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Mutation of the proteolipid protein gene *PLP* in a human X chromosome-linked myelin disorder

(human dysmyelinating disease/Pelizaeus–Merzbacher disease)

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ABSTRACT **Myelin is a highly specialized membrane unique to the nervous system that ensheaths axons to permit the rapid saltatory conduction of impulses. The elaboration of a compact myelin sheath is disrupted in a diverse spectrum of human disorders, many of which are of unknown etiology. The X chromosome-linked human disorder Pelizaeus–Merzbacher disease is a clinically and pathologically heterogeneous group of disorders that demonstrate a striking failure of oligodendrocyte differentiation. This disease appears pathologically and genetically to be similar to the disorder seen in the dysmyelinating mouse mutant jimpy, which has a point mutation in the gene encoding an abundant myelin protein, proteolipid protein (PLP). We report that the molecular defect in one Pelizaeus–Merzbacher family is likewise a point mutation in the *PLP* gene. A single T → C transition results in the substitution of a charged amino acid residue, arginine, for tryptophan in one of the four extremely hydrophobic domains of the PLP protein. The identification of a mutation in this Pelizaeus–Merzbacher family should facilitate the molecular classification and diagnosis of these X chromosome-linked human dysmyelinating disorders.**

Proteolipid protein (PLP) constitutes half of the myelin protein synthesized by oligodendrocytes in the central nervous system (CNS), where it plays a structural role in the architecture of the multilamellar myelin sheath (reviewed in ref. 1). The developmental and tissue-specific expression of PLP is coordinately controlled with the other myelin proteins in the CNS (reviewed in refs. 2–5). In the peripheral nervous system, PLP is the only myelin protein synthesized by nonmyelinating Schwann cells, glial cells that enwrap but do not myelinate axons.§ Moreover, PLP appears to be the only myelin protein produced by myelinating Schwann cells that is excluded from myelin (6, §). These observations have prompted a reevaluation of the roles that PLP must assume in glial cells. Analysis of dysmyelinating animal models has reinforced the hypothesis that PLP performs a unique function in glial cells in addition to its structural role in the myelin sheath. In the jimpy (*jp*) mouse, in which a mutation at a splice site in the *PLP* gene creates aberrantly spliced transcripts (7–12), the resulting absence of PLP has pronounced effects on glial cell differentiation. Few mature oligodendrocytes are found in *jp* CNS, despite the presence of excessive numbers of precursor cells (13, 14). This block in oligodendrocyte maturation cannot be ascribed to the inability to form a compact myelin sheath, since the failure of oligodendrocytes to myelinate in the dysmyelinating shiverer mouse (which has a deletion in the gene encoding myelin basic protein; refs. 15 and 16) does not result in the loss of oligodendrocytes (reviewed in ref. 17). Point mutations in the *PLP* gene of the canine shaking pup and the jimpy^{msd} (*jp^{msd}*) mouse, which result in single amino acid substitutions in PLP, are both associated with a lack of mature oligodendrocytes (18, 19).

The assignment of the *PLP* gene to the human X chromosome (20, 21) focused attention on an X-linked disorder of myelination in man, Pelizaeus–Merzbacher disease. While clinical and genetic heterogeneity for this disease has been described, two well-documented forms, the classical and connatal (Seitelberger) types, are both inherited as X chromosome-linked recessive traits and feature nystagmus, athetosis, tremors, and ataxia (22, 23). Patients with Pelizaeus–Merzbacher disease share many of the traits of the *jp* mutation, including the clinical signs of a dysmyelinating neurological disease, a lack of CNS myelin with a virtual absence of PLP, and a paucity of mature oligodendrocytes (22, 23). In this report we identify a mutation in the *PLP* gene of one such Pelizaeus–Merzbacher family, a four-generation pedigree of the classical type characterized extensively by Koeppen and coworkers by using biochemical and immunocytochemical techniques (24).

