Molecular basis for heterosis for myelin basic protein content in mice

(RNA blot/in vitro translation)

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ABSTRACT Poly(A)+ mRNA was isolated from the brains of C57BL/6J (B6), DBA/2J (D2), and F1 hybrid mice (B6 × D2) of 16–17 days of age. The yield of polysomal RNA, both poly(A)+ and poly(A)−, from the three strains of mice was comparable. When translated in vitro in a reticulocyte lysate system, the mRNA preparations had the same efficiency with respect to stimulation of amino acid incorporation into protein. However, a significant heterotic effect was seen for the production of myelin basic protein (MBP) by the mRNA from the F1 mice. That is, the fraction of protein synthesized as MBP was greater for the F1 hybrid than for either parental strain. The distribution of the form of MBPs was not different among the three strains. We therefore believe that heterosis for brain MBP content in the F1 hybrid may be regulated at the transcriptional or post-transcriptional level.

Heterosis, or hybrid vigor, is an intriguing problem whose genetic basis has been under investigation for many years. Many types of gene and allele interactions can contribute to this phenomenon (1). One approach for studying a heterotic effect is to look for changes in the metabolism of the RNA involved in synthesis of the protein(s) showing the heterotic effect.

We have already reported biochemical evidence of heterosis for brain myelin content in mice (2, 3). Briefly, we showed that the myelin present in the F1 hybrid mice contained elevated levels of several constituents including cerebroside, GM1 ganglioside, 2',3'-cyclic nucleotide, 3'-phosphohydrolase, 5' nucleotidase, and carboxy anhydase. Since the composition of myelin in F1 mice does not change we have used myelin basic protein (MBP) as a marker for myelin. In the present study, we have investigated heterosis for brain MBP content in F1 hybrid mice on a molecular level by using in vitro translation and RNA blotting. With these techniques, we hoped to distinguish between transcriptional and post-transcriptional control and translation or post-translational control. Our data indicate that the brains of F1 hybrid mice contain higher concentrations of mRNA for MBP than either parental strain between 16 and 17 days of age. The results support the view that the heterotic effect is regulated at the transcriptional or post-transcriptional level. We believe these results may imply how the heterosis for myelin itself is regulated. A preliminary report of these data has been presented (4).

MATERIALS AND METHODS

Mice. The C57BL/6J (B6) and DBA/2J (D2) strains of mice were obtained from The Jackson Laboratory. They were maintained in our laboratory by sibling matings. The F1 hybrids (B6 × D2) were also propagated in our laboratory.

Isolation of Polysomal RNA. Polysomal RNA from whole brains of 16- to 17-day-old mice was isolated by the method of Palmiter (5).

Selection of Poly(A)+ RNA. The ethanol-precipitated RNA was pelleted at −10°C by centrifuging at 10,000 rpm for 30 min in a Sorvall SS34 rotor. Poly(A)+ RNA was separated on oligo(dT)-cellulose as described (6). RNA concentration was estimated as 1.0 A260 unit = 40 μg of RNA per ml.

In Vitro Translation. Poly(A)+ RNA was translated in vitro by using the rabbit reticulocyte system (New England Nuclear) with [3H]leucine as the label. Reactions were set up as suggested by the manufacturer. Unless otherwise stated, they contained 0.5 μg of RNA per 25 μl of reaction mixture and were incubated for 1 hr at 37°C. For determination of incorporation of [3H]leucine into protein, the reactions were stopped by the addition of 1 ml of cold 12% trichloroacetic acid. The precipitate was pelleted, solubilized in 1.0 M NaOH, and reproteinated with cold 12% trichloroacetic acid. The precipitate was collected and solubilized again in 1.0 M NaOH. Aliquots of 8 μl were taken, neutralized with HCI, and counted in a Searle scintillation counter.

Immunoprecipitation of MBP. This was performed as described by Rhoads et al. (7) and modified by Taylor and Schimke (8). Anti-MBP IgG was prepared in this laboratory. Immunoprecipitated products were separated on 12% NaDodSO4/urea polyacrylamide gels (3). Gels were sliced and the slices were dissolved in H2O2 at 65°C.

RNA Blotting. The polysomal poly(A)+ RNA was separated on 1% formaldehyde agarose gels and transferred to nitrocellulose filters (BA85, Schleicher & Schuell) as described (6). The filters were prehybridized in 50% formaldehyde/6× SSC (1× SSC = 0.15 M NaCl/0.015 M Na citrate)/0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin at 42°C for 4–24 hr. Hybridization was in the same solution containing 32P-labeled cDNA probe (pMBP-1, a kind gift of A. Roach; labeled by nick-translation) for 2 days at 42°C. The filters were washed four times in 2× SSC/0.1% NaDodSO4 at room temperature and twice in 0.1× SSC/0.1% NaDodSO4 at 42°C. They were exposed to Kodak XAR-5 film at −70°C. Autoradiographic bands were quantitated by densitometry with a Shimadzu CS-910 densitometer.

