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24. This rod has different shapes in different species. In all species, part of the rod runs transverse to the A–P axis of the larval body. The optic axis orientation is given relative the A–P axis of the larval body.
25. I thank D. Raup for kindly allowing me to reexamine his data on apical system crystallography. I am also grateful to D. Cowen for making the Universal stage microscope available; to R. Haugerud for instruction in its use; and to A. Kohn, P. Mace, D. Raup, and R. Strathmann for comments on the manuscript. Supported by NSF grant OCE-8400818 to R. Strathmann and by a 10-week graduate student fellowship from the Smithsonian Institution.

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Assignment of the Gene for Myelin Proteolipid Protein to the X Chromosome: Implications for X-Linked Myelin Disorders

Abstract. *Several inherited disorders in humans and in rodents result in myelin dysgenesis and a deficiency of the molecular constituents of myelin. A complementary DNA to one of the two major myelin proteins, myelin proteolipid protein (also known as lipophilin), has been used with Southern blot analysis of somatic cell hybrid DNA to map the human proteolipid protein gene to the middle of the long arm of the human X chromosome (bands Xq13-Xq22) and to assign the murine proteolipid protein gene to the mouse X chromosome. Comparison of the gene maps of the human and mouse X chromosomes suggests that myelin proteolipid protein may be involved in X-linked mutations at the mouse jimpy locus and has implications for Pelizaeus-Merzbacher disease, a human inherited X-linked myelin disorder.*

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The myelin of the central nervous system (CNS) contains two major membrane proteins, myelin basic protein (MBP) and proteolipid protein (PLP) (also known as lipophilin) (1). The structural role of these proteins in the maintenance of myelin function has been elucidated by extensive biochemical analysis of the purified proteins (*2*). Humans and other mammals are subject to a number of genetically determined demyelinating diseases that occur when one or both of these constituent proteins is deficient (*3*). In mutant mice homozygous for the *shiverer* mutation, the CNS myelin is depleted of MBP as the result of a partial deletion of the autosomal MBP gene; thus genetic defects at the MBP locus can have extreme effects on myelin formation and central nervous system function (*4*). Genetic analyses of the role of PLP in the central nervous system became possible recently, with the molecular cloning and characterization of a complementary DNA (cDNA) for bovine PLP (*5*). As an initial step in such studies, we have used the PLP cDNA to assign the PLP gene to the X chromosome in both man and mouse. In addition, we have localized this gene to the middle of the long arm of the human X chromosome. Comparison of the gene maps of the mouse and human X chromosomes suggests that the PLP gene may have a primary role in several X-linked inherited myelin disorders.

Southern blotting experiments were performed with a ^{32}P-labeled cDNA (pLP1), containing a 0.95–kilobase pair (kbp) insert fragment that includes 444 base pairs of coding sequence corresponding to the carboxyl terminal half of lipophilin and about 500 base pairs of 3′ untranslated sequence (*5*). When hybridized to nitrocellulose filters containing genomic human DNA digested with Eco RI, the PLP cDNA detects two hybridizing bands of lengths 9.0 kbp and 1.3 kbp (Fig. 1). When DNA's were examined from individuals with different numbers of X chromosomes, the hybridization signals of both bands varied in a dose-dependent fashion (Fig. 1, lanes 1, 2, and 3). Thus, the bands detected in DNA from a normal 46,XX female (lane 1) were approximately twice as intense as bands observed in the same amount of DNA from a normal 46,XY male (lane 2). Bands detected in DNA from a 49,XXXXX female were substantially more intense (lane 3). These data suggest that human PLP gene sequences are confined to the X chromosome, since autosomal DNA sequences would be present in identical doses in the three DNA samples.

The chromosomal assignment of the PLP gene made by dosage was confirmed by Southern blot analysis of DNA extracted from 18 human × mouse or human × Chinese hamster somatic cell hybrids, prepared and characterized as described previously (*6*). The ^{32}P-labeled lipophilin cDNA was hybridized to Eco RI–digested DNA from the hybrid lines and from normal mouse and Chinese hamster cells. The two human DNA bands could easily be distinguished from single bands observed either in mouse DNA (8.5 kb) (Fig. 1, lane 4) or in Chinese hamster DNA (20 kb) (Fig. 1, lane 8). Both human DNA bands were present in hybrids containing a normal human X chromosome (Fig. 1, lanes 5, 6, 12, and 13) and absent from hybrids lacking this chromosome (Fig. 1, lane 7). Table 1 summarizes the complete data and the chromosomal content of the 18 hybrids. The X chromosome was the only chromosome with no discordancies; all other human chromosomes were discordant in at least 8 (44 percent) of the 18 hybrids. Hybrids AHA-llaB1 and cl 2D contain the human PLP gene and have a human X chromosome as their only identifiable human material (*7*). In addition, the hybrid pair, A54-8A (which contains the human PLP gene) and A54-8AAz22 (which does not), provide direct evidence for X-linkage of the PLP gene, since they differ only by the absence of the X chromosome from A54-8AAz22, which was derived from A54-8A by back-selection in medium containing 8-azaguanine to select cells that had lost an X chromosome (*6*, *8*).

