The Gene Encoding Peripheral Myelin Protein Zero Is Located on Mouse Chromosome 1

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We have used somatic cell hybrids to map the gene encoding protein zero (P₀), the major structural protein of peripheral myelin. Analysis of Southern blots of DNA obtained from these hybrids allows us to unambiguously assign the P₀ gene to mouse chromosome 1. This observation indicates that mutations in the P₀ gene do not account for *Trembler*, a chromosome 11 mutation that specifically affects myelination in the peripheral nervous system.

The elaboration of myelin, the electrical insulation that surrounds all rapidly conducting axons in vertebrates, involves the induction of a number of glial-specific genes (Lemke, 1988). In the PNS, the most prominent of these induced genes encodes the major structural protein of peripheral myelin, protein zero (P₀). P₀ is a 30-kDa integral membrane glycoprotein that accounts for over 50% of PNS myelin protein (Greenfield et al., 1973; Ishaque et al., 1980). It is restricted in its expression to myelin-forming Schwann cells, the principal glial cells of the PNS: it is not expressed by either CNS glia or by the large number of nonmyelinating Schwann cells that populate many peripheral nerves (Brockes et al., 1980; Lemke, 1988). P₀ exhibits structural similarity to immunoglobulins and immunoglobulin-related cell adhesion proteins (Lai et al., 1987; Lemke et al., 1988) and has been hypothesized to function as a membrane adhesion molecule and to thereby promote and maintain the very tight compaction of apposed membrane surfaces characteristic of myelin (Kirschner and Ganser, 1980; Lemke and Axel, 1985; Lemke et al., 1988).

Like P₀, the mouse mutation *Trembler* is restricted in its expression to the PNS and, most strikingly, to myelin-forming Schwann cells (Falconer, 1951; Henry et al., 1983; Hogan and Greenfield, 1984). Mice carrying this mutation exhibit generalized tremors as a result of hypomyelination of peripheral nerves that, in the wild type, are heavily myelinated (e.g., the sciatic nerve). Although the trembling phenotype is evident in heterozygotes, the mutation is semidominant, in that there are clear gene dosage effects with respect to the extent of hypomyelination

and the steady-state levels of major myelin proteins (Fryxell, 1983). Two alleles of *Trembler*, *Tr* and *Trⁱ*, have been described. *Tr/Tr* homozygotes exhibit a nearly complete lack of peripheral myelin, but are viable under laboratory conditions. *Trⁱ/Trⁱ* homozygotes also exhibit severe hypomyelination, but die 2–3 weeks after birth (Henry and Sidman, 1988). The *Trembler* gene has been genetically mapped to mouse chromosome 11 (Davison and Roderick, 1978), and expression of this gene has been unambiguously demonstrated to be Schwann cell autonomous (Bray et al., 1981). Thus, wild-type Schwann cells myelinate the axons of *Trembler* neurons, but *Trembler* Schwann cells are incapable of myelinating wild-type axons (Aguayo et al., 1977; Bunge et al., 1980). In addition to a hypomyelinating phenotype, *Trembler* Schwann cells exhibit an inability to withdraw from the proliferative state that normally precedes differentiation (Low, 1976; Perkins et al., 1981). This extended proliferation, which often results in the formation of “onion bulb” tumors, is also restricted to myelinating cells: *Trembler* nerves that are largely unmyelinated (e.g., the sympathetic trunk) are populated by a normal number of Schwann cells whose appearance is indistinguishable from wild type (Perkins et al., 1981).

The parallel expression of P₀ and the *Trembler* gene product has led to the suggestion that they are identical. We have tested this hypothesis directly. We have used a cloned rat P₀ cDNA, together with a set of DNAs prepared from mouse-hamster and mouse-rat somatic cell hybrids, to map the P₀ gene to mouse chromosome 1 and to exclude the possibility of a second P₀ gene located on mouse chromosome 11. This finding demonstrates that the PNS-specific *Trembler* mutation does not involve any direct alteration in the structure of the major PNS-specific myelin gene, but must instead result from the alteration of an as-yet-unidentified gene uniquely expressed by myelinating Schwann cells.

Materials and Methods

Probes. Southern blots of genomic DNA from mouse-hamster and mouse-rat hybrid lines were probed with the 1.85-kb EcoRI insert of a nearly full-length clone of the rat P₀ cDNA (pSN63) (Lemke and Axel, 1985). This DNA was labeled to high specific activity by nick translation or random hexamer priming using α^{32} P-deoxynucleotides, and hybridized at high stringency to Southern blots according to standard procedures (Maniatis et al., 1982).

