Volume 232, number 2, 323–327 FEB 05873 May 1988

Developmentally regulated alternative splicing of brain myelin-associated glycoprotein mRNA is lacking in the quaking mouse

Nobuya Fujita, Shuzo Sato, Tadashi Kurihara*, Takashi Inuzuka, Yasuo Takahashi* and Tadashi Miyatake

Departments of Neurology and **Neuropharmacology, Brain Research Institute, Niigata University, Niigata 951, Japan*

Received 19 February 1988; revised version received 25 March 1988

Evidence is presented that expression of the two myelin-associated glycoprotein mRNAs is developmentally regulated in mouse brain. In quaking mouse, the mRNA without a 45-nucleotide exon portion was scarcely expressed throughout development. We conclude that the mechanism of splicing out the 45-nucleotide exon portion is lacking in quaking mouse.

Myelin; Myelin-associated glycoprotein; Alternative splicing; Dysmyelination; Developmental regulation; (Quaking mouse)

1. INTRODUCTION

The myelin-associated glycoprotein (MAG), a glycoprotein with a molecular mass of about 100 kDa, is a quantitatively minor component of central nervous system myelin, but is believed to play an important role in oligodendrocyte-axon interactions [1]. In vitro translation revealed two developmentally regulated polypeptides with molecular masses of 72 and 67 kDa, respectively [2], which are supposed to correspond to the two forms of mRNA clarified recently by cDNA cloning [3,4]. The two forms of rat MAG mRNA are produced by alternative splicing of a 45-nucleotide (nt) exon portion that contains a termination codon [3,4].

Quaking mouse is a mutant with impaired myelination [5], the mutation being located on chromosome 17 [6]. Early fucose-labeling experiments suggested anomalies of MAG in quaking mouse [7]. It was particularly noted that MAG in quaking mouse has a higher molecular mass than that in the normal control. In vitro translation with mRNA from quaking mouse, however, produced mainly a polypeptide at the same position in SDS-polyacrylamide gel electrophoresis as the smaller polypeptide that was normally found in older animals [8].

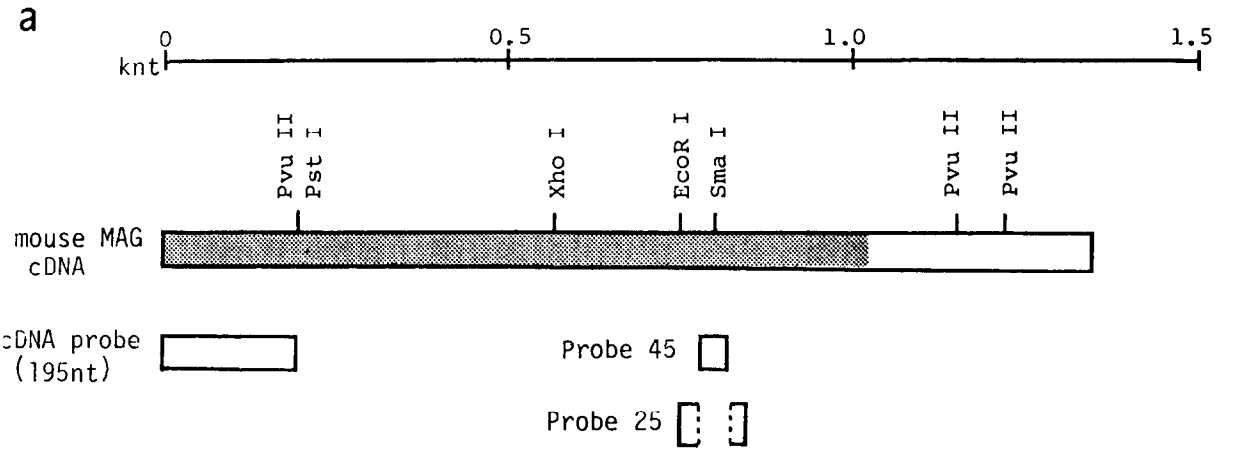
A cDNA of mouse MAG was isolated here and sequenced, and two synthetic probes were prepared to distinguish the two forms of mouse MAG mRNA. Evidence is presented that expression of the two forms of MAG mRNA is developmentally regulated in mouse brain. One form of mRNA had a peak in expression at the time of most active myelination and the other form had a peak at a later stage of development. In quaking mouse, only the latter form of mRNA was expressed throughout development; developmentally regulated alternative splicing of MAG mRNA is thus lacking in quaking mouse.

