Journal of Neuroscience Research 36:508–519 (1993)

Characterization of the *cis*-Acting Elements of the Mouse Myelin P_2 Promoter

V.A. Bharucha, K.W.C. Peden, B.R. Subach, V. Narayanan, and G.I. Tennekoon
Departments of Pediatrics and Neurology, Section of Pediatric Neurology, University of Michigan Medical Center, Ann Arbor (V.A.B., B.R.S., G.I.T.); Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland (K.W.C.P.); Department of Pediatrics, University of Pittsburgh, Pittsburgh, Pennsylvania (V.N.)

Myelin P_2 is a basic protein of an apparent molecular weight of 14,800. Expression of P_2 has been found largely in the cytosol of Schwann cells in the peripheral nervous system. Although the function of P_2 is unknown, its striking homology to a family of fatty acid binding proteins has led to the idea that P_2 may function as a fatty acid transport molecule. To investigate the DNA elements that control the expression of P_2, sequences 5′ to the coding region were cloned upstream of the *cat* reporter gene. A series of 3′ and 5′ promoter mutants was constructed and their activity determined following transfection into secondary Schwann cells and the MT_4H1 Schwann cell line. Using this strategy, we have identified a 217 bp silencer region and a 142 bp positive regulatory region. In addition, we have localized the 5′ flanking sequences in the promoter that are responsive to cAMP induction and to the transcription factor CCAAT/enhancer binding protein (C/EBP).

Key words: deletion mutants, silencer, cAMP responsive elements, CAAT/enhancer binding protein

INTRODUCTION

The formation of myelin is dependent upon the developmentally regulated expression of myelin genes. In the peripheral nervous system (PNS), myelin is synthesized by Schwann cells and in the central nervous system (CNS) the myelin forming cells are the oligodendroglia. Myelin itself is comprised of both lipid (~70%) and protein (~30%) components, as are the precursor plasma membranes of myelin forming cells (Agrawal and Hartman, 1980). In the CNS, the major proteins consist of proteolipid protein (PLP), myelin basic protein (MBP), and the Wolfgram proteins (including 2′,3′-cyclic nucleotide 3′ phosphodiesterase) (see review by Campagnoni, 1988). The major myelin proteins of the PNS include P_0, MBP, and P_2.

Attempts to characterize the peripheral myelin proteins have led to postulated roles for the P_0 and MBP in maintaining both structural integrity and in bringing about myelin compaction (Omlin et al., 1982; Lemke and Axel, 1985). Myelin P_2 protein is a 14,800 dalton (14.8 kDa) basic protein, which has been shown immunocytochemically to be localized to the cytoplasmic surface of compact myelin and in actively myelinating Schwann cells (Trapp et al., 1984). Based on its striking sequence homology with other fatty acid binding proteins (FABPs), P_2 has been hypothesized to be involved in the transport of intracellular fatty acids (Narayanan et al., 1988). Further evidence supporting this role comes from X-ray crystallographic data, which show a fatty acid located centrally within a flattened barrel structure of P_2 (Jones et al., 1988). These data have provided vital information regarding the functional characteristics of the protein and have resulted in a postulated role for P_2 during the myelination process.

One interesting observation regarding the expression of P_2 protein is that it is found in varying amounts in myelin of different species. In rodents it comprises 1–2% of total myelin protein, in rabbits it makes up 6%, and in humans it makes up about 14% (Greenfield et al., 1973). Moreover, the distribution of P_2 is also found to vary among different species. In rodents P_2 protein is confined to the PNS, whereas in rabbits, cattle, and humans the protein is found in both the PNS and CNS (Greenfield et al., 1982). It is unclear what controls the expression of P_2, since very little is known about the regulatory sequences of the P_2 gene that might be responsible for this tissue and species specific variation.

A cDNA clone of P_2 from rabbit and a genomic clone of mouse P_2 have been isolated and characterized by Narayanan et al. (1988, 1991). Both cDNA and genomic clones for myelin P_0 (Lemke and Axel, 1985),

Received March 24, 1993; revised and accepted May 26, 1993.

