Translational Regulation by Steroids

IDENTIFICATION OF A STEROID MODULATORY ELEMENT IN THE 5'-UNTRANSLATED REGION OF THE MYELIN BASIC PROTEIN MESSENGER RNA*

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Although steroids have been implicated in post-transcriptional regulation, their effects on mRNA translation rates have been uncertain. We have used a cell-free translation system programmed with synthetic messages to show that steroids can alter the translation rates of a number of myelin protein mRNAs and the mRNA encoding a non-myelin protein, the estrogen receptor. Through the use of deletion analysis, site-directed mutagenesis, and chimeric mRNAs we have identified a 9-nucleotide segment in the 5'-untranslated region of one myelin protein mRNA that is necessary for steroid action. Steroid-mediated translational regulation is discussed in terms of myelination where subtle developmental changes in protein composition of the membrane have significant consequences on its morphology and function. We propose that the modulation of mRNA translation rates by steroids is a more general phenomenon that may serve as another mechanism by which steroids can regulate gene expression.

Steroids are generally considered to exert their control of gene expression at the transcriptional level, through a well studied receptor-mediated mechanism (Beato et al., 1987; Evans, 1988; Evans and Arnaiz, 1989). However, a number of studies indicate that steroids also may regulate gene expression post-transcriptionally. For example, progesterone has been reported to be capable of selectively increasing the initiation of translation of ovalbumin mRNAs in chick oviduct (Pennequin et al., 1978), glucocorticoids have been reported to inhibit the translation of some, but not all, ribosomal proteins in lymphosarcoma cells (Meyuhas et al., 1987), and estradiol has been reported to cause a significant reduction in estrogen receptor (ER)1 protein and mRNA levels 48 h after administration to MCF-7 cells, despite normal rates of transcription of the ER gene (Saceda et al., 1988). The post-transcriptional action of steroids could occur by altering the stability of the mRNAs (Shapiro et al., 1985; Paek and Axel, 1987) and/or by altering the rates of translation of responsive mRNAs (Pennequin et al., 1978; Labate et al., 1985). In this regard, steroid receptors recently have been found to interact with RNA (Ali and Vedeckis, 1987) and steroids and their receptors have been shown to interact with other components of the translation system (Liao et al., 1980; Labate et al., 1986).

In the mammalian central nervous system, the oligodendrocyte expresses the major myelin protein genes under a tightly controlled developmental program (Campagnoni and Macklin, 1988). The major myelin protein genes include those encoding the myelin basic proteins (MBP), the myelin proteolipid protein (PLP), 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase), and the myelin-associated glycoprotein. All of the myelin protein genes are alternatively spliced, with the MBP gene undergoing the most complex pattern of splicing to produce at least five MBP isoforms which vary in mass from 14 to 21.5 kDa in the mouse. None of the myelin protein genes appear to be transcriptionally regulated by steroids, but evidence has been reported recently which suggests post-transcriptional regulation by steroids (Kumar et al., 1989; Verdi et al., 1989). In primary oligodendrocyte cultures, hydrocortisone stimulates the expression of the MBPs and PLP, without any increase in the transcription rates of these genes (Kumar et al., 1989). Other studies have shown that the translation of MBP and PLP mRNAs could be stimulated by hydrocortisone and other steroids in cell-free systems (Verdi et al., 1989).

The purpose of the work reported here was to determine whether steroids could exert a direct effect of the translation of specific mRNAs and to determine if sequences within the MBP mRNA were involved in this phenomenon. We have observed that steroids can alter the initial rates of translation of several mRNAs in both a positive and negative fashion, and that an element in the 5'-untranslated (UT) region of one of those messages, the MBP mRNA, appears to be required for steroid action.

MATERIALS AND METHODS

Preparation of Variant Clones—From full length MBP cDNAs encoding either the 14- or 18.5-kDa MBPs, a series of MBP cDNA clones with altered 5'-UT regions were generated by polymerase chain reaction. Sense primers were used that started at various sites within the 5'-UT region (−48, −98, −23) and, in some cases, contained "site-directed" mismatches in the region between −32 and −37. These primers were used in tandem at their 5' ends. The 3' end of the antisense primer was matched to nucleotides into the 3' UT region and contained two mismatched bases that created a BamHI site 43 nucleotides downstream of the translation termination codon. The polymerase chain reaction conditions employed were those recommended by the manufacturer (Cetus/U.S. Biochemical Corp.), except that the concentration of Mg2+ was 1.5 mM, and the annealing temperature was