MATERIALS AND METHODS

A human genomic EMBL3 library constructed from a normal donor was obtained from John Weis and was screened for PLP-containing clones with human PLP cDNA probes {PLP1.5 and PLP1.3 from ref. 6, nick-translated with [α-^{32}P]dCTP (Amersham)}. Of 10^6 plaques screened, 7 were mapped in detail, as shown below the *PLP* gene in Fig. 1 (λ52, λ91, λ92, λ51Δ, λ332, λ271, λ322). Exon-containing regions were subcloned into pTZ18R and pTZ19R vectors (Pharmacia) for sequencing of the double-stranded plasmid DNA with either the Klenow fragment or reverse transcriptase (GEM-SEQ; Promega Biotec) as described by the manufacturer (sequences not shown but available).¶

Two distinct pedigrees of Pelizaeus–Merzbacher families are analyzed in this report. From the pedigree described by Koeppen *et al.* (24), both the proband (designated PM2), who died at the age of 18, and his affected nephew (PM3), who displayed abnormalities at birth, were examined for mutation at the *PLP* locus. From the pedigree originally reported by Watanabe *et al.* (27), a 20-year-old affected male (J.H.;

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Abbreviations: PLP, proteolipid protein; CNS, central nervous system; PCR, polymerase chain reaction.

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§Ono, K., Friedrich, V., Hudson, L., Lazzarini, R. & Dubois-Dalcq, M., Proceedings of the 2nd International Conference on Charcot-Marie Tooth Disease, June 28–July 1, 1987, Harriman, NY, in press.

¶Sequences (not shown here) have been deposited in the GenBank data base (accession nos. M27110 and M27111).

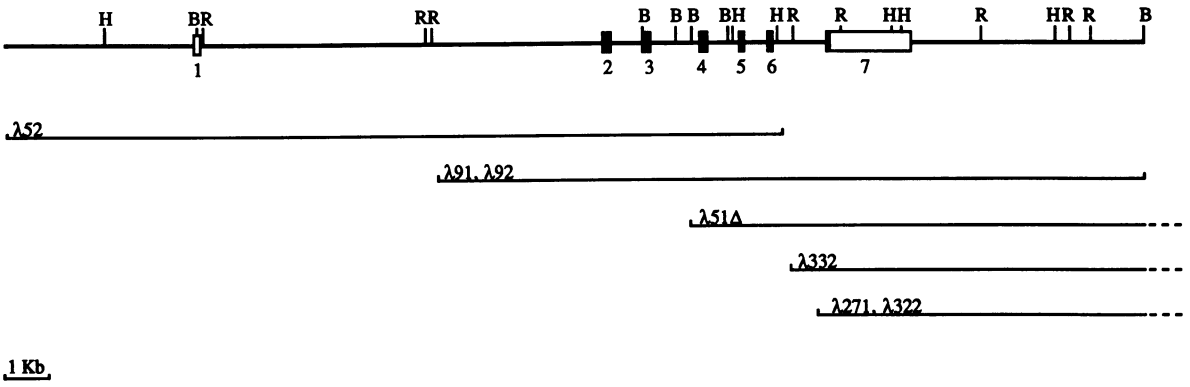


FIG. 1. Structure of the human *PLP* gene. B, *Bam*HI; R, *Eco*RI; H, *Hin*dIII. The intron/exon junctions in man and mouse were identical (19, 25, 26).