Address reprint requests to G.I. Tennekoon, Departments of Pediatrics and Neurology, Section of Pediatric Neurology, R6060 Kresge II, Box 0570, University of Michigan Medical Center, Ann Arbor, MI 48109-0570.

MBP (Takahashi et al., 1985; deFerra et al., 1985), and PLP (Milner and Sutcliffe, 1983; Nave et al., 1987) have been isolated and characterized. Comparison of their protein and DNA sequences has shown some homologies between P_0 and MBP (Lemke, 1988; Lemke et al., 1988) and modest homology between MBP and PLP (Macklin et al., 1987; see review by Mikoshiba et al., 1991). In contrast, P_2 shares no significant homology with the other three myelin specific proteins, either at the protein or DNA level, including their respective 5′ flanking regions (Narayanan et al., 1988, 1991).

One common inducible pathway in the expression of these genes has been identified recently. Raising intracellular cAMP levels by treatment with forskolin and other cAMP analogues has been shown to result in the induction of P_2, MBP, and P_0 mRNA expression (Lemke and Chao, 1988; Monuki et al., 1989, 1990; Gandelman et al., 1989). This is surprising considering the absence of a consensus cAMP responsive element (CRE) (Montminy et al., 1986) and a lack of significant homologies among the 5′ elements of these genes. Thus, in an attempt to define better the 5′ regulatory elements within the promoter region of the myelin P_2 gene necessary for expression, we have constructed a series of 5′ and 3′ deletion promoter mutations and have examined their effect on promoter activity with a reporter gene. Using this strategy, we have identified a 217 bp silencer region and a positive regulatory region. In addition, we have characterized the 5′ flanking sequences that are responsive to cAMP induction and have identified the sequences that are regulated by the transcription factor CCAAT/enhancer binding protein (C/EBP).

MATERIALS AND METHODS

Plasmids

The adenylate cyclase α subunit expression vector, pCMV-Cα, was obtained from M. Uhler and C/EBP expression vector, pMSV-C/EBP, was obtained from S. McKnight.

Cell Culture

MT_4H1 rat Schwann cell line (Peden et al., 1989) and secondary Schwann cells used for transfections were grown in 10 cm dishes in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (DMEM0-10). MT_4H1 cells were maintained without the addition of $ZnCl_2$.

Construction of pP_2*cat*

A schematic representation of the strategy used to construct pP_2*cat* expression plasmid is shown in Figure 1. Briefly, the 4 kb P_2 gene (Narayanan et al., 1991) was cleaved with restriction endonucleases EcoRI and HindIII. The 1 kb fragment was isolated on an agarose gel and subsequently gel purified. This fragment contained the ATG of P_2, but the presence of this ATG did not alter chloramphenicol acetyltransferase (Cat) activity. The fragment was then ligated to the 3.6 kb p*cat* fragment (K. Peden, unpublished data) obtained following digestion with EcoRI and HindIII (Fig. 1). This construct was next used to obtain a series of 5′ and 3′ deletion mutants.

