treated with En^{3}Hance, dried, and exposed to Kodak XAR-5 film at −50°C for 2 weeks. Those areas of the gel that contained basic protein were cut out, swelled in 60 μl water and 300 μl Protosol overnight, mixed with 8 ml of a toluene-based scintillation fluid (3.6 g/L 2,5-diphenyloxazole (PPO), 0.4 g/L *p*-bis-[2-(4-methyl-5-phenyloxazolyl)] benzene (dimePOPOP), and counted.

TABLE 1. *Characterization of ribosomes*

Genotype	Yield		
	A_{260}/brain	A_{260}/g brain	A_{260}/A_{280}
C57BL/6	13.2	31.7	1.82 ± 0.01
shi/+	13.7	33.0	1.88 ± 0.03
shi/shi	14.6	34.5	1.85 ± 0.03
mld littermates	13.3	29.7	1.85 ± 0.03
mld/mld	14.6	32.8	1.84 ± 0.04
qk/+	13.8	30.6	1.79 ± 0.03
qk/qk	14.2	32.5	1.81 ± 0.05
jimpy littermates	12.1	30.4	1.78 ± 0.05
jp/Y	14.2	34.7	1.81 ± 0.04

RESULTS

Total polyribosomes were isolated from the brains of 16–20-day C57BL/6 mice, the four neurological mutants, and four heterozygote or littermate controls. As shown in Table 1, no significant differences were observed among the nine genotypes in either the yields of polyribosomes or their purity, as assessed by the A_{260}/A_{280} ratio. The average yield of polyribosomes from all genotypes was 13.7 ± 0.3 A_{260}/brain or 32.2 ± 0.6 A_{260}/g brain and the average A_{260}/A_{280} ratio of these preparations was 1.82 ± 0.01. The *in vitro* protein synthetic activities of these polysomes were examined and, as shown in Table 2, no differences were noted in the capacity of these preparations to incorporate [^{35}S]methionine into TCA-precipitable protein. In these and all the experiments to be described, incorporation of mutant or littermate polysomes was always measured relative to a minimum of one control (C57BL/6) incubation done at the same time, to eliminate variations in total incorporation that occurred from experiment to experiment. The average total incorporation by the C57BL/6 polysomes was 12.4 ± 0.8 × 10^6 dpm/100 μl incubation.

Figures 1 and 2 are fluorograms of SDS-polyacrylamide gel separations of the BPs immunoprecipitated with affinity-purified anti-BP antibodies. Identification of the four BPs (21.5K, 18.5K, 17K, and 14K) was performed as described previously (Yu and Campagnoni, 1982). There was essentially no incorporation of [^{35}S]methionine into the four BPs by either the homozygous *shi* or *mld* polyri-

TABLE 2. *Incorporation of [^{35}S]methionine into total TCA-precipitable protein*

Genotype	% C57BL/6 control
shi/+	103 ± 11
shi/shi	106 ± 12
mld littermates	94 ± 7
mld/mld	89 ± 10
qk/+	96 ± 8
qk/qk	102 ± 11
jimpy littermates	126 ± 23
jp/y	102 ± 19

Average incorporation into C57BL/6 controls was 12.4 ± 0.8 × 10^6 dpm/100 μl incubation.

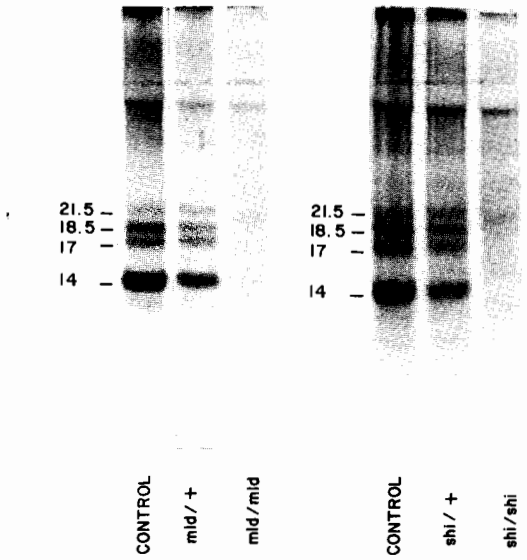

FIG. 1. Fluorograms of immunoprecipitated BPs synthesized *in vitro* using polyribosomes isolated from C57BL/6 (control), *mld/mld*, *mld* littermate, *shi/shi*, and *shi/+* brains. The four myelin BPs are identified by the numbers, corresponding to their apparent molecular weights. In this figure and Fig. 2 the prominent band migrating in the high-molecular-weight region of the gels is probably not related to the BPs and results from nonspecific binding of a radioactive protein to the Pansorbin. Unlike the four BPs this band is not competed out with unlabeled BP. Using an alternative procedure involving more rigorous washing steps, it is possible to eliminate this band from the fluorograms. This alternative procedure, however, was not developed until after these experiments were completed.