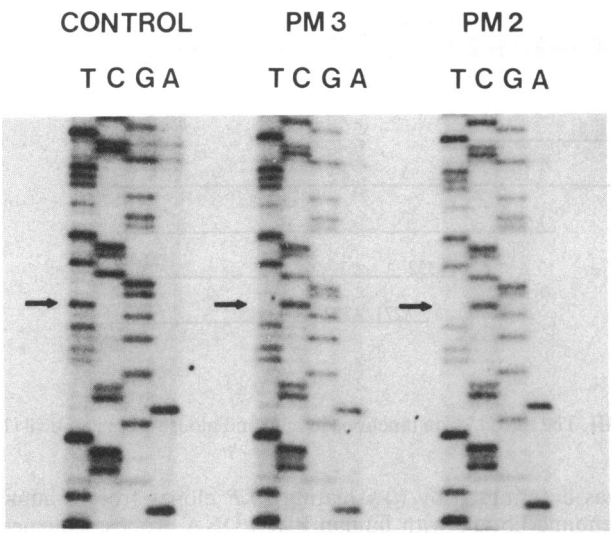
designated PM1 in this report), who upon examination by D. Farrell displayed disturbances in electroencephalograms and abnormal evoked responses (28), was characterized. DNA from Pelizaeus–Merzbacher patients was prepared from fibroblasts donated by D. Farrell for PM1 (J.H.), and lymphoblasts were obtained from the Coriell Institute (Camden, NJ) for PM2 (GM7157) and PM3 (GM74478). Genomic libraries from PM1 and PM3 DNA were constructed in the phage λEMBL3 vector. Of 1.8×10^6 recombinants screened in the PM1 library, 4 *PLP*-positive clones were obtained. One of these, λ56 containing all seven exons, was mapped and subcloned into pTZ18R for sequencing. Of 10^6 recombinants screened in the PM3 library, 2 *PLP*-positive clones were selected, and the one containing exon 4, λ93, was subcloned into pTZ18 for sequencing. Sequencing of double-stranded plasmid DNA was carried out with the modified phage T7 DNA polymerase (Sequenase; United States Biochemical) as detailed by Tabor and Richardson (29). With the exception of approximately 150 bp following the terminal polyadenylylation site, which was amplified by the polymerase chain reaction (PCR) technique prior to sequencing, the *PLP* exons, intron/exon junctions, and 1 kilobase (kb) of 5′ flanking sequence were sequenced from plasmids subcloned from λ56. Only exons 3 and 4 were sequenced from λ93.

Exons 1–7 were amplified manually from normal or patient genomic DNA by the PCR as described by Erlich and coworkers (30, 31) with the following modifications using 20-mer oligonucleotide primers prepared on an Applied Biosystems synthesizer. Each 100-μl reaction mixture included bovine serum albumin (170 μg/ml) instead of gelatin; instead of overlaying samples with mineral oil, we centrifuged the samples after each denaturation step. Each cycle of a 30-cycle program included a 1-min 95°C denaturation step, followed by a 2-min, 55°C annealing step, and finally a 70°C extension step, which was carried out for 2–6 min, depending on the size of the amplified fragment. *Thermus aquaticus* polymerase (Taq I) was purchased from Stratagene. The amplified fragments ranged in size from 236 to 512 base pairs (bp); a detailed map of their positions will be published elsewhere (36). Primers were separated from amplified fragments on Centricon 30 filtration units (Amicon) and were sequenced by the method of Higuchi *et al.* (31) with a second set of internal primers [20-mers that were phosphorylated with [γ-^{32}P]ATP (Amersham)] and Sequenase (United States Biochemical). To eliminate possible errors due to the Taq I polymerase, samples were amplified in duplicate and both samples were sequenced.

RESULTS AND DISCUSSION

Single Base Change in the *PLP* Gene of a Pelizaeus–Merzbacher Patient. The structure of the normal *PLP* gene was determined by (*i*) isolating *PLP* clones from a human genomic library with human PLP cDNA probes sequenced previously (6), (*ii*) mapping the positions of the seven exons, and (*iii*) sequencing all exons and the intron/exon junctions (Fig. 1). PLP is extremely conserved within and between species both at the protein and DNA level (6, 7), which is reflected in the absence of base changes between our sequence of the human *PLP* genomic DNA and the over 1300 bp published by Stoffel and coworkers (26) and by the scarcity of restriction fragment length polymorphisms. Moreover, no differences have been observed in the coding or noncoding regions of four unrelated individuals whose *PLP* gene was sequenced from a genomic library, from a cDNA library (6), or from PCR-amplified DNA (data not shown). The degree of preservation of the *PLP* gene is striking and makes two predictions: first, little variation in the amino acid sequence of this integral membrane protein would be tolerated; and second, perhaps the DNA or RNA encoding PLP directly plays a critical regulatory role in glial cells. The former prediction has been borne out by analysis of the *jp^{msd}* mutation, in which a conservative amino acid substitution in an α-helical hydrophobic domain of PLP has drastic effects on myelination (19).