Exonuclease III Digestion

A schematic representation of the strategy used to construct the 3′ and 5′ deletion mutants is shown in Figure 2. For these studies, 5 μg of pP_2*cat* DNA was used per reaction. Exo-Mung Bean reagents were obtained from Stratagene (La Jolla, CA). The reaction buffer contained 50 mM Tris, pH 8.0, 5 mM $MgCl_2$, 10 μg/ml tRNA, and 10 mM β-mercaptoethanol. To each reaction, 1 μl exonuclease III (100 U/μl) was added. Timed digestion was done at 23°C. (At 23°C, the exonuclease III removes 125 bp/min.) The reaction was placed on ice and mung bean nuclease buffer (30 mM sodium acetate, pH 5.0, 50 mM NaCl, 1 mM $ZnCl_2$, and 5% glycerol) was added. The reaction was heated at 68°C for 15 min. To each tube, 15 U of mung bean nuclease was added and incubated for 30 min at 30°C. The reaction was stopped by addition 55 μl of a solution containing 20% sodium dodecyl sulfate (SDS), 0.2 M EDTA, and 10 mg/ml proteinase K. After another incubation at 37°C for 30 min, the sample was extracted with phenol:chloroform and the DNA precipitated. For the 3′ deletions, HindIII linkers were added. For the 5′ deletions, the pP_2*cat* plasmid was linearized with AatII, which leaves a 3′ overhang and makes it unsuitable as a substrate for exonuclease III. The plasmid was blunt-ended using the 3′-5′ exonuclease activity of the Klenow fragment, followed by repairing the ends with the same enzyme and adding cold dNTPs. XhoI linkers were added to the two ends and digested with XhoI. Some of this plasmid was used to create the 5′ deletions via the exonuclease III/mung bean nuclease strategy outlined above. After mung bean nuclease treatment, the various deletions were separated on a 1.2% low melting temperature agarose gel. The different 5′ deletion fragments were excised from a 1.2% agarose gel and purified by GeneClean (Bio 101, La Jolla, CA), and XhoI linkers were added. The 5′ P_2 promoter deletions were isolated as XhoI-HindIII fragments. These fragments were ligated into a pP_2*cat* derivative whose AatII site had been converted to an XhoI site by linker addition between the XhoI and HindIII sites. All the 3′ and 5′ deletions were sequenced by the dideoxy sequencing protocol using Sequenase (USB, Cleveland, OH) (Sanger et al., 1977). For the 5′ deletions, the primer used was a 21-mer from nucleotides 4241–4261 of pBR322 (5′-AAACAAAT-

