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An AG→GG transition at a splice site in the myelin proteolipid protein gene in jimpy mice results in the removal of an exon

Wendy B. Macklin*, Minnetta V. Gardinier*+, Kit D. King* and Kathy Kampf*

*Mental Retardation Research Center, Department of Psychiatry, UCLA Medical Center, 760 Westwood Plaza, Los Angeles, CA 90024 and +Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, 1901 Perdido St, New Orleans, LA 70112, USA

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The myelin proteolipid protein gene was characterized in jimpy mice to identify the specific mutation that produces dysmyelination, oligodendrocyte cell death, and death of the animal by 30 days of age. Exon 5 and flanking intron segments were isolated from jimpy proteolipid protein genomic clones and sequenced. A single nucleotide difference was noted between the normal and jimpy proteolipid protein genes: the conversion of an AG/GT to a GG/GT in the splice acceptor signal preceding exon 5, which apparently destroys the splice signal. Thus, exon 5 of the mouse myelin proteolipid protein gene is skipped during the processing of mRNA, producing a shortened proteolipid protein mRNA.

Myelin; Proteolipid protein; Splicing; Dysmyelination; (Jimpy mice)

1. INTRODUCTION

Myelin protein gene expression has recently become one of the most intensively studied developmental systems in neurobiology [1,2]. This results in part from the overall abundance of the myelin membrane constituents within the nervous system, as well as their relatively simple composition, e.g. two genes, the myelin basic protein gene and the myelin proteolipid protein (PLP) gene produce 80% of the CNS myelin membrane protein [3,4]. In addition, the expression of the myelin genes is tightly developmentally regulated, with the major peak of expression in mice occurring between 10 and 25 days after birth. Several dysmyelinating mouse mutants have been characterized, and the genetic defect in one of these, the shiverer mutant, has been identified as a deletion of exons 2–7 of the myelin basic protein gene [5].

Correspondence address: W.B. Macklin, Mental Retardation Research Center, Dept of Psychiatry, UCLA Medical Center, 760 Westwood Plaza, Los Angeles, CA 90024, USA

The jimpy (*jp*) mouse carries a recessive X-linked dysmyelination mutation, which produces tremors beginning approx. 11 days after birth, followed by seizures and death by day 30 [6]. The myelin PLP gene is the putative site of this mutation. This conclusion was reached because (i) PLP is found exclusively in CNS myelin, and the dysmyelination in *jp* mice is restricted to CNS myelin; (ii) the PLP gene maps to the same region of the X chromosome as the *jp* locus [7,8]; and (iii) PLP mRNA in *jp* mice is smaller than PLP mRNAs from normal mice [9–12]. Nave et al. [11] definitively demonstrated, by sequencing a *jp* PLP cDNA, that the difference in size resulted from an internal 74 base deletion. They proposed that the specific alteration of the PLP mRNA results from a splicing defect in the processing of the PLP mRNA. This proposal was further supported by

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subsequent information on the human and mouse proteolipid protein genes, which demonstrated that the excised segment of the *jp* PLP mRNA corresponds exactly to exon 5 of the PLP gene [13,14]. The current studies were undertaken to identify the specific mutation within the PLP gene in *jp* mice that causes this splicing defect.

2. MATERIALS AND METHODS

2.1. *Preparation and screening of* jp *genomic library*

A *jp* genomic DNA library was obtained from A.T. Campagnoni, UCLA. It was prepared from a partial *Mbo*I digest of genomic *jp* DNA, by ligation to *Bam*HI-cut λ J1 [15]. Clones containing the *jp* PLP gene were isolated by screening the library with a PLP-specific cDNA, BAS1013 [16], which contains sequences encoding the 3′-end of exon 2 to the 5′-end of exon 7, encompassing most of the coding region of the PLP mRNA.

2.2. *Southern blot analyses*

The *jp* PLP genomic clones were further characterized by restriction mapping. The following probes were used to map these clones: BAS1013; pJD13A (a rat PLP cDNA containing the 3′-end of exon 4 to the 5′-end of exon 7) [9]; and p27 (a full-length rat cDNA) [17]. Probes were labeled with [^{32}P]dATP or -dCTP by nick translation [18] or random priming [19]. Clones were digested with restriction endonucleases and separated on 0.8% agarose gels. Southern blots were prepared, hybridized with probe overnight, washed and autoradiographed as in [9].