Four of the hybrids examined contain only portions of the human X chromosome as a result of X;autosome translocations in the human cells used to prepare the hybrids. Examination of DNA from these hybrids provided information on the regional localization of the PLP gene on the X chromosome. PLP gene sequences were present in a hybrid containing the distal two-thirds of the long arm of the X (Xq13-Xqter) (Fig. 1, lane 11), and absent from hybrids containing the entire short arm of the X, the short arm and the proximal third of the long arm (Xpter-Xq13) (Fig. 1, lane 9), or the distal half of the long arm (Xq22-qter) (Fig. 1, lane 10). Thus, PLP sequences can be localized to region Xq13-Xq22, in the middle of the long arm of the human X chromosome.

The mammalian X chromosome represents an ancient conserved linkage group (*9*). Genes localized to the X chromosome in one mammalian species are usually found to be X-linked in other species. To determine whether the PLP gene is X-linked in mouse as well as in humans, we examined DNA from a mouse × Chinese hamster hybrid, EV1-5, which contains the mouse X chromosome as its only identifiable mouse material (*10*). DNA from this hybrid contained both hamster and mouse PLP sequences (Fig. 2, lane 1). DNA from hybrid EV1-5A-1a, a counterselected derivative of EV1-5 that no longer contains the mouse X, did not contain the 8.5-kb mouse DNA band. Thus, these data indicate, as expected, X-linkage of the murine PLP gene.

The chromosomal assignment of the human and mouse PLP genes contributes to our understanding of inherited demyelinating disorders in these two species. In mouse, several myelin-deficient mutants have been described (*3*, *4*, *11*). In addition to a number of autosomally inherited defects, there are two allelic X-linked mutations, jimpy (*jp*) and myelin synthesis deficiency (*msd*) (*11*). The CNS myelin fraction of the brains of these mutants is severely depleted of a number of myelin constituents, including PLP. Study of the *jp* mutation in particular has contributed to the understanding of myelin biochemistry, in both normal and disease states (*3*). Nonetheless, despite extensive investigation, identification of the primary defect has remained elusive. Our finding that the PLP gene is X-linked indicates that this gene locus may contribute to these two X-linked disorders.

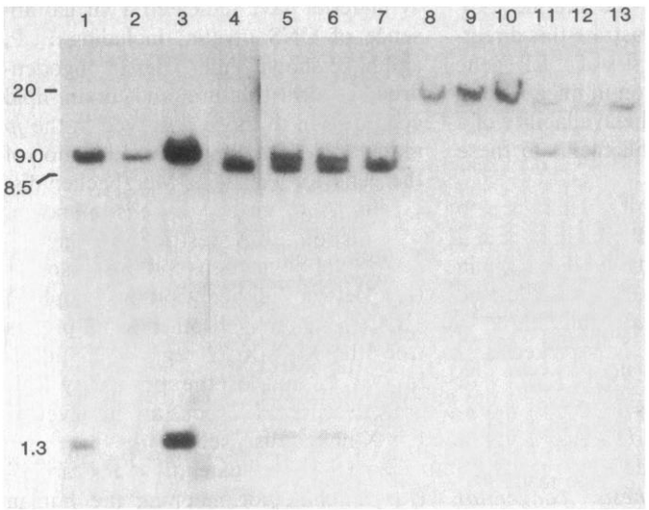


Fig. 1. Hybridization of myelin proteolipid protein cDNA to human × mouse and human × Chinese hamster hybrid cell DNA's. Hybrids were prepared and characterized as described (*6*). Equal amounts of DNA that had been digested with Eco RI were applied to all lanes, and Southern blots were prepared according to standard procedures (*19*). Hybridization to ^{32}P-labeled insert from pLP1 was carried out as described (*20*). (Lanes 1, 2, and 3) Human cell lines with 46,XX, 46,XY, and 49,XXXXX karyotypes, respectively; (lane 4) mouse cell line; (lanes 5 and 6) human × mouse hybrid cell lines containing the human X chromosome; (lane 7) human × mouse hybrid cell line missing human X chromosome; (lane 8) Chinese hamster cell line; (lanes 9 through 13) human × Chinese hamster hybrid cell lines W4-3AAz2, W53-5Bc15, W4-1A, cl 2D, and W44-14A, respectively (see Table 1). Human band at 1.3 kbp is visible in lanes 2, 11, 12, and 13 on original autoradiograph.