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‡ The abbreviations used are: ER, estrogen receptor; MBP, myelin basic protein; PLP, 30-kDa myelin proteolipid protein; CNPase, 2',3'-cyclic nucleotide 3'-phosphodiesterase; HC, hydrocortisone; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; UT, untranslated; SDS, sodium dodecyl sulfate.
Steroid Modulatory Element in MBP mRNA

50 °C. Fifty cycles of amplification was used. Following precipitation, the polymerase chain reaction-generated fragments were directionally cloned into the EcoRI/BamHI sites of the transcription vector pGEM such that the sense RNA would be produced from the T7 promoter. In every case, the product, after subcloning into pGEM, was sequenced to verify the nucleotide sequence of the insert. Fig. 1 presents a flow diagram illustrating the construction of the altered clones. The sequences of primers employed to generate the altered MBP clones were as follows:

(46) GAATTGAAATTCGAGCCACATCCGAGAAGACCCGG
GACGCTCTCCGGACCGCT
(20) GAATTGGAATTCGAGCCACATCCGAGAAGACCCGG
GACGCTCTCCGGACCGCT
(−23) GAATTGGAATTCGAGCCACATCCGAGAAGACCCGG
GACGCTCTCCGGACCGCT

The sequences of 5' primers used to create site-directed changes within the region between −52 and −37 of the 5'-UT region of MBP were as follows (altered bases shown in bold type):

GAATTGGAATTCGAGCCACATCCGAGAAGACCCGG
GACGCTCTCCGGACCGCT

In all cases the 3' primer was used in identical every case. The sequence of which was TTTAGGCGATATATAGGATCCGA. All primers were generated at the Molecular Biology Institute and UCLA Jonsson Comprehensive Cancer Center Fermenter/Preparation Core Facility.

The sense primer used for the construction of the AGAAGA +tyrosine hydroxylase clone contained a tandemly repeated EcoRI restriction site at the 5' and upstream of AGAAGA+14 nucleotide of the 5'-UT region of tyrosine hydroxylase. The antisense primer began 24 nucleotides downstream of the translation termination codon and contained two BamHI recognition sequences in tandem at their 5' ends. The cDNAs for CNPase, myelin-associated glycoprotein, tyrosine hydroxylase, and ER were the generous gifts of Drs. D. Colman (Columbia University, New York), R. Milner (Scripps Research Institute, La Jolla, CA), B. Kaplan (Western Psychiatric Institute, Pittsburgh, PA), and G. Greene (University of Chicago), respectively.

In Vitro Transcription—Capped transcripts were generated according to the manufacturer's specifications (Stratagene) with T7 RNA polymerase, except that the reaction was performed for 1 h at 37 °C with 3.5 mM GTP and 2 units of RNase Block (Stratagene). After 1 h, 2 units of RNase-free DNase (Stratagene) was added and the reaction was allowed to proceed for an additional 15 min to digest the template DNA. Synthetic RNAs were stored in sterile water for no longer than 90 days at −20 °C.

In Vitro Translation—Twenty-microliter reactions using rabbit reticulocyte lysates purchased from Bethesda Research Laboratories were performed according to published procedures except that the amount of tRNA in the reaction was 5 μg (Roth et al., 1985; Verdi et al., 1989). Steroids were added to the system in 125 nCi of [35S]methionine hydroxylase, and ER were the generous gifts of Drs. D. Colman (Columbia University, New York), R. Milner (Scripps Research Foundation, La Jolla, CA), B. Kaplan (Western Psychiatric Institute, Pittsburgh, PA), and G. Greene (University of Chicago), respectively.

Steroids Can Modulate the Translation of mRNAs—Previous work in this laboratory indicated that MBP mRNA was translated less efficiently than brain mRNAs as a whole (Campagnoni et al., 1987). During the course of that work we found that a protein synthesis inhibitor, fusidic acid, stimulated the synthesis of MBP in cell-free translation systems at concentrations well below those at which there was any effect on total protein synthesis. Because fusidic acid shares the same five-membered cyclopentanophenanthrene ring structure as steroid hormones, we examined the effect of other steroids on the translation of MBP mRNAs. We found that addition of 10−8 M hydrocortisone (HC) to reticulocyte lysates programmed with mouse brain poly(A) + RNA produced a 1.7-fold increase in the synthesis of MBP, with no measurable effect on total protein synthesis.