2. MATERIALS AND METHODS

Quaking mouse was obtained from Jackson Laboratory (Bar Harbor, ME, USA).

Correspondence address: N. Fujita, Department of Neurology, Brain Research Institute, Niigata University, Niigata 951, Japan

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50



b

```
5'
GCC TAT GGC CAG GAC AAC CGC ACG GTG GAG CTG AGT GTC ATG TAT GCA CCT TGG AAG CCC ACA GTG AAT
Ala Tyr Gly Gln Asp Asn Arg Thr Val Glu Leu Ser Val Met Tyr Ala Pro Trp Lys Pro Thr Val Asn

GGG ACG GTG GTG GCC GTA GAG GGG GAG ACT GTC TCT ATC CTG TGT TCC ACA CAG AGC AAC CCG GAC CCC
Gly Thr Val Val Ala Val Glu Gly Glu Thr Val Ser Ile Leu Cys Ser Thr Gln Ser Asn Pro Asp Pro

ATC CTT ACC ATC TTC AAG GAG AAG CAG ATC CTA GCC ACG GTC ATC TAT GAG AGT CAG
Ile Leu Thr Ile Phe Lys Glu Lys Gln Ile Leu Ala Thr Val Ile Tyr Glu Ser Gln
```

c

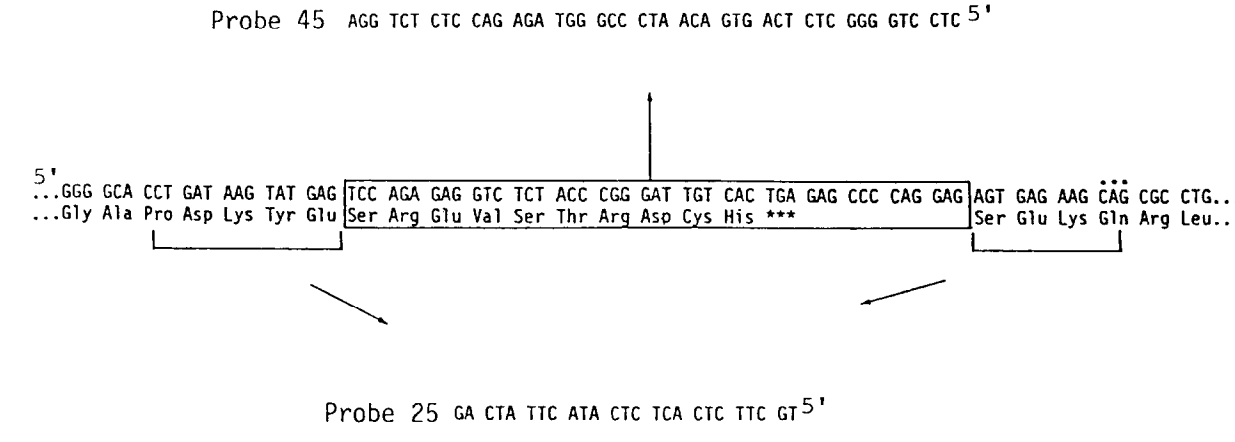


Fig.1. Mouse MAG cDNA and probes. (a) Restriction map of mouse MAG cDNA. The region sequenced is shadowed. The positions of the probe sequences are indicated under the restriction map. (b) Sequence of the 195-nt cDNA. The sequence is completely identical with that of rat MAG cDNA except for the 7 nucleotides indicated by dots. (c) Sequence of the 45-nt exon and adjacent portions. The sequence is completely identical with that of rat MAG cDNA except for the 3 nucleotides indicated by dots. Probe 45 is complementary to the sequence of the 45-nt exon portion; probe 25 is complementary to the sequences of both sides adjacent to the 45-nt exon portion.

A cDNA library (λgt11) for mouse brain was obtained from Clontech Laboratories (Palo Alto, CA, USA) and screened with anti-mouse MAG serum as described [9]. Anti-mouse MAG serum was prepared and characterized as described previously for anti-rat MAG serum [10]. The cDNA was subcloned into pSPT18. DNA sequence was determined by the procedure of Maxam and Gilbert [11].