RESULTS

RNA Isolation. Polysomal RNA was isolated from the brains of 16- to 17-day-old mice of three strains: C57BL/6J (B6), DBA/2J (D2), and the F1 hybrid (B6 × D2). Table 1 shows the recoverable yield of this RNA and the poly(A)+ RNA fractionated from it. It can be seen that, on the basis of μg per g wet weight of brain, there is no significant difference among the three strains.

Abbreviation: MBP, myelin basic protein.

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Table 1. Polysomal RNA content of 16-day-old mouse brains

<table>
<thead>
<tr>
<th>Strain</th>
<th>RNA after phenol extraction, µg per g wet wt</th>
<th>Poly(A)+ RNA, µg per g wet wt</th>
<th>% poly(A)+</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6</td>
<td>1078.68 ± 64.0 (n = 11)</td>
<td>27.95 ± 2.34 (n = 11)</td>
<td>2.61 ± 0.2</td>
</tr>
<tr>
<td>D2</td>
<td>1127.70 ± 89.8 (n = 6)</td>
<td>21.81 ± 2.27 (n = 6)</td>
<td>1.97 ± 0.18</td>
</tr>
<tr>
<td>F1</td>
<td>971.95 ± 57.0 (n = 6)</td>
<td>28.62 ± 1.76 (n = 6)</td>
<td>2.88 ± 0.24</td>
</tr>
</tbody>
</table>

Polysomal RNA was prepared from the brains of B6, D2, and F1 mice. Poly(A)+ RNA was separated out on oligo(dT)-cellulose. Concentration is estimated as 1.0 A260 unit = 40 µg of RNA per ml. Amounts shown represent mean ± SEM.

In Vitro Translation of Poly(A)+ RNA. To test whether the heterotic effect for MBP content seen in the F1 hybrids was occurring at the level of transcription or translation, poly(A)+ RNA from the two parental strains and the F1 hybrid were translated in vitro in a rabbit reticulocyte lysate system. Fig. 1 shows a time course of incorporation of [3H]leucine into protein. The saturating concentration of poly(A)+ RNA under these conditions was 0.5 µg of poly(A)+ RNA per 25 µl of reaction mixture (Fig. 2). Based on the data in Figs. 1 and 2, standard assay conditions were picked at 60 min and at 0.5 µg of mRNA.

Under these conditions, the amount of total protein synthesis directed by the RNA from these strains was examined. Table 2 shows that the amount of [3H]leucine incorporated into protein was nearly the same in all three strains.

The above results indicate that there are no quantitative differences in protein translation between the parental strains and the F1 hybrid that might account for the hypermyelination seen in the F1. That is, the amount of translatable poly(A)+ RNA does not differ among the three strains.

Immunoprecipitation of MBP. Incorporation of [3H]leucine into MBP was measured by immunoprecipitation of the protein produced in vitro with anti-MBP IgG. Table 2 shows that the MBP production was significantly higher in the F1 hybrid mice. MBP synthesized in the in vitro translation reaction mixture was subjected to further analysis by Na-DodSO4/polyacrylamide gel electrophoresis after immunoprecipitation to determine whether any differences occurred in the class of MBPs being produced by the three strains (Fig. 3). An equal number of radioactive counts was added to each lane. The specific radioactivity of each of the four MBPs was quite similar in all three strains. In addition, the S/L ratios (S, small basic protein; L, large basic protein) did not vary greatly.

RNA Blotting. To determine whether the increased amount of myelin in the F1 mice in vivo could be correlated with a greater number of mRNAs for MBP present in the polysomes, poly(A)+ RNA isolated from the polysomes was blotted to a cDNA probe for MBP. Fig. 4 shows an autoradiograph of a blot of the polysomal poly(A)+ RNA from the two parental strains and the F1 mice. A single band of ~2000 bases is seen in all three strains. There appears to be relatively more MBP mRNA present in the polysomes of F1 mice. Quantitation of these bands by densitometry gives the results shown in Table 3. This result indicates that the increased myelin content of F1 mice, as reflected in MBP content, is being regulated at the transcriptional or post-transcriptional level. That D2 has a greater amount of mRNA for MBP than B6 supports the contention that D2 mice have a more heavily myelinated central nervous system than do B6 mice (2, 3).