To eliminate the possibility that HC was acting on some other message in the poly(A) + population whose product might be stimulating MBP mRNA translation, synthetic RNAs produced from cDNAs subcloned into transcription vectors were used to program the cell-free translations. This also permitted a direct examination of the effects of hydrocortisone on the translation of a number of cloned messages, independent of its effects at the transcriptional level.

No effects of HC were observed on the translation of a number of mRNAs, including those encoding myelin-associated glycoprotein, tyrosine hydroxylase, and globin (Table I). In contrast, the translation of three myelin protein mRNAs were altered by the steroid. The translation of MBP and PLP mRNAs were stimulated by HC, and the translation of the

**TABLE I**

<table>
<thead>
<tr>
<th>Message encoding</th>
<th>% control ± SEM</th>
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<tr>
<td>Myelin basic proteins</td>
<td>202 ± 35</td>
</tr>
<tr>
<td>Proteolipid protein</td>
<td>233 ± 57</td>
</tr>
<tr>
<td>CNPase</td>
<td>45 ± 10</td>
</tr>
<tr>
<td>Myelin-associated glycoprotein</td>
<td>86 ± 7</td>
</tr>
<tr>
<td>Tyrosine hydroxylase</td>
<td>91 ± 13</td>
</tr>
<tr>
<td>Globin</td>
<td>102 ± 8</td>
</tr>
</tbody>
</table>

*Percent of control incubations performed in the absence of hydrocortisone. Presented are the means (±SEM) of 30 experiments performed in triplicate for MBP and 3-4 experiments with other transcripts.
CNPase mRNA was inhibited. The dose response for the stimulation of MBP mRNA translation by HC (Fig. 2) was determined and found to be maximal at $10^{-8}$ M, a physiologically relevant concentration. In other experiments with estradiol, the translation of one non-myelin protein message, the ER mRNA, was found to be significantly inhibited (to ~55% of control) by this sex steroid. The dose-response curve for estradiol on the translation of the ER mRNA is also shown in Fig. 2. The response was similar, but in opposite direction to that exerted by HC on the translation of MBP mRNA. Maximal inhibition occurred at $10^{-9}$ M estradiol.

The effects of a number of steroids, including glucocorticoids, mineralocorticoids, and sex hormones, were examined on the translation of the MBP and CNPase mRNAs (Fig. 3) since these represented myelin protein messages whose translation were modulated in both a positive and negative fashion. Glucocorticoids exerted a greater effect on the translational efficiencies of MBP and CNPase mRNAs than did the sex hormones, and no effect was observed with aldosterone, cortisol 21-acetate, or the sterols, cholesterol and desmosterol (data not shown). In general, there was an inverse correlation in the effects of the steroids on the translation of the two messages. Those steroids that most effectively stimulated the translation of the MBP mRNA tended to be most effective at inhibiting the translation of the CNPase message. This reciprocal pattern of regulation is illustrated in Fig. 4 where an MBP mRNA and CNPase mRNA have been translated together in the presence and absence of steroid. Under normal conditions the CNPase mRNA is translated more efficiently than the MBP mRNA, but in the presence of steroid the synthesis of MBP is stimulated and the CNPase is inhibited, such that the levels of the two proteins produced have been reversed.

**Steroids Modulate the Rates of Translation of Responsive mRNAs**—The standard incorporation time of 1 h, used in these studies, was well within the linear incorporation period of $[^{35}S]$methionine into protein for both the control and steroid-supplemented translations. Thus, it was unlikely that the apparent modulation of translation was due to changes in the stabilization of the messages by the steroids over the course of the incubation. Nonetheless, initial incorporation rates were calculated from double-reciprocal plots of the kinetic data for the stimulation of MBP mRNA translation by HC and the inhibition of ER mRNA translation by estradiol (Fig. 5). The rates for the translation of MBP mRNA were $4.23 \pm 0.7$ fmol/min for the control (minus HC) and $14.9 \pm 4.8$ fmol/min in the presence of $10^{-9}$ M HC. Initial rates for the translation of ER mRNA performed in the absence of estradiol were $57 \pm 14$ and $17 \pm 4.8$ fmol/min for translations containing $10^{-9}$ M estradiol. These results indicate that the effects of the steroids were on the rates of translation of the mRNAs. As a further control, radiolabeled MBP mRNA was synthesized and added to translation reactions in the presence and absence of HC. The reactions were incubated for up to 2.5 h, samples withdrawn at 15–30-min intervals, and the labeled RNA analyzed by agarose gel electrophoresis. There was no detectable difference in the stability of the RNA over the time period of the incubation (data not shown).