A detailed analysis of the *PLP* gene in the Pelizaeus–Merzbacher patients was undertaken both by the construction of genomic libraries and by PCR amplification of genomic DNA and sequencing of the amplified products. The proband (PM2) and nephew (PM3) of the pedigree described by Koeppen *et al.* (24) had only a single base change in their *PLP* gene, a T → C transition in exon 4, which would substitute an arginine (CGG) residue for tryptophan (TGG) at amino acid 162 (Fig. 2). This alteration was confirmed by sequencing a subclone from the phage λ clone (λ93) containing exon 4, which precludes the possibility that an error in fidelity by the Taq I polymerase could account for the base change observed in PCR-amplified DNA. An arginine substitution at this site may disrupt the structure of PLP, as the introduced charged amino acid is located in one of the hydrophobic domains that is devoid of charged amino acids. PLP has four such hydrophobic α-helical domains, whose interactions may be critical to the correct folding of the protein and subsequent compaction of the myelin sheath (32). The introduction of the charged arginine residue into one of the hydrophobic α-helices may perturb these interactions. Although we have not yet directly established that the single base change in exon 4 creates the dysmyelinating phenotype observed in this Pelizaeus–Merzbacher family, several lines of evidence argue that the Trp-162 → Arg change is responsible. The lack of polymorphism at the *PLP* locus and the marked interspecies conservation of the amino acid sequence (i.e., man, mouse, and rat are identical for the amino acid sequence of PLP (6, 7, 33), whereas cow (34) and dog (18)

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EXON 4

TTT GTG GGC ATC ACC TAT GCC CTG ACC GTT GTG
Phe Val Gly Ile Thr Tyr Ala Leu Thr Val Val

C
TGG CTC CTG GTG TTT GCC TGC TCT GCT GTG CCT
Trp Leu Leu Val Phe Ala Cys Ser Ala Val Pro
Arg

GTG TAC ATT TAC TTC AAC ACC TGG ACC ACC TGC
Val Tyr Ile Tyr Phe Asn Thr Trp Thr Thr Cys

CAG TCT ATT GCC TTC CCC AGC AAG ACC TCT GCC
Gln Ser Ile Ala Phe Pro Ser Lys Thr Ser Ala

AGT ATA GGC AGT CTC TGT GCT GAC GCC AGA ATG
Ser Ile Gly Ser Leu Cys Ala Asp Ala Arg Met