Synthetic DNA probes (probe 45 and probe 25) were prepared by solid-phase synthesis on an Applied Biosystems DNA synthesizer. The purified probes were labeled at the 5′-end with [γ-^{32}P]ATP by T_4 polynucleotide kinase [12]. The 195-nt cDNA probe (fig.1b) was labeled with [α-^{32}P]dCTP by a random-primed DNA labeling kit (Boehringer Mannheim, Mannheim, FRG).

Total RNA was prepared by the guanidinium thiocyanate/cesium chloride procedure [13]; poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose chromatography [14]. For Northern blot analysis, poly(A)$^+$ RNA was subjected to electrophoresis on a 1.5% agarose gel containing 2.2 M formaldehyde, and transferred by capillary blotting to nitrocellulose filters. A Bio-Dot SF blotting apparatus (Bio-Rad Laboratories, Richmond, CA, USA) was used for slot-blot analysis of total RNA. RNA was denatured in 2.2 M formaldehyde at 60°C for 10 min before electrophoresis or slot-blotting.

Filters were hybridized with the cDNA probe or with probe 45 under the conditions described [15]. Filters were hybridized with probe 25 for 20 h at 60°C in 0.9 M NaCl, 0.09 M Tris-HCl (pH 7.5), 6 mM EDTA, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin and 0.1% SDS. They were washed twice for 20 min each at 65°C in 0.9 M NaCl, 0.09 M sodium citrate (pH 7.0) and 0.1% SDS, and exposed to an X-ray film with an intensifying screen at −70°C. For reprobing, the filters were washed twice for 1 h each in 0.05% sodium pyrophosphate, 5 mM Tris-HCl (pH 7.5) and 0.2 mM EDTA at 70°C.

3. RESULTS AND DISCUSSION

One positive clone containing approx. 1350-nt cDNA (fig.1a) was isolated from a mouse brain cDNA library by immunoscreening. The sequenced 1020 nt of the cDNA had 96% identity with the corresponding region of the previously reported rat MAG cDNA. Two forms of rat MAG mRNA are produced by alternative splicing of a 45-nt exon portion [3,4]. The 45-nt exon portion of the mouse MAG cDNA was identical with that of the rat MAG cDNA. To distinguish the two forms of mouse MAG mRNA, we prepared two synthetic probes (fig.1c). Probe 45 is complementary to the sequence of the 45-nt exon portion and specific for the mRNA which contains the 45-nt exon portion.