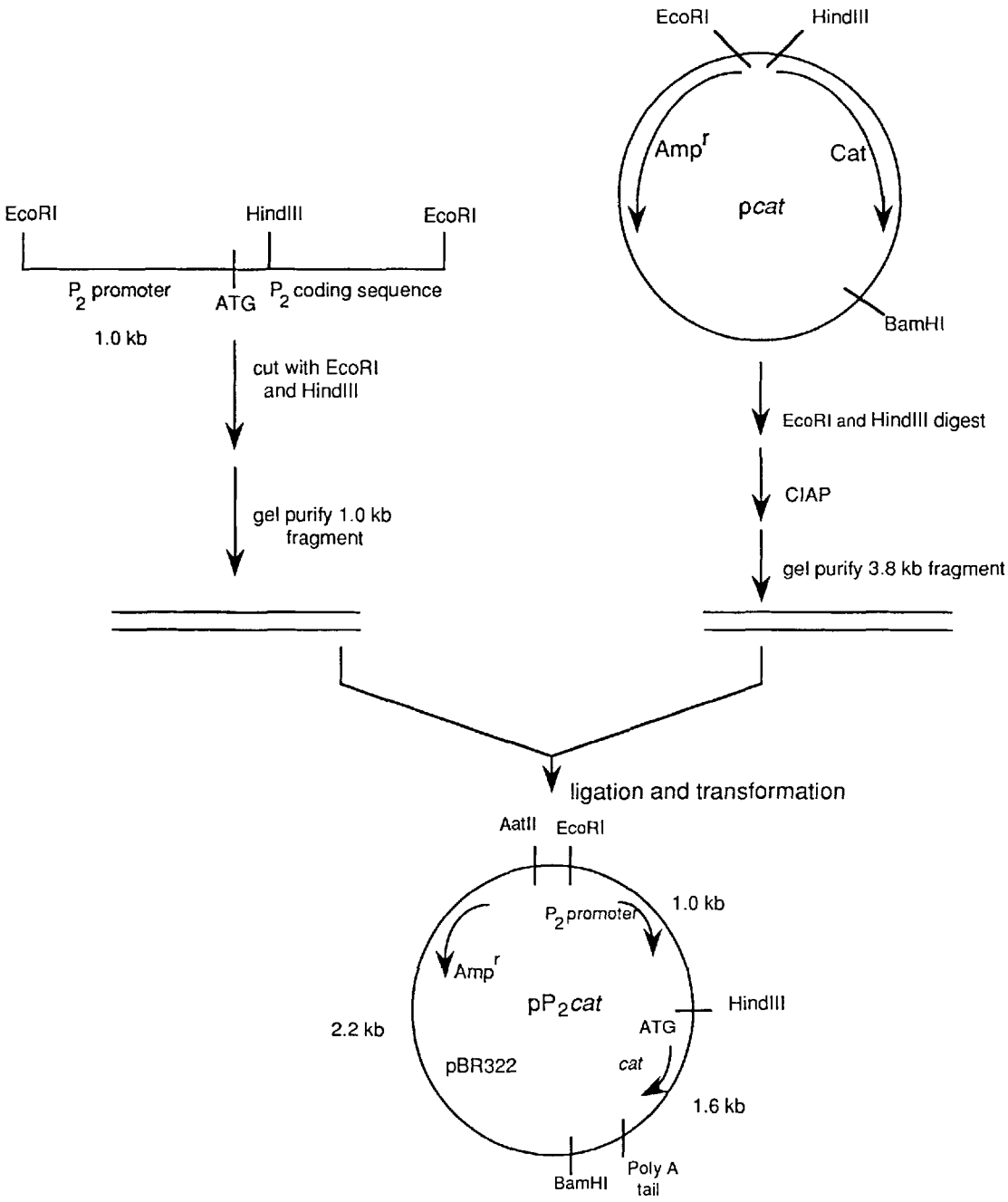


Fig. 1. Construction of pP_2cat plasmid.

AGGGGTTCCGCGC-3′). For the 3′ deletions, a 31-mer primer downstream from the HindIII site in the *cat* gene was used (nucleotides 4921–4951; 5′-GCCATTGGG ATATATCAACGGTGGTATATCC-3′).

Transient Transfection Assays

All transfections were done using the calcium phosphate precipitation method (Gorman et al., 1982). A total of 10 μg of DNA was transfected into Schwann cells or MT_4H1 cell lines. The precipitate was removed 12 hr later and the cells were rinsed once with phosphate buffered saline (PBS), followed by addition of fresh mediums. Cells were harvested 48 hr later and assayed for Cat activity and β-galactosidase activity.

Cat Activity and β-Galactosidase Activity

To correct for differences in transfection efficiencies from experiment to experiment, β-galactosidase as-