TAT G
Tyr G(ly)
```

FIG. 2. The *PLP* gene of patients PM2 and PM3 contains a point mutation. Arrows note the sequence difference found in the genes of patients PM2 and PM3, where a cytidine residue is evident in the position of a thymidine residue in the normal control (S.G.). The exon 4 DNA sequence is shown with the encoded amino acids below, illustrating the Trp → Arg substitution that would result in abnormal *PLP* in patients PM2 and PM3. The PM2 and PM3 DNA amplified from exon 4 has an *Fnu*4HI site (5′-GCNGC-3′), which was absent from the normal control.

differ by two and one conservative amino acids, respectively) suggest that any amino acid change may affect PLP function. Moreover, PLP was absent in the PM2 patient, while low levels of the other myelin proteins (myelin basic protein, myelin-associated glycoprotein, and 2′,3′-cyclic-nucleotide 3′-phosphodiesterase) were detectable (24). Finally, both the biochemical course and clinical course of the disease in this Pelizaeus–Merzbacher family mimic those observed in the *jp* mouse, in which the splice site mutation in the *PLP* gene creates an aberrant PLP protein (7–12).

Diagnosis of Other X Chromosome-Linked Dysmyelinating Disorders. Prenatal diagnosis of this untreatable degenerative disorder could be made available for the PM2/PM3 family, either by taking advantage of the fact that the mutation creates a new restriction site (*Fnu*4HI) or by PCR-amplifying and sequencing exon 4. Unfortunately, the conserved nature of the *PLP* gene and the experience with *jp*, jp^{msd}, and shaking pup, in which each mutation occurs at a distinct site of the *PLP* gene, suggest that each Pelizaeus–Merzbacher pedigree may have a different mutation in the *PLP* gene. Indeed, an altered profile of *PLP*-positive bands on Southern blots has been reported for another candidate of Pelizaeus–Merzbacher disease (35), and an unrelated family displayed a novel point mutation in the *PLP* gene (36), which hints at a genetic heterogeneity that may complicate the analysis of the putative mutation in any pedigree. However, the modest size of the *PLP* gene should permit the analysis of all mutations affecting this gene and clarify whether Pelizaeus–Merzbacher disease is as genetically heterogeneous as the half-dozen variants of the disease would imply. Historically, pedigrees with X-linked (classical, connatal types), autosomal recessive (variant with Cockayne's disease, variant with patchy demyelination), or autosomal dominant (adult Lowenberg–Hill type) patterns of inheritance have been lumped into the Pelizaeus–Merzbacher category based on the pleiotropic clinical findings (22, 23). The discovery of an altered *PLP* gene in the classical form of Pelizaeus–Merzbacher disease (Fig. 2) invites inspection of the *PLP* gene in some of these variant forms of Pelizaeus–Merzbacher disease. The molecular dissection of the *PLP* gene would be especially welcome for cases with sex-linked inheritance that lack a pathological analysis because, even when magnetic resonance imaging is available to illuminate the lack of myelin, only an invasive biopsy or autopsy can document the reduction in differentiated oligodendrocytes and the sparse myelin islands characteristic of Pelizaeus–Merzbacher disease.

We have analyzed the *PLP* gene from one such variant of Pelizaeus–Merzbacher disease, a six-generation Pelizaeus–Merzbacher family with over 23 affected males originally described by Watanabe *et al.