2.3. *Subcloning of a* jp *genomic clone*

Two DNA fragments containing exon 5 were prepared isolated by digesting a *jp* PLP genomic clone with *Pvu*II and *Bam*HI (a 1.1 kb fragment containing the 5′-end of exon 5), or with *Pvu*II and *Bgl*II (a 1.4 kb fragment containing the 3′-end of exon 5). These fragments were isolated in 1% agarose gels, electroeluted, and subcloned into M13mp18, which had been digested with *Hin*cII and *Bam*HI. Insert-containing clones were identified, isolated, and sequenced by standard dideoxy techniques using 6 and 8% denaturing acrylamide gels [20].

3. RESULTS AND DISCUSSION

Approx. 7 different PLP-specific clones were identified and isolated from a *jp* genomic library. These clones were characterized by restriction analysis, and two clones appeared to encompass most of the *jp* PLP gene (fig.1). These clones had essentially identical restriction maps relative to the normal mouse PLP gene [14], except for truncated ends caused by the cloning.

Two restriction fragments containing the ends of exon 5 were isolated from clones KKjp7 and KKjp9, and subcloned into M13 for sequencing. These fragments were selected because a *jp* PLP cDNA sequence indicated that the site of the *jp* PLP gene mutation was likely to be in the splice acceptor segment of the mouse PLP exon 5 [11,14]. Thus, exon 5 was sequenced, and the sequence indicates that there is a single base alteration in the splice acceptor sequence of this exon (fig.2). The sequence of the normal intron sequence upstream from exon 5 is GCTGCTTTTATGTATCTTAG/GT [14], and the sequence of this region in the *jp* PLP gene is GCTGCTTTTATGTATCTTGG/GT. The underlined nucleotide appears to be the only nucleotide difference between these two PLP genes around exon 5. This alteration of an AG/GT to a GG/GT apparently destroys this splice signal and eliminates this exon from the mature PLP mRNA in *jp* mice. The consensus signal for a splice acceptor signal is $(Py)_nNCAG/G$, and the underlined AG are the only nucleotides that are invariant in all splice acceptor sequences identified to date [21]. Thus, a mutation producing an AG to GG conversion of the splice acceptor sequence would be expected to destroy the splice signal.

These data are consistent with a recent report by Nave et al. ([22]; personal communication), who compared their data on the *jp* mouse PLP exon 5 with the human PLP exon 5. It is possible in the current study to compare more of the intron sequence, because the *jp* PLP gene sequence is directly compared to the normal mouse PLP gene, rather than the human gene. With this comparison, it is possible to establish that there is no other sequence difference in 160 nucleotides upstream or downstream of the mouse PLP exon 5. Thus, this mutation appears to be a single nucleotide alteration in the mouse PLP gene.

PLP mRNA levels in 20 day *jp* mice are 5–10%

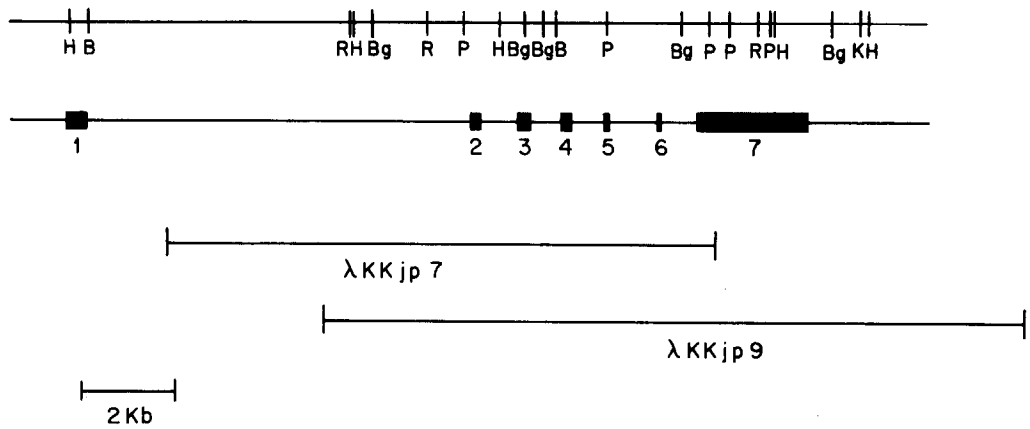

Fig.1. Restriction map of the myelin PLP gene. Two isolated jimpy PLP genomic clones are shown in relationship to the normal PLP gene. Restriction enzymes utilized: *Hin*dIII, H; *Bam*HI, B; *Eco*RI, R; *Bgl*II, Bg; *Pvu*II, P; *Kpn*I, K.