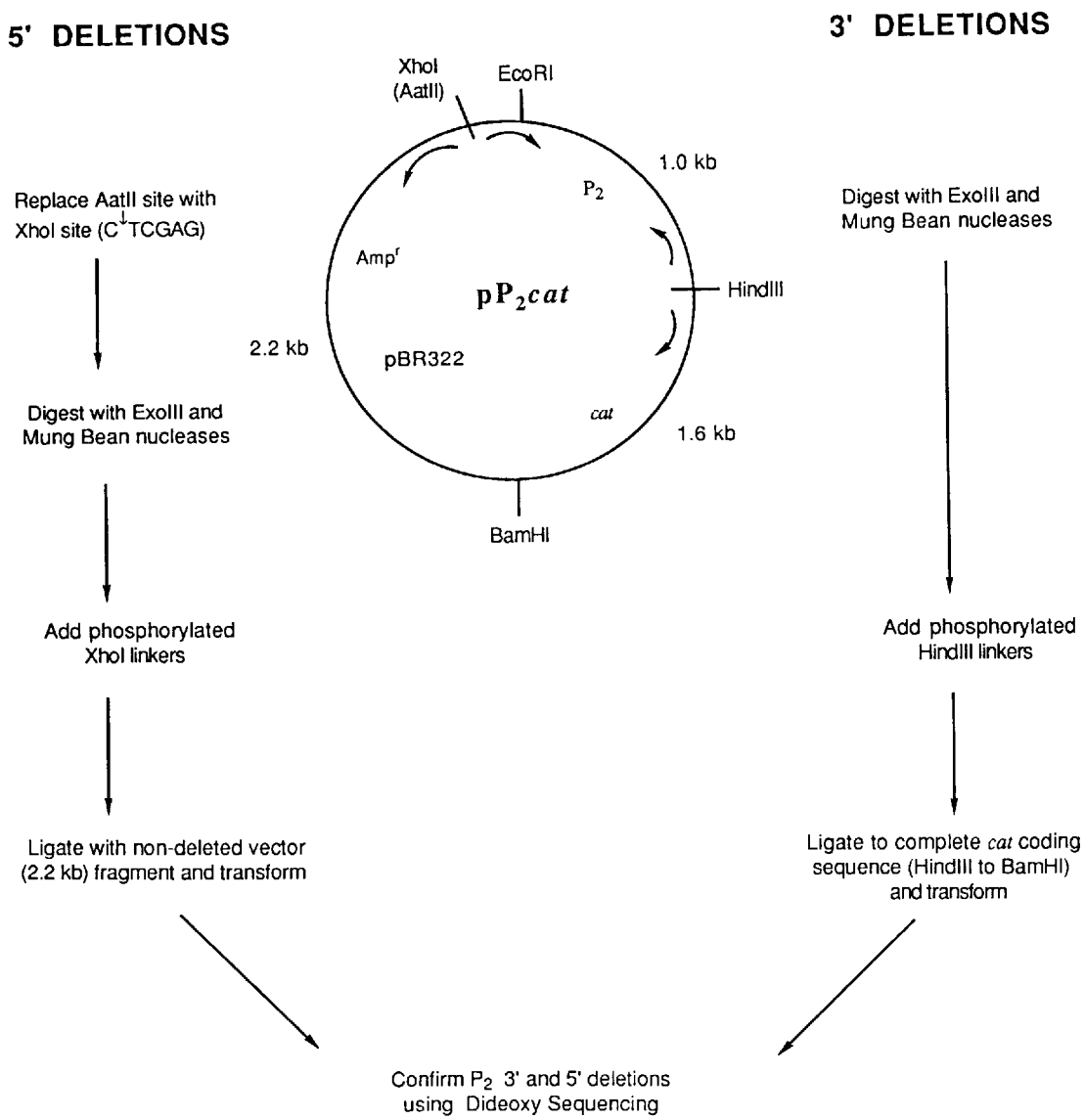


Fig. 2. Schematic representation of the construction of 3′ and 5′ pP_2cat deletion mutants.

says were performed (Miller, 1972). In this assay, o-nitrophenol-β-galactoside is converted by β-galactosidase to galactose and o-nitrophenol, and the amount of the latter compound is determined spectrophotometrically. The reaction was stopped by the addition of concentrated Na_2CO_3 solution, which shifts the pH to 11 and thereby inactivates β-galactosidase. The assay for β-galactosidase was done at 37°C for 30 min to 2 hr depending on the transfection efficiency. The amount of β-galactosidase was determined by the formula: number of units = 1,000 [OD_{420}–1.75 OD_{550}/hr]. Cat activity was assayed according to the method of Gorman et al. (1982). The conversion of [^{14}C]chloramphenicol to the mono- and diacetyl forms was monitored by thin layer chromatography and autoradiography. Cat activity was computed as a percentage of conversion to the acetylated forms and normalized to 100 units of β-galactosidase.