Although it is possible that X-linkage of the mouse mutants and of the PLP gene are unrelated to each other, the hypothesis that the *jp* and *msd* mutations occur at the PLP locus on the X is strengthened by consideration of the comparative gene maps of the mouse and human X chromosomes (Fig. 3) (*12*). The *jp* and *msd* genes have been mapped genetically to within 2 centimorgans of the murine α-galactosidase locus (*11*, *12*), to a region within which the relative order of the X-linked genes for testicular feminization, phosphoglycerate kinase, and α-galactosidase is the same in mouse and man (Fig. 3). This conserved region encompasses bands Xq12-Xq22 on the human X. That the human PLP gene maps to this region strongly supports the

Table 1. Assignment of the myelin proteolipid protein (PLP; lipophilin) gene to the human X chromosome, region Xq13-Xq22.

Hybrids	Human chromosomes																						Human PLP gene
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	
A60-1A	−	−	−	−	+	−	+	−	−	−	−	−	−	+	−	−	−	−	−	+	+	+	+	+
A60-2A	−	+	−	+	+	−	+	−	−	−	−	−	−	+	−	−	+	−	−	−	+	+	+	+
L23-4B	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	+	+	−	+	+	−	+	+
L23-4C	+	+	−	+	+	+	+	−	−	+	+	−	+	+	+	−	+	+	+	+	+	−	+	+
A23-1A	+	−	+	−	−	−	+	+	−	+	+	+	−	+	+	+	−	−	+	+	−	−	+	+
L23-1B	+	−	+	−	+	+	+	−	−	−	−	+	+	+	−	+	+	−	−	+	+	−	−	−
A23-2E1	−	+	+	−	−	−	−	−	−	−	−	+	+	+	−	−	+	−	−	+	−	−	+	+
A54-2A	+	+	+	−	+	+	+	+	−	−	+	+	+	+	−	−	+	+	+	+	+	−	+	+
A54-8A*	+	+	+	+	+	−	−	−	−	+	+	−	−	−	+	−	−	+	+	−	+	−	+	+
A54-8AAz22*	+	+	+	+	+	−	−	−	−	+	+	−	−	−	+	−	−	+	+	−	+	−	−	−
W44-14A	+	−	+	+	−	−	−	−	+	−	+	+	−	+	−	+	−	−	+	−	−	−	+	+
cl 2D	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
AHA-11aB1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
A48-1G†	−	−	−	−	+	−	+	−	−	+	+	−	−	−	−	−	−	+	−	+	+	−	+	+
A48-1GAz44†	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	+	−	+	+	−	Xpter-cen:	−
W4-1A†	−	−	−	+	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	:Xq13-qter	+
W4-3AAz2†	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	Xpter-q13:	−
W53-5Bc15†	−	−	+	−	+	+	−	−	−	−	+	+	+	+	+	+	+	−	−	−	+	+	:Xq22-qter	−
Percent Discordancy	56	50	61	56	56	72	44	61	67	56	44	61	61	56	67	72	56	56	50	44	56	67	0	

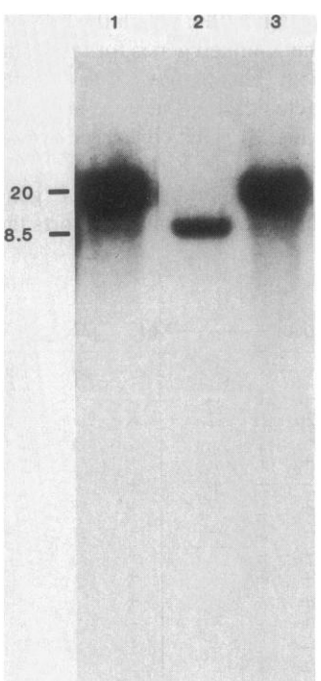
*Hybrids A54-8A and A54-8AAz22 both contain a t(4;10) present in the human parental cells (*6*). †A48-1G contains both reciprocal products of a parental t(X;11)(cen;p11) and thus contains a complete X chromosome (*21*). A48-1GAz44 was back-selected in medium containing 8-azaguanine and contains only the Xp/11p chromosome (*21*). Hybrids W4-1A and W4-3AAz2 contain the 14q$^+$ and Xp$^-$ derivatives of KOP2 cells, respectively, which have a t(X;14) translocation (*22*). W53-5Bc15 contains the 14q$^+$ derivative of a different parental t(X;14)(q22;q24.3) translocation (*23*).