that of normal as measured by in vitro translation and by dot-blot analysis [9,23]. The fact that these levels are so low is important because at present, there is no clear correlation between these low levels of *jp* PLP mRNA and the specific mutation within the PLP gene in *jp* mice. The low levels presumably arise either from increased turnover of the PLP mRNA or from decreased transcription of the PLP gene in *jp* mice. Presently, it is not clear whether a 74-nucleotide deletion in the protein coding segment of a 3200-nucleotide mRNA would alter mRNA stability, but clearly this must be tested. The other possibility, i.e. that PLP gene transcription is reduced in *jp* mice, may in fact occur because of specific aspects of this splicing defect. This splicing defect deletes an exon containing 74 nucleotides, which produces a frameshift in the protein coding region. The *jp* PLP mRNA would be translated into a protein containing the first 206 amino acids of PLP and then 34 amino acids unrelated to PLP. By S_1 nuclease mapping, mRNAs for both PLP and DM20 (an alternatively spliced PLP gene product [14,24]) are found in *jp* mice [10,12], so presumably low levels of both PLP and DM20 proteins containing the altered C-terminus can be synthesized in these animals. The possibility exists that the production of defective PLP and DM20 protein could feedback and reduce the transcription of the gene.

Such a possibility is somewhat supported by studies on the early expression of PLP mRNA in *jp* mice. PLP mRNA levels in young *jp* animals appear to be near normal levels [9]. Jimpy mice at 3 and 7 days of age have 89 and 49% of the PLP mRNA of age-matched controls, respectively, and

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                                                    Gly Val Leu Pro Trp
catgcctcta gccttatgaa gtttactctg gctgctttta tgtatcttgg GT GTT CTC CCA TGG
                                                       a

Asn Ala Phe Pro Gly Lys Val Cys Gly Ser Asn Leu Leu Ser Ile Cys Lys Thr
AAT GCT TTC CCT GGC AAG GTT TGT GGC TCC AAC CTT CTG TCC ATC TGC AAA ACA