RESULTS

P_2 Promoter Contains a Functional TATA Box

To determine the functionality of the TATA box, we constructed a total of nine 3′ deletion mutants of the mouse P_2 promoter. Cat activity was determined following transfection into secondary rat Schwann cells and the MT_4H1 Schwann cell line. These deletions extended from +120 to −230 bp (Fig. 3), thereby inactivating the TATA box (3′ −83 through 3′ −175) and CAAT boxes (3′ −230). Cat activity for all the deletion constructs measured in the two cell types was not significantly dif-

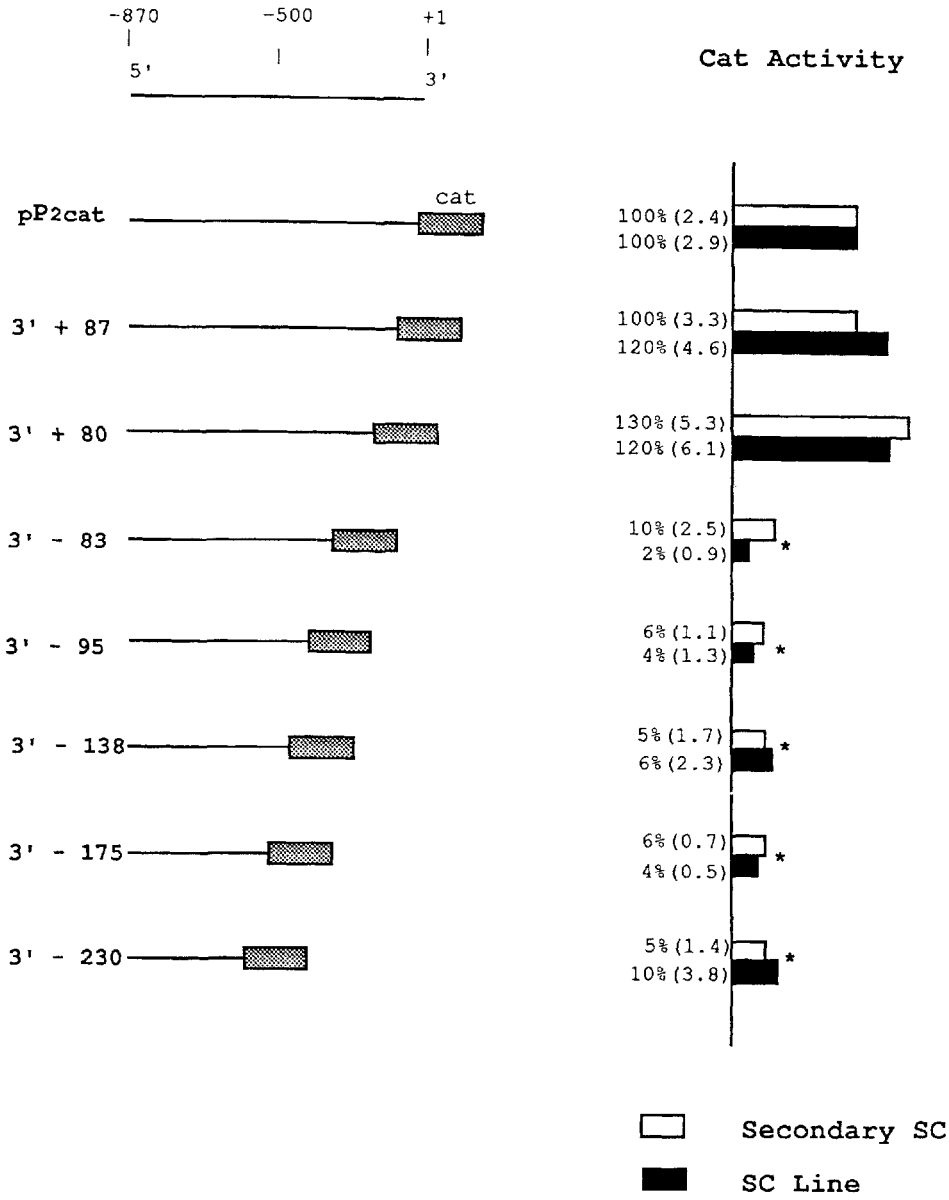


Fig. 3. 3′ deletion analysis of the P_2 promoter region reveals a functional TATA box. Secondary Schwann cells (open bars) and the MT_4H1 cell line (solid bars) were transiently transfected with 5 μg of pP_2cat and 3′ deletion clones. To account for transfection efficiencies, 2 μg of the β-galactosidase gene driven by the RSV promoter was cotransfected. For each construct, Cat activity was calculated as the percentage of the value obtained for pP_2cat (= 100%), normalized for transfection efficiency by 100 units of β-galactosidase. Results are the average of 5 separate measurements for each construct and the standard error of measurement (± SEM) for each construct is shown in parentheses. Constructs significantly different [determined by analysis of variance (ANOVA)] from pP_2cat are indicated by an asterisk ($P \leq 0.001$). The RNA start site of the P_2 promoter is indicated as +1.

ferent. However, Cat activity of deletion mutants 3′ −83 through 3′ −230, when compared to the full length P_2 promoter (pP_2cat), indicated that expression from the promoter was completely eliminated by the removal of the TATA and CAAT boxes, such that Cat activity expressed was no different from background as measured by p*cat*. Similarly, deletions beyond the TATA and CAAT boxes showed negligible Cat expression. These data indicated that expression from the mouse P_2 promoter requires a functional TATA box.

5′ Flanking Sequences of the Mouse P_2 Promoter Contain Positive and Negative Regulatory Elements

To identify the transcriptional regulatory sequences within the 5′ flanking region of the mouse P_2 promoter, we progressively deleted promoter sequences from position −870 to −293. The deletion mutants were then cloned upstream of the *cat* reporter gene and the resulting deletion mutants were transfected in secondary rat Schwann cells and the MT_4H1 Schwann cell line. The results showed that the