To determine if the classic glucocorticoid receptor was involved in the stimulation of MBP mRNA by HC, translations were performed in the presence of the antagonist RU38486. At no concentration tested did RU38486 antagonize the stimulation observed by HC on MBP mRNA translation, even when the antagonist was in 100-fold molar excess (Table II). This indicates that the stimulation of MBP translation does not involve the classic glucocorticoid receptor.

**Localization of the Responsive Element in MBP mRNA**—In previous studies we observed that the translation of all alternatively spliced forms of the MBP mRNAs transcribed from...
Fig. 5. Double-reciprocal plots of time course data showing a increase in the initial rate of MBP synthesis (top) and a decrease in the initial rate of ER synthesis (bottom) in the presence of $10^{-8}$ and $10^{-6}$ M HC and estradiol, respectively. A composite of three experiments measuring the synthesis of MBP and four experiments measuring the synthesis of ER by $[^{35}S]$methionine incorporated into trichloroacetic acid precipitable protein are shown. Slope $^{-1}$ (MBP control) = 0.236 and slope $^{-1}$ (MBP HC) = 0.066; slope $^{-1}$ (ER control) = 0.0196 and slope $^{-1}$ (ER estradiol) = 0.058.

**TABLE II**

Effect of the glucocorticoid receptor antagonist RU38486 on HC stimulation of MBP mRNA translation

<table>
<thead>
<tr>
<th>Addition</th>
<th>Incubation</th>
<th>dpm incorporated</th>
<th>% stimulation</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>70.3 ± 3.4</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>$10^{-8}$ M RU38486</td>
<td>70.6 ± 4.6</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>$10^{-6}$ M HC</td>
<td>89.6 ± 3.5</td>
<td>127</td>
<td></td>
</tr>
<tr>
<td>$10^{-8}$ M HC + $10^{-8}$ M RU38486</td>
<td>86.7 ± 5.4</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>$10^{-6}$ M HC</td>
<td>113.9 ± 3.0</td>
<td>162</td>
<td></td>
</tr>
<tr>
<td>$10^{-8}$ M HC + $10^{-8}$ M RU38486</td>
<td>109.6 ± 2.8</td>
<td>156</td>
<td></td>
</tr>
<tr>
<td>$10^{-6}$ M HC</td>
<td>137.4 ± 4.4</td>
<td>193</td>
<td></td>
</tr>
<tr>
<td>$10^{-8}$ M HC + $10^{-8}$ M RU38486</td>
<td>135.4 ± 2.3</td>
<td>193</td>
<td></td>
</tr>
</tbody>
</table>

$[^{35}S]$Methionine incorporated into trichloroacetic acid-precipitable protein. Presented are the means of incorporation (±S.D.) of incubations performed in triplicate.

This prompted us to investigate further the role of the 5'-UT region in this phenomenon. A series of cDNA clones encoding the 14- and 18.5-kDa MBP isomers, but which differed in the lengths of their 5'-UT regions, were generated using the polymerase chain reaction and subcloned into pGEM. The transcribed RNAs were examined in the cell-free translation system in the presence and absence of HC. Removal of the 5'-UT sequence between -29 and -37 resulted in the loss of responsiveness of both MBP mRNAs to HC. A compilation of these results is shown in Table II, along with a polyacrylamide gel illustrating the results for the 18.5-kDa MBP mRNA. The gels show translations of several modified mRNAs encoding the 18.5-kDa MBP that began at -28 and the 5'-UT region. The smaller band in each lane represents internal initiation 57 nucleotides downstream of the normal MBP start site. The NM lane contains the products of control incubation in which no message was present.

The result was supported by studies in which the translation of an MBP mRNA, which contained little of the 3'-UT region but a normal 5'-UT region, was stimulated by HC (Fig. 6).
TABLE III
Effect of the oligonucleotide AGAAGACCC on HC stimulation of MBP mRNA translation

<table>
<thead>
<tr>
<th>Molar ratio oligonucleotide:MBP mRNA</th>
<th>% control*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>205 ± 35</td>
</tr>
<tr>
<td>0.01</td>
<td>189 ± 50</td>
</tr>
<tr>
<td>0.10</td>
<td>148 ± 8</td>
</tr>
<tr>
<td>1.0</td>
<td>134 ± 19</td>
</tr>
<tr>
<td>10.0</td>
<td>113 ± 21</td>
</tr>
<tr>
<td>100.0</td>
<td>103 ± 4</td>
</tr>
<tr>
<td>Control oligonucleotide*</td>
<td>249 ± 85</td>
</tr>
</tbody>
</table>

*a Percent of control incubations performed in the absence of hydrocortisone. The means (± S.D.) of three experiments are presented. The concentration of HC was 10^{-9} M.