Cell lines and DNAs. Fifteen different hybrid cell lines, prepared by fusion of the E36 Chinese hamster cell line with the CMS4TG and MethA mouse sarcoma cell lines, were used, together with 1 line prepared from microcell fusions of C57BL/6J mouse embryo fibroblasts with rat FTO-2B hepatoma cells. The preparation and karyotyping of these lines have been described previously (Pravtcheva et al., 1983; Killary and Fournier, 1984). The chromosome composition of the mouse-

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Figure 1. P_0 gene is located on mouse chromosome 1. Southern blot hybridization of the rat P_0 cDNA to genomic DNA isolated from mouse-hamster hybrid cell lines. Genomic DNA (20 μ g each lane) was isolated from the indicated mouse-hamster hybrid cell lines, digested with BamHI, resolved on an agarose gel, Southern-blotted, and probed with a full-length, nick-translated rat P_0 cDNA probe. Size markers are in kilobases and mark the migration of λ HindIII standards.

hamster cell lines used in this study is summarized in Table 1. The mouse-rat line used—F(11)J—contains only mouse chromosome 11 on a complete background of rat chromosomes (Killary and Fournier, 1984). Genomic DNA (20 μ g) from each mouse-hamster line was digested with BamHI, resolved on agarose gels, and blotted onto nylon (Hybond) membranes for Southern analysis. Restriction digests using BamHI, EcoRI, HindIII, PstI, and XbaI (10 μ g each digest) were similarly analyzed for the F(11)J rat-mouse hybrid line.

Results and Discussion

We analyzed BamHI restriction digests of genomic DNA prepared from the 15 mouse-hamster hybrid cell lines described in Table 1, together with DNA from the CMS4TG parental mouse and E36 parental Chinese hamster lines. BamHI was chosen for these digests since it generates a number of distinctive

Figure 2. P_0 gene is not located on mouse chromosome 11. *A*, Southern blot hybridization of a rat P_0 cDNA to genomic DNA isolated from either C57BL/6J mouse liver (*M*), F(11)J rat-mouse hybrid cells (*R/M*), or back-selected FB(11)J cells (*R*). F(11)J cells contain mouse chromosome 11 on a complete rat background, while FB(11)J cells carry only rat chromosomes. DNAs were cut with the indicated restriction enzymes and probed as described for Figure 1. Note that for each enzyme *R/M* and *R* lanes exhibit identical hybridization patterns, which are clearly distinguished from the patterns present in *M* lanes. Size markers are in kilobases and mark the position of λ HindIII standards. *B*, Secondary control Southern hybridization of a rat *c-erbA* cDNA to the HindIII, PstI, and XbaI digests from the blot in *A*. The *c-erbA* gene has previously been shown to reside on mouse chromosome 11. Arrowheads indicate restriction fragments shared between *M* and *R/M* lanes, but not present in *R* lanes. The large (~20 kb) molecular weight fragment present in the *M* lanes and absent from the *R/M* lanes in the HindIII and PstI digests results from partial digestion of the C57BL/6J mouse genomic DNA. *C*, Secondary control Southern hybridization of a rat IL-3 cDNA probe to the same panel of digests displayed in *B*. Arrowheads indicate restriction fragments shared between *M* and *R/M* lanes, but not present in *R* lanes.

A

B

C

Table 1. Summary of hybridization data for mouse-hamster hybrid cell lines

Hybrid cell line	Mouse chromosome composition																			Mouse P_0 hybridization	
mFE11	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X	+
mFE2/1/1	1	2	3	4		6	7	8	9	10		12	13		15		17	18	19	X	+
mFE2/1/7	1	2	3			6	7	8	9			12	13		15		17		19	X	+
mFE2/1/2		2	3	4		6	7	8	9	10		12	13	14	15		17	18	19	X	–
MACH2aC2	1	2	3	4			7	8	9	10		12	13		15	16	17	18	19	X	+
C17B	1	2	3	4			7		9			12			15		17		19	X	+
C11	1	2	3	4	5	6	7	8 ^a	9	10 ^a	11 ^a	12	13		15	16	17	18 ^b	19	X	+
TuCE12G/1					5	6 ^c		8 ^c				12	13	14		16	17		19	X	–
TuCE12G/7		2			5	6 ^c		8 ^c		10		12	13	14	15	16	17	18	19	X	–
4B31AZ3		2					7					12	13 ^c		15	16 ^b			19		–
mc8	1 ^b	2	3 ^b	4			7		9			12	13	14 ^b					19	X	+
mAE19	1																			X	+
CEC						6 ^c														X	–
ma10b												12								X	–
R44																	17	18			–

^a Present in 10–15% of hybrid cells.^b Present in <10% of hybrid cells.^c Rearranged chromosome.

lower-molecular-weight restriction fragments for the mouse P_0 gene (Lemke et al., 1988), but only a single very-high-molecular-weight fragment for the hamster gene.

The segregation of mouse P_0 hybridization with mouse chromosome composition is indicated in Table 1, and a typical Southern blot from which these data were derived is illustrated in Figure 1. The segregation pattern indicated in Table 1 unequivocally maps the P_0 gene to mouse chromosome 1. For example, DNA from the mAE19 hybrid line, which contains only mouse chromosomes 1 and X, yields a mouse P_0 hybridization signal. In contrast, DNA from the ma10b hybrid line, which contains just mouse chromosomes X and 12, yields only the background hamster P_0 hybridization signal. There is no discordance in the data presented in Table 1: the only lines that yield a mouse P_0 hybridization signal are those that contain mouse chromosome 1.