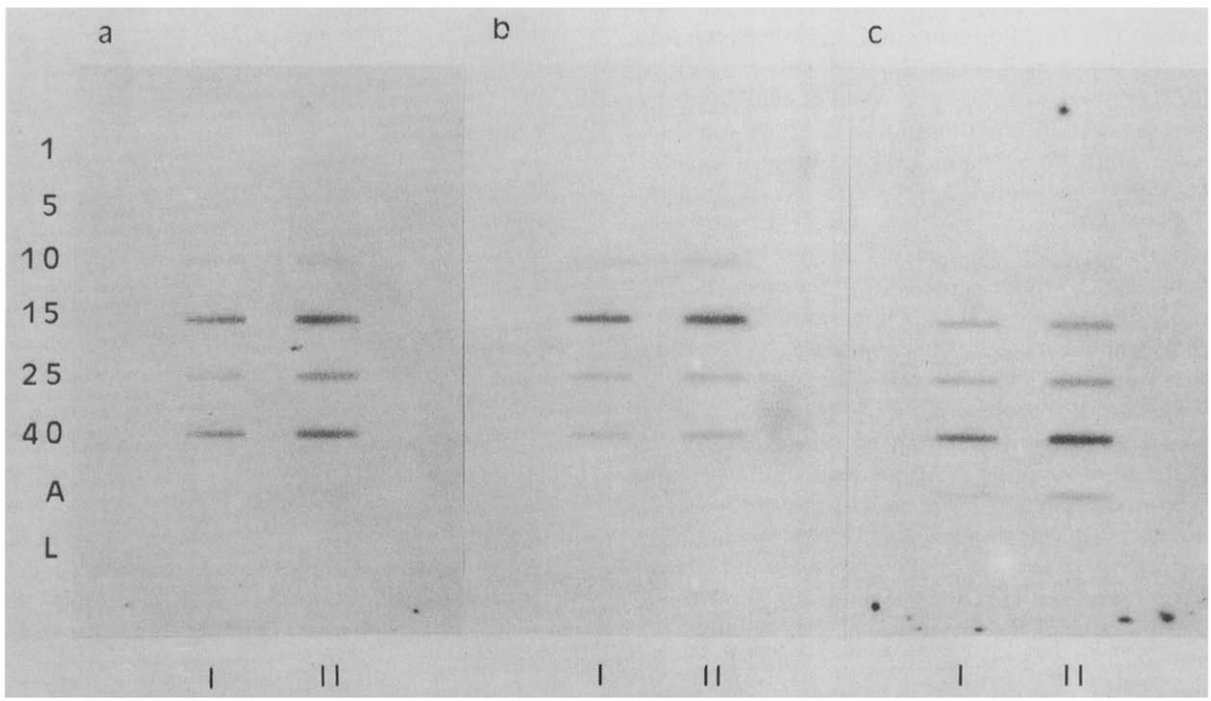


Fig.2. Slot-blot analysis of MAG mRNA in the normal mouse. Total RNA (15 μg each in I and 40 μg each in II) from 1, 5, 10, 15, 25, 40-day-old and adult (A) ddY mouse brain was blotted; total RNA from adult ddY mouse liver (L) was also blotted. The filter was probed first with probe 25 (b), second with probe 45 (c), and finally with the 195-nt cDNA probe (a).

Probe 25 is complementary to the sequences of both sides adjacent to the 45-nt exon portion and specific for the mRNA which does not contain the 45-nt exon portion. The 195-nt cDNA probe (fig.1b) was used to estimate both forms of MAG mRNA.

The MAG cDNA hybridized at approx. 2500 nt in Northern blots of both poly(A)$^+$ RNA and total RNA. The developmental changes of MAG mRNAs in normal mouse brain were examined by slot-blot hybridization of total RNA using the two synthetic probes and the cDNA probe (fig.2). Probe 25 had a peak in mRNA expression at 15 days postpartum (fig.2b), probe 45 at 40 days (fig.2c), and the cDNA probe had two peaks at 15 and 40 days (fig.2a). These results indicate that expression of the two MAG mRNAs in normal mouse brain is developmentally regulated: one form of mRNA without the 45-nt exon portion is expressed mainly at the time of most active myelination, while the other form containing the 45-nt exon portion is expressed mainly in later stages.

The developmental changes of MAG mRNAs in quaking mouse were examined by Northern blot analysis (fig.3). In quaking mouse, little hybridization occurred with probe 25 throughout development (fig.3b). Probe 45 and the cDNA probe hybridized more intensely in quaking mouse than in the control (fig.3a,c). Slot-blot analysis of total RNA gave the same results. The results indicate that the mRNA without the 45-nt exon portion is scarcely expressed in quaking mouse. The other form of mRNA containing the 45-nt exon portion was over-expressed in quaking mouse. We conclude that the mechanism of splicing out the 45-nt exon portion is lacking in quaking mouse.

This study together with the previous in vitro translation analysis [8] suggest that the MAG in the brain of quaking mouse consists mainly of the smaller polypeptide produced by the mRNA containing the 45-nt exon portion. The MAG in quaking mouse is known to have a higher molecular mass than that in normal mouse [7]. This is probably due to the difference in the carbohydrate portion of the MAG molecule. It should be noted that the amount of MAG is greatly reduced in the brain of quaking mouse [16] though the mRNA for the smaller polypeptide is over-expressed.