Translation performed with a 100-fold molar excess of a synthetic oligonucleotide differing in two bases, AGAACGCC.

III, when the molar ratio of nonamer to message was increased, there was a decrease in the observed stimulation by HC. (The ability of HC to stimulate the translation of MBP mRNA was completely abolished when the oligonucleotide was present in the 10-fold excess or greater.) A mutated oligonucleotide of the same size, but with two alterations in the sequence (AGAACGCC), was unsuccessful in competing out the effect of HC stimulation, even when it was present in 100-fold molar excess (Table III).

Site-directed Mutagenesis of the MBP mRNA Confirms the Importance of the Sequence—Site-directed mutagenesis of the nonamer region within the MBP mRNA was performed to provide additional evidence that bases within the −29 to −37 region of the message were important for the steroid mediated modulation of translation. These results are presented in Fig. 7. Modifications were made at three bases in the nonanucleotide region of the MBP message. A single base substitution at −37 (UGAAGACCC) or a double base substitution at bases −32, −33 (AGAAGCCGCC) rendered the translation of the message nonresponsive to HC. A single base change at −33 (AGAAAGCCC) reduced the stimulation of translation by over 80%. Hence, base changes in three of the nucleotides within the nonamer region of one MBP message resulted in a significant reduction and/or elimination of the stimulatory response of HC on the translation of MBP mRNA.

The HC Modulatory Element Can Be Transferred to a Normally Nonresponsive Message—To determine if the HC modulatory sequence in the MBP mRNA could confer HC responsiveness on another, normally nonresponsive message, the mRNA for tyrosine hydroxylase was engineered with the AGAAGACCC sequence at its 5′ end. When the altered tyrosine hydroxylase mRNA was translated in the presence and absence of HC, a significant (147 ± 10.2%) stimulation was observed in the amount of tyrosine hydroxylase synthesized (Fig. 8). Although the stimulation was not as great as that observed with the MBP mRNA, the data indicate that addition of the sequence to the 5′-UT of a nonresponsive message can render that message responsive to HC.

DISCUSSION

There have been a number of reports indicating that sex steroids or glucocorticoids can regulate gene expression at a post-transcriptional level by affecting, either positively or negatively, specific steps in protein synthesis (Liao et al., 1980; Rannels et al., 1978). These effects have been reported on general protein synthesis in responsive tissues as well as on the translation of specific mRNAs. For example, there is evidence that steroids can alter peptide chain initiation in skeletal muscle (Rannels et al., 1978), chick oviduct (Palmiter, 1972; Pennequin et al., 1978), and prostate (Liang and Liao, 1978). It also has been reported that steroids can selectively affect the synthesis of ovalbumin mRNAs in chick oviduct (Pennequin et al., 1978) and that glucocorticoids can selectively inhibit the translation of some, but not all, ribosomal proteins in lymphosarcoma cells (Meyuhas et al., 1987). Generally, in these studies the steroid was administered in vivo or in culture, where direct effects of the steroid on rates of translation could not easily be assessed.

In the work reported here, a cell-free translation system programmed with synthetic mRNA was used to determine the effects of steroids on the translation of specific synthetic mRNAs. The use of this system had some advantages in that it permitted (a) an assessment of the effect of the steroid on translation, independent of effects on transcription, (b) a direct assessment of the effect of steroids on the rates of translation of specific mRNAs, and (c) an examination of possible regulatory sequences in the 5′-UT region by programming the system with structurally modified transcripts.

The post-transcriptional modulation of gene expression by steroids has generally been proposed to occur either through messenger RNA stabilization or through direct effects on translation. In the system reported here, the kinetic data clearly indicate that steroids were affecting the translation rates of responsive messages. A number of results indicate that the mechanism by which hydrocortisone is acting in this translation system is different from that which occurs at the transcriptional level involving the classic glucocorticoid receptor. 1) An antagonist of the glucocorticoid receptor, RU38486, did not inhibit the stimulation of MBP mRNA translation by glucocorticoids. 2) The order of effectiveness among the glu-
was different from that of the glucocorticoid receptor-mediated effect on transcription. 3) The dose-response curves are more complex than that observed with the classic glucocorticoid receptor, reaching maximum in the physiologically relevant range of $10^{-8}$ to $10^{-4}$ M steroid. 4) The nucleotide sequence of the element in the MBP mRNA that is responsive to HC is unlike that of the glucocorticoid responsive element in genes that are transcriptionally regulated by this steroid.