As noted above and illustrated in Figure 1, hybridization to the mouse P_0 gene is easily distinguished from hybridization to the hamster gene. Given the exon-intron configuration of the cloned mouse gene (Lemke et al., 1988), BamHI bands of approximately 1.3 and 1.0 kb are predicted to yield the strongest hybridization signal when BamHI digests of mouse genomic DNA are analyzed with a full-length, nick-translated P_0 cDNA probe. (These bands contain the longest stretches of exon DNA colinear with the cDNA.) As illustrated in Figure 1, this is the result we obtained. The position of additional higher-molecular-weight bands in some of the digests (e.g., CMS4TG) is, based on the BamHI restriction map of the cloned mouse P_0 gene (Lemke et al., 1988), consistent with partial fragmentation generated by incomplete digestion. In all of the BamHI digests performed, the background hamster gene is present as a large band of approximately 15 kb. In general, the intensity of hybridization to the mouse P_0 gene is well correlated with the relative level at which mouse chromosome 1 is represented in a given hybrid population. Thus the mc8 line, in which mouse chromosome 1 is represented in less than 10% of the cells, yields a hybridization signal significantly lower than that for the

MACH2aC2 line, in which this chromosome is present in approximately 70% of the cells.

These data unambiguously identify chromosome 1 as the carrier of the mouse P_0 gene. However, they do not exclude the formal possibility that an additional, identically configured P_0 gene is also present on mouse chromosome 11. Since this chromosome is among the first to be lost in mouse-hamster hybrids, lines containing mouse chromosome 11, but not mouse chromosome 1, cannot be readily generated. In order to exclude the formal possibility of a second P_0 gene on mouse chromosome 11, we analyzed the segregation of mouse P_0 hybridization in a mouse-rat hybrid cell line containing only mouse chromosome 11 on a fixed, complete rat background (Killary and Fournier, 1984). The results of this analysis are shown in Figure 2. The hybridization pattern observed with restriction digests of DNA prepared from the F(11)J rat-mouse hybrid line, which contains mouse chromosome 11, is identical to that of DNA prepared from a second line, FB(11)J. This line, a derivative of F(11)J in which loss of mouse chromosome 11 has been achieved through back-selection with bromodeoxyuridine, contains only rat chromosomes. The F(11)J/FB(11)J pattern is easily distinguished from the P_0 profile observed with digests of genomic DNA prepared from C57BL/6J mice (Fig. 2A), clearly indicating that mouse P_0 sequences are not present in the F(11)J DNA.

The presence of mouse chromosome 11 in the F(11)J line was verified in 2 ways. First, the F(11)J cells used to isolate the DNA analyzed in Figure 2 were grown in HAT medium to select for the presence of the thymidine kinase (TK) gene. This gene is located on mouse chromosome 11 and is uniquely provided by this chromosome in the F(11)J mouse-rat hybrid, since the parental rat line used to generate the hybrid is TK[–] (Killary and Fournier, 1984). (TK[–] cells are killed in HAT medium.) Second, the blot shown in Figure 2A was stripped and secondarily hybridized with cDNA probes for the c-erbA and interleukin-3 genes, which have been mapped to distal and proximal regions of mouse chromosome 11, respectively, and which flank the Trembler locus (Zabel et al., 1984; Barlow et al., 1987; Thomp-

son et al., 1987). These secondary hybridizations also indicated that mouse chromosome 11 DNA was in fact present in the F(11)J DNA analyzed. Figure 2, *B* and *C*, illustrates the hybridization patterns observed for c-erbA and IL-3, respectively.

These results demonstrate that the *P₀* gene resides on mouse chromosome 1, and that *P₀* sequences are absent from mouse chromosome 11. This finding in turn indicates that the PNS-specific *Trembler* mutation does not result from an alteration in the structure of the *P₀* gene, which encodes the major PNS-specific myelin protein. *Trembler* should therefore be distinguished from myelination mutations such as *jimpy* and *shiverer*, which result from alterations in the structure of the genes encoding the major myelin proteins proteolipid protein and myelin basic protein, respectively (Roach et al., 1985; Nave et al., 1987). Indeed, the multifaceted phenotype exhibited by *Trembler* mice is more consistent with mutation of a regulatory, as opposed to structural, gene. An example of this sort of mutation is provided by *sevenless* (Banerjee et al., 1987), a mutation in *Drosophila*. In flies, the differentiation of 1 set of developing photoreceptor cells is controlled by their interaction with a second set of developing cells in a manner similar to that seen for the axonal control of Schwann cell differentiation and myelination. *Sevenless* perturbs this interaction much as *Trembler* perturbs the interaction of axons and Schwann cells. Given that *Trembler* has been precisely mapped and has been shown to be expressed in a Schwann cell-autonomous fashion, we hope to characterize the molecular basis of this important mutation by identifying novel Schwann cell-specific mRNAs transcribed from genes located on mouse chromosome 11.

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