Table 2. In vitro translation of polysomal poly(A) RNA

<table>
<thead>
<tr>
<th>Strain</th>
<th>Total incorporation, cpm × 10^3</th>
<th>% MBP of total incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6</td>
<td>1.60 ± 0.21 (n = 9)</td>
<td>1.6 ± 0.1 (n = 3)</td>
</tr>
<tr>
<td>D2</td>
<td>2.77 ± 0.71 (n = 4)</td>
<td>1.7 ± 0.1 (n = 4)</td>
</tr>
<tr>
<td>F1</td>
<td>1.88 ± 0.31 (n = 9)</td>
<td>4.33 ± 0.9* (n = 3)</td>
</tr>
</tbody>
</table>

Polyosomal poly(A)+ RNA (0.5 µg per 25-µl reaction mixture) was translated in vitro using [3H]leucine (17.8 μCi per 25-µl reaction mixture; 1 Ci = 37 GBq). Incorporation is trichloroacetic acid-precipitable counts ± SEM.

* P < 0.05, with respect to both B6 and D2.
The aim of this investigation was to determine the molecular level at which regulation of heterosis for brain MBP content in mice occurs. In the present study, we chose to use in vitro translation to help us determine whether the heterotic effect on myelination in F1 hybrid mice was controlled on a transcriptional or a translational level. The heterosis for myelin content does not appear to influence total RNA content (3) or the amount of polysomal or poly(A)+ RNA (Table 2). This is not unusual, as other investigators have reported that the amount of poly(A)+ RNA in quaking, jimpys, and myelin synthesis-deficient mutant mice did not differ from normal mice (10). It is important to note that our system for studying myelination in mice does not depend on mutant mice, but rather on the overproduction of myelin in normal mice during a critical period of active myelination (14–40 days; see ref. 3).

Using the in vitro reticulocyte lysate translation system, we tested the biological activity of the total polysomal poly(A)+ RNA. The ability of the reticulocyte lysate system to translate the RNA from all three strains just about equally indicates that the basis for the heterotic effect does not lie in a greater biological activity of RNA from the F1 hybrid.

The distribution of the four MBPs (11) produced by the three different genotypes in vitro showed no detectable qualitative differences (Fig. 3). However, the amount of RNA coding for MBP present in the polysomes of the three strains did differ significantly. The greater the myelination of the central nervous system in vivo, the greater the amount of MBP mRNA present in the polysomes. Thus, F1 mice had the greatest amount of MBP mRNA in the polysomes, B6 mice had the least, and D2 mice had an intermediate level. These results suggest that the molecular basis for the increased MBP synthesis seen in the F1 hybrid mice is most likely at the level of transcription. However, since the increase in the amount of mRNA for MBP is not as great as the increased amount of MBP produced in vitro, it is possible that some of the regulation is translational. We cannot determine from the above data specifically how the control is exerted. We already know that the amount of RNA coding for MBP that appears in the polysomes of F1 mice is increased. This may be accomplished in several ways. First, there may be a greater amount of mRNA for MBP produced in the F1 hybrid during this developmental stage. Second, the same proportion of MBP transcripts may be produced, but a greater number may be post-transcriptionally modified in F1 mice such that they are better transported to the cytoplasm. Third, the transcripts in F1 mice could also be modified in such a way as to have a longer half-life.

The increase in the amount of MBP mRNA present in the F1 mice at 17 days of age may also be due to a shift in the developmental program of these mice. The peak of myelination could occur earlier than in either parental strain, leading to increased mRNA levels for myelin constituents, such as MBP, at a different age for the F1 mice. We are currently

### Table 3. Quantitation of MBP RNA in polysomes

<table>
<thead>
<tr>
<th>Strain</th>
<th>RNA, average %</th>
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<tbody>
<tr>
<td>B6</td>
<td>100</td>
</tr>
<tr>
<td>D2</td>
<td>127.7 ± 7.30</td>
</tr>
<tr>
<td>F1</td>
<td>144.0 ± 6.41</td>
</tr>
</tbody>
</table>

The amount of RNA is shown as % ± SEM. The value for B6 was arbitrarily assigned to be 100%. The difference between B6 and D2 and between B6 and F1 is significant at the 0.01 and 0.001 levels, respectively. The difference between D2 and F1 is not statistically significant.
testing this idea by using the techniques described here on mRNA from the three strains of mice at various ages.

Our results indicate that the hypermyelination seen in the F1 hybrid mice is reflected in a greater production of MBP by these mice. MBP constitutes \sim 30\% of the central nervous system myelin protein (9, 12, 13). Understanding the regulation of MBP synthesis should be an important step toward understanding the regulation of myelination in the central nervous system.

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