We have been able to localize a nine base region within the 5'-untranslated region of MBP mRNA between -29 and -37 that appears to be necessary for the stimulation of translation by HC. Several lines of evidence indicate the importance of this region: 1) removal of this region of the message eliminates the translatable response to steroid; 2) site-directed mutagenesis of several bases within this region reduces or eliminates the response; 3) a synthetic oligonucleotide of the same sequence can entirely compete out the response at a 10:1 molar ratio of oligonucleotide:message, but no such competition is observed with an oligonucleotide containing only two base changes; and 4) transference of the sequence to a nonresponsive message renders the translation of that mRNA responsive to HC.

The fact that the stimulation of translation could be competed out with a synthetic oligonucleotide suggests that this region of the MBP mRNA is involved in binding to one or more factors involved in the response. However, the sequence alone probably does not account for the entire translational response to steroid since transference of the sequence to the 5'-UT region of the MBP mRNA that is important in this regulation is likely to be necessary for the stimulation of translation by steroids, and the regulation of the genes encoding the major myelin glycoproteins has been shown to be modulated at both the transcriptional and translational levels by steroids.

It is also possible that the effects of steroids on message stability and rates of translation might be related mechanistically. For example, a steroid-induced increase in polypeptide chain initiation would be expected to result in increased ribosomal density per message, thereby leading to increased message stability. The opposite would be true for messages whose translation was inhibited by steroids, where ribosome density would be expected to decrease in the presence of steroids. It has been shown that transcripts that are not undergoing active translation or have fewer ribosomes per message are good substrates for degradation (Schneider et al., 1978; Kelly et al., 1987).

Do these results have physiological significance? In vivo, serum levels of HC closely parallel the onset and time course of myelination in rodent brain (Hafez et al., 1990). Several studies have demonstrated the importance of steroids in myelination or the expression of myelin components in primary gial cultures in vitro (Dawson and Kernes, 1979; Preston and McMorris, 1984). In a recent study (Kumar et al., 1989), HC was found to increase MBP and PLP gene expression 2-3 fold, without affecting the transcription rates of these genes, in primary oligodendrocyte cultures.

The magnitude of the effects of steroids upon the post-transcriptional modulation of myelin protein gene expression is not large, only 2-3-fold. Nonetheless, the importance of this form of regulation should not be underestimated. During most active myelination, the oligodendrocyte produces prodigious amounts of myelin over a relatively short period of time in the mouse (10-40 days). Estimates have been made that during the period of maximal myelin accumulation, oligodendrocytes synthesize an amount of myelin more than three times the weight of their cell bodies each day (Morell and Toews, 1984). During this period, the expression of the myelin protein genes is extremely active and the myelin proteins produced, when incorporated into myelin, are quite stable, with low turnover rates (Benjamin, 1984). Over this same developmental period the myelin membrane itself undergoes significant compositional changes, with the proportion of MBP and PLP increasing significantly relative to CNPase. These protein compositional changes appear to correlate with morphological changes as the myelin membrane becomes tightly wrapped around the axons. Consequently, a 2-3-fold increase in the synthesis of the MBPs and PLP, concomitant with an equivalent decrease in the expression of CNPase can produce a profound shift in the composition of the membrane during this developmental period. The phenomenon shown in Fig. 4 illustrates this phenomenon and reflects the type of compositional change noted in myelin with maturation (Benjamin, 1984). In such a biological system, even though the effects on translation of the messages are relatively small they are multiplicative and the relative levels of individual proteins can become increasingly disparate in a geometric progression with such stable end products.

In summary, the unique characteristics of myelination and the regulation of the genes encoding the major myelin proteins have revealed three genes within this system that are regulated at the translational levels by steroids. For at least one of these mRNAs there appears to be a steroid modulatory element in the 5'-UT region that is important in this regulation. This form of regulation is likely to be present in other...
systems (e.g. estrogen receptor gene expression) with analogous steroid modulatory regions present in responsive messages.

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