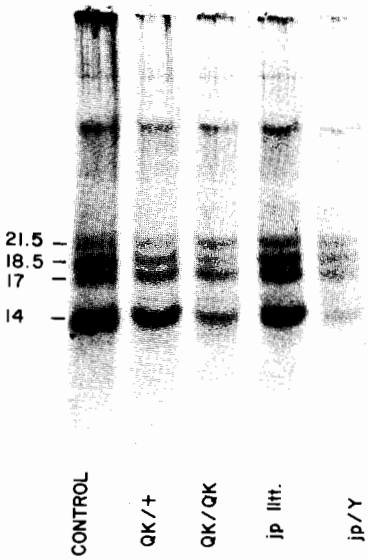

FIG. 2. Fluorograms of immunoprecipitated BPs synthesized *in vitro* using polyribosomes isolated from C57BL/6, *qk/qk*, *qk/+*, *jp*/Y, and *jp* littermate brains. The four myelin BPs are identified by the numbers, corresponding to their apparent molecular weights.

bosomes (Fig. 1), whereas significant incorporation of label was observed into the four BPs by *jp*/Y and *qk/qk* polyribosomes (Fig. 2). To obtain a semiquantitative estimate of the relative levels of incorporation of [^{35}S]methionine into each of the four BPs as a function of the mutation, after fluorography each BP band was cut out from the dried gel, hydrolyzed with Protosol, and counted. It should be noted that such a procedure is only semiquantitative and fairly large deviations resulted. Nonetheless, the counts provided a rough estimate of the levels of BP synthesis, and the data are presented in Table 3. We have consistently noted that the 17K and 21.5K BP are heavily labeled in the *in vitro* translation systems when [^{35}S]methionine is used as a precursor. This is likely to be due to the presence of additional methionine in these BPs over that present in the 14K and 18.5K BPs (Agrawal et al., 1981, 1982; P. R. Carnegie, personal communication). With respect to the mutants, *mld* and *shi* homozygote polysomes appeared to synthesize lower levels of all four BPs than could be detected by our technique. The *jp*/Y polysomes synthesized roughly half the levels of the 17K, 18.5K, and 21.5K BPs, and very low levels of the 14K BP, indicating that of the four BPs it was the most severely affected in this mutation. In contrast, homozygous quaking polysomes synthesized close to normal levels of the 18.5K BP and, perhaps, slightly lower levels of the 14K BP (the large error in these data prevents an unambiguous interpretation). Incorporation into the 17K and 21.5K BPs by the *qk/qk* polysomes was actually higher than the controls. This same trend was noted in the quaking heterozygote (*qk/+*) polysomes, with incorporation into these two BPs being significantly higher than that obtained with the control polysomes. Thus, very little synthesis of the four BPs was noted by *mld/mld* and *shi/shi* polyribosomes; and substantial synthesis of the four BPs occurred on *qk/qk* polyribosomes. Synthesis of the 14K BP was most severely reduced on *jp*/Y polysomes, with the other three BPs synthesized in significantly higher amounts, but still substantially below control levels. Syntheses of BPs by *shi/+* and *mld* littermate polysomes were roughly half the levels of the C57BL/6 controls. Polysomes from the jimpy littermates synthesized approximately normal levels of the 17K, 18.5K, and 21.5K BPs, but only about two-thirds of the control levels of the 14K BP, consistent with the more severe reduction in the synthesis of the 14K BP by the hemizygous jimpy (*jp*/Y) polysomes.

DISCUSSION

In this study, we chose to use a "run-off," homologous brain polysome system because [^{35}S]-methionine incorporation into each of the four BPs should be proportional to the numbers of active mRNAs for those proteins being translated at the time the polysomes were isolated. In previous work

TABLE 3. *Incorporation into BP as percentage of control*

Genotype	14K	17K	18.5K	21.5K
C57BL/6	100	100	100	100
qk/+	102 ± 19	129 ± 11	97 ± 16	151 ± 19
jimpy littermates	66 ± 13	90 ± 9	83 ± 12	86 ± 40
mld littermates	49 ± 6	70 ± 3	53 ± 7	29 ± 21
shi/+	42 ± 10	64 ± 12	51 ± 15	42 ± 21
qk/*qk*	70 ± 28	115 ± 18	87 ± 24	148
jp/Y	7 ± 5	48 ± 7	43 ± 7	46 ± 17
mld/*mld*	—	—	—	—
shi/shi	—	—	—	—

Average incorporation into each of the four basic proteins in the C57BL/6 controls was: 14K, 1.8 ± 0.1 × 10^4 dpm; 17K, 8.8 ± 0.9 × 10^3 dpm; 18.5K, 8.5 ± 0.9 × 10^3 dpm; and 21.5K, 3.6 ± 1.0 × 10^3 dpm.

we provided evidence indicating that the four BPs are not metabolically related and are coded by four separate mRNAs (Yu and Campagnoni, 1982). This study extends those findings and suggests that in the mutants the levels of the individual mRNAs associated with the polyribosomes (i.e., "active," translating messengers) can vary considerably. For example, the levels of synthesis of each of the four BPs were not always altered identically with respect to the controls within a given mutation (e.g., *jp*), suggesting that synthesis of the four BPs may not be coordinately regulated. The data also indicate that overall protein synthesis is apparently unimpaired in the mutants examined.