Ala Glu
GCT GAG gtaagtgaat gagaagagtg ctttttaaaa aatagattgg ctagacatgg
```

Fig.2. Sequence of exon 5 and flanking intron segments of the myelin PLP gene in *jp* mice. The single nucleotide difference between exon 5 of *jp* mice and normal mice is underlined, and the correct base for the normal PLP gene is identified below.

it drops as the animals age, to 12.5% of normal 10-day animals and 5–6% of normal 20–25-day animals. These data would suggest that PLP mRNA levels in very young *jp* mice are close to normal levels, but that at the age when normal animals begin to increase significantly their level of PLP mRNA (between 10 and 25 days), the *jp* mice maintain their same low levels of PLP mRNA expression. Thus, the early transcription rate of the PLP gene may be close to normal, and it is possible that when defective PLP and DM20 proteins are translated from these mRNAs in the young animals, their presence triggers a cellular response to prevent the normal increase in PLP gene transcription.

In summary, the current study identifies the specific nucleotide alteration within the PLP gene that produces the *jp* mRNA defect. This mutation causes a splicing defect that eliminates exon 5 from the PLP mRNA, and produces aberrant PLP and DM20 protein. This single base mutation causes oligodendrocyte cell death, astrocyte hypertrophy and essentially the total absence of CNS myelin. Neither *jp* mice nor jp^{msd} mice [25], which are allelic to *jp* and presumably also have an altered PLP gene, survive past 30 days of age. Thus, it appears that alterations in the expression of the myelin PLP gene are lethal at a very early age. In comparison, in other mouse dysmyelination mutants, alterations in the myelin basic protein gene and presumably other myelin protein genes grossly reduce the amount of myelin produced; these animals also have tremors and seizures throughout their lifetime. Nevertheless, these mutants have less severe clinical problems, as compared to the *jp* mutant. For example, shiverer mice, which carry a deletion of the myelin basic protein gene [5]; myelin-deficient mice, which carry a duplication of the myelin basic protein gene, and produce very low levels of myelin basic protein [26]; and quaking mice, which carry an undefined genetic defect, yet are quite deficient in myelin production, all have much longer lifespans, somewhat more myelin, and are generally healthier than *jp* mice. In quaking mice, the dysmyelination has no effect on lifespan, and adult females are even fertile. Why an inappropriately spliced PLP mRNA in *jp* mice should produce early astrocyte changes, oligodendrocyte cell death, and the very premature death of the animal is an important question that is currently under active investigation.

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REFERENCES

[1] Sutcliffe, J.G. (1987) Trends Genet. 3, 73–76.
[2] Campagnoni, A.T. and Macklin, W.B. (1987) Mol. Neurobiol., submitted.
[3] Eng, L.F., Chao, F.C., Gerstl, B., Pratt, D. and Tavaststjerna, M.G. (1968) Biochemistry 7, 4455–4465.
[4] Gonzalez-Sastre, F. (1970) J. Neurochem. 17, 1049–1056.
[5] Roach, A., Takahashi, N., Pravtcheva, D., Ruddle, F. and Hood, L. (1985) Cell 42, 149–155.
[6] Sidman, R.L., Dickie, M.M. and Appel, S.H. (1964) Science 144, 309–310.
[7] Willard, H.F. and Riordan, J.R. (1985) Science 230, 940–942.
[8] Mattei, M.G., Alliel, P.M., Dautigny, A., Passage, E., Pham-Dinh, D., Mattei, J.F. and Jolles, P. (1986) Hum. Genet. 72, 352–353.
[9] Gardinier, M.V., Macklin, W.B., Diniak, A.J. and Deininger, P.L. (1986) Mol. Cell. Biol. 6, 3755–3762.
[10] Morello, D., Dautigny, A., Pham-Dinh, D. and Jolles, P. (1986) EMBO J. 5, 3489–3493.
[11] Nave, K.-A., Lai, C., Bloom, F.E. and Milner, R.J. (1986) Proc. Natl. Acad. Sci. USA 83, 9264–9268.
[12] Hudson, L.D., Berndt, J.A., Puckett, C., Kozak, C.A. and Lazzarini, R.A. (1987) Proc. Natl. Acad. Sci. USA 84, 1454–1458.
[13] Diehl, H.-J., Schaich, M., Budzinski, R.-M. and Stoffel, W. (1986) Proc. Natl. Acad. Sci. USA 83, 9807–9811.
[14] Macklin, W.B., Campagnoni, C.W., Deininger, P.L. and Gardinier, M.V. (1987) J. Neurosci. Res., in press.
[15] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning, pp.269–308, Cold Spring Harbor Laboratory, NY.
[16] Sorg, B.J.A., Smith, M.M. and Campagnoni, A.T. (1987) J. Neurochem., in press.

[17] Milner, R.J., Lai, C., Nave, K.-A., Lenoir, D., Ogata, J. and Sutcliffe, J.G. (1985) Cell 42, 931–939.
[18] Rigby, P.W.J., Dieckmann, M., Rhodes, C. and Berg, P. (1977) J. Mol. Biol. 113, 237–251.
[19] Feinberg, A.P. and Vogelstein, B. (1983) Anal. Biochem. 132, 6–13.
[20] Sanger, F., Coulson, A., Barrell, B., Smith, A. and Roe, B. (1980) J. Biol. Chem. 143, 161–178.
[21] Padgett, R.A., Grabowski, P.J., Konarska, M.M., Seiler, S. and Sharp, P.A. (1986) Annu. Rev. Biochem. 55, 1119–1150.
[22] Nave, K.-A., Lai, C., Bloom, F.E. and Milner, R.J. (1987) J. Neurochem. 48, S130.
[23] Sorg, B.J.A., Agrawal, D., Agrawal, H.C. and Campagnoni, A.T. (1986) J. Neurochem. 46, 379–387.
[24] Nave, K.-A., Lai, C., Bloom, F.E. and Milner, R.J. (1987) Proc. Natl. Acad. Sci. USA 84, 5665–5669.
[25] Meier, C. and MacPike, A.D. (1970) Exp. Brain Res. 10, 512–525.
[26] Popko, B., Puckett, C., Lai, E., Shine, H.D., Readhead, C., Takahashi, N., Hunt, S.W. iii, Sidman, R.L. and Hood, L. (1987) Cell 48, 713–721.