The fact that the MAG mRNA without the 45-nt exon portion is expressed mainly at the time of most active myelination suggests that the mechanism of splicing out the 45-nt exon in the early stage of development is important for myelination. We suppose that deficiency of this mechanism is involved in the pathogenesis of quaking mouse. D'Eustachio et al. [17] have recently shown that the MAG coding sequence

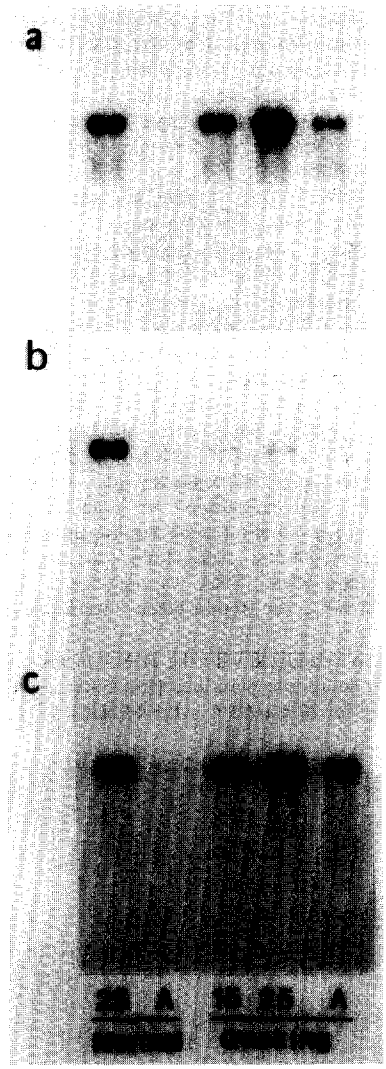


Fig.3. Northern blot analysis of MAG mRNA in quaking mouse. Poly(A)$^+$ RNA (6 μg each) from 15, 25-day-old and adult (A) quaking mouse brain was electrophoresed. Poly(A)$^+$ RNA (6 μg each) from 25-day-old and adult (A) ddY mouse brain was also electrophoresed. The filter was probed first with probe 25 (b), second with probe 45 (c), and finally with the 195-nt cDNA probe (a).

resides on chromosome 7; thus the genetic locus of MAG is different from that of the quaking mutation. It is unknown how the quaking mutation causes the abnormal MAG mRNA expression described here.

Acknowledgements: We thank Dr S. Tsuji for his suggestion on the use of probe 25. We thank Drs K. Sakimura, R. Kuwano and H. Usui for their help and suggestions. This work was supported in part by grants-in-aid from the Ministry of Education, Science and Culture of Japan and the Ministry of Health and Welfare of Japan.

REFERENCES

[1] Quarles, R.H. (1984) Dev. Neurosci. 6, 285–303.
[2] Frail, D. and Braun, P. (1984) J. Biol. Chem. 259, 14857–14862.
[3] Salzer, J.L., Holmes, W.P. and Colman, D.R. (1987) J. Cell Biol. 104, 957–965.
[4] Lai, C., Brow, A.B., Nave, K.A., Noronha, A.B., Quarles, R.H., Bloom, F.E., Milner, R.J. and Sutcliffe, J.G. (1987) Proc. Natl. Acad. Sci. USA 84, 4337–4341.
[5] Sidman, R.L., Dickie, M.M. and Appel, S.H. (1964) Science 144, 309–311.
[6] Davisson, M.T. and Roderick, T.H. (1986) in: Manipulating the Mouse Embryo (Hogan, B. et al. eds) pp.285–298, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[7] Matthieu, J.-M., Brady, R.O. and Quarles, R.H. (1974) J. Neurochem. 22, 291–296.
[8] Frail, D. and Braun, P. (1985) J. Neurochem. 45, 1071–1075.
[9] Huynh, T.V., Young, R.A. and Davis, R.W. (1985) in: DNA Cloning (Glover, D.M. ed.) vol.1, pp.49–78, IRL Press, Oxford.
[10] Quarles, R.H., Johnson, D., Brady, R.O. and Sternberger, N. (1981) Neurochem. Res. 6, 1115–1127.
[11] Maxam, A.M. and Gilbert, W. (1980) Methods Enzymol. 65, 499–560.
[12] Chaconas, G. and Van de Sande, J.H. (1980) Methods Enzymol. 65, 75–85.
[13] Chirgwin, J.M., Przybyla, A.E., McDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294–5299.
[14] Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. USA 69, 1408–1412.
[15] Thomas, P.S. (1980) Proc. Natl. Acad. Sci. USA 77, 5201–5205.
[16] Inuzuka, T., Johnson, D. and Quarles, R.H. (1987) J. Neurochem. 49, 597–602.
[17] D'Eustachio, P., Colman, D.R. and Salzer, J.L. (1988) J. Neurochem. 50, 589–593.