Myelin-deficient and shiverer mutations

Using the *in vitro* system described in this study we were unable to detect significant synthesis of any mouse BP in the *mld* and *shi* homozygotes. The inability to detect synthesis of the BPs does not mean that the mRNAs for these proteins are not there but rather they are present at very low levels (<5% of controls). Since a small amount of immunoreactive BP can be detected in the brains of these mutants (Dupouey et al., 1979; Ganser and Kirschner, 1980; Matthieu et al., 1980) it is unlikely that our antibodies are not recognizing the mutant BPs, although this possibility cannot be rigorously excluded.

These studies indicate that the BP defect in these two mutations lies prior to the translation of the message, suggesting that the BP mRNAs do not reach the ribosome. The reason for this is unclear but it could involve either the lack of transcription of the BP gene(s) or altered processing of the BP mRNAs. The fact that the *shi*/+ animals synthesized approximately half the levels of each of the four BPs makes it unlikely that this represents a mutation in a regulatory gene [i.e., a mutation in a gene that controls the transcription of the BP gene(s)].

Quaking mutation

The quaking mutant appears to be very different from the *mld* and *shi* mutants with respect to BP synthesis. Synthesis of all four BPs occurred at least at normal rates, yet the steady state level of BP in the brain is only 5–20% of normal. This suggests that most of the newly synthesized BPs are unable to be incorporated into the myelin membrane as has been postulated by others (Brostoff et al., 1977; Greenfield et al., 1977).

The *qk* mutation has been described as an "arrest" of myelin maturation because the ultrastructure and composition of adult *qk* myelin resembles "immature" normal myelin (Hogan and Joseph, 1970; Wisniewski and Morell, 1971). One of the characteristics of *qk* myelin and "immature" myelin is a lower 14K/18.5K BP ratio (Greenfield et al., 1971; Morell et al., 1972; Nussbaum and Mandel, 1973; Druse and Hogan, 1974; Magno-Sumbilla and Campagnoni, 1977; Matthieu et al., 1978). Since normal levels of all the BPs are being synthesized in the mutant, then proportionately less of the 14K BP must be entering the membrane relative to the 18.5K protein. The implications of this are not clear. It is possible that myelin maturation and assembly occurs in two stages, as suggested by Burkhart et al. (1981) and that in the later stage either the 14K BP is assembled into the membrane by a different mechanism or its normal assembly mechanism is selectively disrupted. At the very least there appears to be a difference in the assembly of the 14K BP into the membrane relative to that of the 18.5K protein in this mutant.

Jimpy mutation

The jimpy mutation appears to be more complicated with respect to the synthesis of BPs than the other three mutations and, therefore, it is not easy to categorize as either a "synthesis" or an "assembly" defect. In this mutation the synthesis of the 14K BP appears to be more reduced than that of the other three BPs. While synthesis of the 17K, 18.5K, and 21.5K BP is lower than normal, in *jp* it is still substantially greater than the levels of BP found in *jp* brains relative to controls (3–12%). Several groups have attempted to examine membrane fractions that might contain or correspond to jimpy "myelin," and there is a striking reduction in the amounts of the 18.5K and 14K BPs in these preparations (Nussbaum and Mandel, 1973; Matthieu et al., 1974; Matthieu and Waehneldt, 1978). This is a much greater reduction than can be explained by the reduction in the synthesis of the proteins. Thus, it appears that the jimpy mutation is unable to incorporate the newly synthesized BP into myelin. The reduction in the synthesis of the 14K BP observed in this study is in accord with the findings of Carnow et al. (1982). They concluded that there was very little mRNA for the 14K BP in the brains of *jp* mice based on translation of mRNA in a reticulocyte protein-synthesizing system. Our finding that *jp*/Y polysomes synthesize significantly lower than normal levels of the four BPs is not in agree-

ment with the earlier studies of Carson et al. (1975), although both studies agree that more total BP is synthesized than is incorporated into myelin.

It is clear that the four neurological mutations examined represent a spectrum of genotypes capable of synthesizing vastly different levels of each of the four BPs. It is interesting that the "normal" littermates in many cases (e.g., *shi* and *mld*) are synthesizing significantly less BP than C57BL/6 mice, yet apparently functional myelin is formed.

The intent of this study was to attempt to define in general terms the points at which BP metabolism was altered in these four mutations and to determine if all four BPs were involved to the same extent. BP metabolism in the *shi* and *mld* mice appears to be abnormal prior to or during synthesis of the proteins, and all four proteins are affected. BP metabolism in quaking mice is altered subsequent to protein synthesis such that all the BP synthesized is not incorporated into myelin and incorporation of the 14K BP into the membrane seems to be reduced to a greater extent than the 18.5K BP. In the case of the jimpy mutation both synthesis and incorporation of the four BPs appear to be altered, with synthesis of the 14K BP most seriously affected. We believe that this information will be useful in focusing future work on BP metabolism in these mutants to specific phases of BP synthesis and assembly into myelin.

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