* (27) and further studied by Wilkus and Farrell (28). This pedigree is a textbook case of Pelizaeus–Merzbacher disease both clinically and genetically, but a curious pathology was noted in a 3-month-old affected infant from this family. Apparently normal myelin was present, but a significant amount of the myelin sheaths were organized into ball-like structures in the oligodendrocyte perikarya and terminal processes (27). The *PLP* gene from this pedigree (PM1) was unaltered for over 4 kb of coding and noncoding sequence. In addition, analysis by Southern blot hybridization of restriction digests of PM1 genomic DNA probed with human PLP cDNA failed to reveal any differences from the normal gene (Fig. 3). A detailed restriction map of a phage λ genomic clone (λ56) from patient PM1, which contained exons 1–7, also failed to uncover any differences from the normal gene (data not shown). Subcloning and sequencing of this λ clone (λ56) showed that the *PLP* gene of the PM1 patient was identical to the normal gene (data not shown) in all coding regions, in intron/exon junctions, in polyadenylylation sites, and for >1 kb 5′ to the translation initiation site. These results indicate that there is another locus on the X chromosome affecting myelination in addition to the *PLP* locus. None of the other major myelin genes (myelin basic protein; myelin-associated glycoprotein, P_0) are known to map to the X chromosome, leaving open for consideration a less abundant glial-specific protein whose function would be essential for the maintenance of normal myelin sheaths. The pedigree of Watanabe *et al.* (27) presumably contains a mutation at this second locus on the X chromosome, which would define genetically an X-linked dysmyelinating disease distinct from the classical and connatal forms of Pelizaeus–Merzbacher disease.

Nosology of the Inborn Errors of Myelin Metabolism. Myelin disorders with autosomal inheritance (22) are candidates for mutation in any of the genes encoding myelin proteins other than PLP. These human dysmyelinating disorders would be expected to present with overlapping clinical symptoms, but pathologically would not display the hallmark of mutation at the *PLP* locus, which is the lack of mature oligodendrocytes. Now that many of the genes encoding myelin proteins have been cloned (*PLP*, myelin basic protein, myelin-associated glycoprotein, P_0, P_2, and 2′,3′-cyclic-

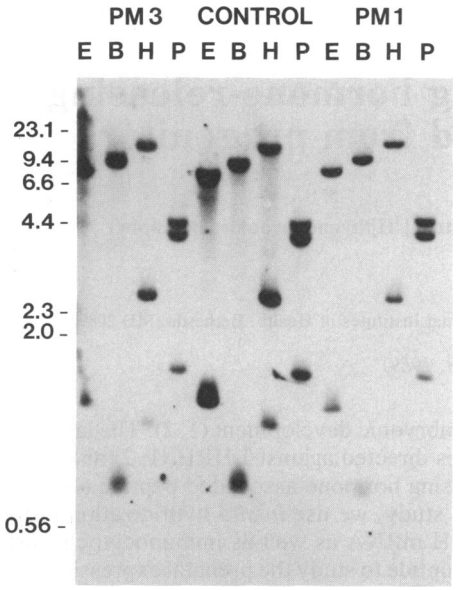

FIG. 3. The structure of the *PLP* gene is preserved in two Pelizaeus–Merzbacher pedigrees. DNA (15 μg per slot) from Pelizaeus–Merzbacher patients or an unaffected control (S.G.) was digested with *Eco*RI (lanes E), *Bam*HI (lanes B), *Hin*dIII (lanes H), or *Pst* I (P), electrophoresed on a 0.6% agarose gel, blotted onto nitrocellulose, and probed with a ^{32}P-labeled, nick-translated PLP cDNA of 1.5 kb (ref. 6). The sizes in kb of the phage λ *Hin*dIII marker are shown on the left. The PM3 (*Left*) and PM1 (*Right*) samples generate the same pattern of bands as the normal control (*Center*).

nucleotide 3′-phosphodiesterase; refs. 2–5) and mapped to their respective human chromosomes, molecular biological techniques may be applied where scant biochemical data is available. In particular, the further molecular analysis of the sex-linked disorders of myelination, clinically and pathologically classified as Pelizaeus–Merzbacher disease, offers the potential for accurate diagnosis and prenatal testing in affected families. The X-linked inborn errors of myelin metabolism represent at least two loci on the X chromosome critical to myelination, one of which encodes PLP. These two disorders can be readily discriminated pathologically by the extent of myelination. Authentic Pelizaeus–Merzbacher patients should present with only residual amounts of sudanophilic cells (mature oligodendrocytes that have elaborated a myelin sheath, which absorbs the Sudan black dye). Our results predict that all patients with the genetics, clinical history, and pathology of the classical type of Pelizaeus–Merzbacher disease will have a mutation at the *PLP* locus. We propose restricting the term Pelizaeus–Merzbacher to these patients to reduce the confusion attendant with grouping a diverse array of myelin disorders under the Pelizaeus–Merzbacher rubric.