possibility that the *jp* and *msd* mutations are mutant alleles at the murine PLP locus. Efforts to demonstrate this directly will require analysis of PLP gene structure and expression in mice with *jp* and *msd* mutations. The availability of a cDNA for PLP (*5*) will facilitate these analyses.

Similarly, it would be interesting to investigate aspects of PLP structure and function in X-linked myelin disorders in other animal species. For example, the myelin deficiency (*md*) mutation in a strain of Wistar rats is X-linked and displays many of the biochemical and ultrastructural features of the *jp* mutation in the mouse (*3*, *13*). An X-linked trait in pigs involving defective myelination (*hypomyelinogenesis congenita*) (*14*) may also represent a defect at this locus.

The human X-linked disorder Pelizaeus-Merzbacher disease, which has many features suggestive of an inborn failure of myelination, may be analogous to the murine *jp* mutation (*15*). Affected male infants show signs of slowly progressive neurological disease. Morphological and biochemical studies on autopsy material have indicated a virtual absence of CNS myelin, including PLP, and have shown similarities in oligodendrocyte ultrastructure and brain lipid composition to those observed in the *jp* mutant (*15*, *16*). Although the location of the gene for Pelizaeus-Merzbacher disease on the X chromosome is unknown, analysis of DNA restriction-fragment-length polymorphisms in this disorder (*17*), detected either with the lipophilin cDNA itself or with other DNA probes from the Xq13-Xq22 region, should allow evaluation of the possibility that defects at the PLP locus are involved in this X-linked disease.

Our study illustrates the "utilitarian" (*18*) rationale for mapping the human genome. Establishing the map location of a previously unassigned gene can serve, in some cases, to highlight a relation between genes or between a gene and a disease and to formulate, thereby, testable hypotheses. Our data suggest a possible basis for several inherited defects of the X-linked PLP gene, and also provide a genetic framework within which to consider the role of myelin and its constituent proteins in the mammalian nervous system.



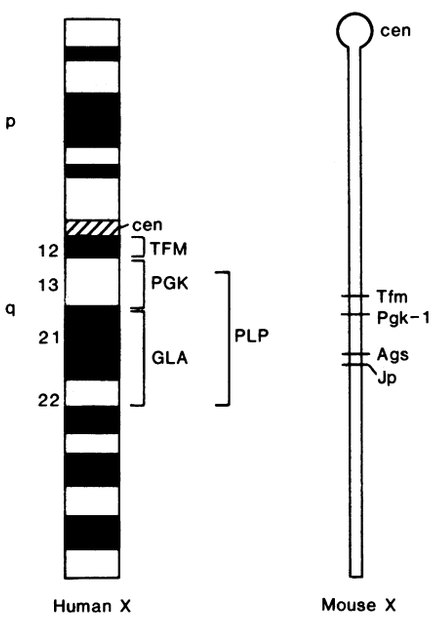


Fig. 2 (left). Hybridization of myelin proteolipid protein cDNA to mouse × Chinese hamster hybrid cell DNA. Hybrid cell line EV1-5, containing only a mouse X chromosome, has been described (10). Eco RI–digested DNA was applied, and a Southern blot prepared and hybridized as described in legend to Fig. 1. (Lane 1) EV1-5 hybrid; (lane 2) mouse cell line; (lane 3) Chinese hamster cell line. The Chinese hamster parent of EV1-5 is line E36, which gives a much more intense hybridization signal to the probe used than does wg3H, the Chinese hamster parent of hybrids shown in Fig. 1. The basis for this apparent difference in copy number is unknown. Fig. 3 (right). Schematic representation of the human X chromosome (left) and the mouse X chromosome (right). Comparative physical map (human) and genetic map (mouse) are shown. Designations of relevant human bands are given at the left (p, short arm; q, long arm). Abbreviations are cen, centromere; TFM and Tfm, human and mouse testicular feminization gene loci, respectively; PGK and Pgk-1, human and mouse phosphoglycerate kinase loci, respectively; GLA and Ags, human and mouse α-galactosidase loci, respectively; PLP, human myelin proteolipid protein locus; Jp, mouse jimpy locus.

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