Note Added in Proof. A third Pelizaeus-Merzbacher family was recently found by M. E. Hodes and coworkers (37) to have a distinct mutation at the *PLP* locus, a proline → leucine substitution at amino acid 14.

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1. Braun, P. E. (1984) in *Myelin*, ed. Morell, P. (Plenum, New York), pp. 97–116.
2. Sutcliffe, J. G. (1987) *Trends Genet.* **3,** 73–76.
3. Lemke, G. (1988) *Neuron* **1,** 535–543.
4. Campagnoni, A. (1988) *J. Neurochem.* **51,** 1–14.
5. Campagnoni, A. & Macklin, W. (1988) *Mol. Neurobiol.* **2,** 41–89.
6. Puckett, C., Hudson, L., Ono, K., Friedrich, V., Benecke, J., Dubois-Dalcq, M. & Lazzarini, R. A. (1987) *J. Neurosci. Res.* **18,** 511–518.
7. Hudson, L. D., Berndt, J., Puckett, C., Kozak, C. A. & Lazzarini, R. A. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 1454–1458.
8. Nave, K.-A., Lai, C., Bloom, F. E. & Milner, R. J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 9264–9268.
9. Nave, K.-A., Bloom, F. E. & Milner, R. J. (1987) *J. Neurochem.* **49,** 1873–1877.
10. Macklin, W. B., Gardinier, M. V., King, K. D. & Kampf, K. (1987) *FEBS Lett.* **223,** 417–421.
11. Morello, D., Dautigny, A., Pham-Dinh, D. & Jolles, P. (1986) *EMBO J.* **5,** 3489–3493.
12. Ikenaka, K., Furuichi, T., Iwasaki, Y., Moriguchi, A., Okano, H. & Mikoshiba, K. (1988) *J. Mol. Biol.* **199,** 587–596.
13. Skoff, R. (1982) *Brain Res.* **248,** 19–31.
14. Matthieu, J. M., Widmer, S. & Herschkowitz, N. (1973) *Brain Res.* **55,** 403–412.
15. Molineaux, S., Engh, H., deFerra, F., Hudson, L. & Lazzarini, R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7542–7546.
16. Roach, A., Takahashi, N., Pravtcheva, D., Ruddle, F. & Hood, L. (1985) *Cell* **42,** 149–155.
17. Hogan, E. & Greenfield, S. (1984) in *Myelin*, ed. Morell, P. (Plenum, New York), pp. 489–534.
18. Nadon, N., Duncan, I. & Hudson, L. (1988) *J. Neurosci.* **14,** 829A (abstr.).
19. Gencic, S. & Hudson, L. D. (1989) *J. Neurosci.*, in press.
20. Willard, H. F. & Riordan, J. R. (1985) *Science* **230,** 940–942.
21. Mattei, M. G., Alliel, P. M., Dautigny, A., Passage, E., Pham-Dinh, D., Mattei, J. F. & Jolles, P. (1986) *Hum. Genet.* **72,** 352–353.
22. Seitelberger, F. (1970) in *Handbook of Neurology*, eds. Vinken, P. J. & Bruyn, G. W. (North-Holland, Amsterdam), pp. 150–202.
23. Zeman, W., DeMyer, W. & Falls, H. F. (1964) *J. Neuropathol. Exp. Neurol.* **23,** 334–354.
24. Koeppen, A. H., Ronca, N. A., Greenfield, E. A. & Hans, M. B. (1987) *Ann. Neurol.* **21,** 159–170.
25. Macklin, W. B., Campagnoni, C. W., Deininger, P. L. & Gardinier, M. V. (1987) *J. Neurosci. Res.* **18,** 383–394.
26. Diehl, H.-J., Schaich, M., Budzinski, R.-M. & Stoffel, W. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 9807–9811.
27. Watanabe, I., Patel, V., Goebel, H., Siakotos, A., Zeman, W. & Dyer, J. S. (1973) *J. Neuropathol. Exp. Neurol.* **32,** 313–333.
28. Wilkus, R. J. & Farrell, D. F. (1976) *Neurology* **26,** 1042–1045.
29. Tabor, S. & Richardson, C. C. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4767–4771.
30. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239,** 487–491.
31. Higuchi, R., von Beroldingen, C., Sensabaugh, G. & Erlich, H. (1988) *Nature (London)* **332,** 543–546.
32. Hudson, L. D., Friedrich, V., Behar, T., Dubois-Dalcq, M. & Lazzarini, R. (1989) *J. Cell Biol.* **109,** 717–727.
33. Dautigny, A., Alliel, P. M., d'Auriol, L., Pham-Dinh, D., Nussbaum, J.-L., Galibert, F. & Jolles, P. (1985) *FEBS Lett.* **188,** 33–36.
34. Naismith, A. L., Hoffman-Chudzik, E., Tsui, L.-C. & Riordan, J. R. (1985) *Nucleic Acids Res.* **13,** 7413–7425.
35. Fahim, S. & Riordan, J. R. (1986) *J. Neurosci. Res.* **16,** 303–310.
36. Gencic, S., Abuelo, D., Ambler, M. & Hudson, L. D. (1989) *Am. J. Human Genet.* **45,** 435–442.
37. Trofatter, J., Dlouhy, S., DeMyer, W., Conneally, P. M. & Hodes, M. E. (1989) *Proc. Natl. Acad. Sci. USA* **86,** in press.