transcriptional activity of some of the deletion mutants was significantly higher (5′ −590 through 5′ −398) in the absence of upstream 5′ flanking sequences; however, there were no significant differences in Cat activity measured in the two cell types (Fig. 4). Specifically, clones that did not contain sequences from −739 to −570 (5′ −739 through 5′ −570) displayed 2–3-fold higher Cat activity than the full length promoter. This region contains a 217 bp TC tract, from position −739 to position −520 (see Fig. 8), and upon complete deletion of this tract (5′ −435), Cat activity increased by 5-fold (Fig. 4). A 1.3-fold drop in promoter activity was observed when only 26 bp downstream of clone 5′ −435 were deleted (−435 to −409), and a further drop when 11 bp were removed from −409 (mutant 5′ −409) to −398 (mutant 5′ −398). This suggests that this 37 bp region contains positive regulatory sequences. When a further 104 bp were deleted downstream of position −389 (mutant 5′ −293), Cat activity was no more than background, although the TATA box and CAAT boxes were intact. This 30-fold reduction in promoter activity demonstrates that strong positive regulatory elements are present in this region. Thus, these data indicate that the 5′ flanking region of the P_2 promoter contains sequences that both negatively and positively regulate the expression of P_2.

Forskolin Treatment Transactivates the Myelin P_2 Promoter

Several myelin genes have been reported to be induced by forskolin treatment, presumably due to increased cAMP levels. To test if the myelin P_2 promoter contains sequences that may be regulated by forskolin and to map the sequences responsible, we transfected pP_2cat and the 3′ and 5′ deletion mutants into secondary rat Schwann cells. Twenty-four hours following transfection, forskolin was added to the medium to a final concentration of 4 μM, and Cat activity was assayed on the following day.

Forskolin treatment was found to significantly activate the P_2 promoter 2.4-fold in secondary Schwann cells, thus demonstrating that the promoter for this myelin gene is responsive to increased cAMP levels. Deletion of the sequences downstream to the TATA box within the 3′ mutants had no effect on the forskolin response (3′ +87 and 3′ +80). As expected, though, the 3′ deletion mutants lacking the TATA box and the CAAT boxes did not show any change in basal Cat expression following forskolin treatment (data not shown). On the other hand, deletion of 5′ sequences (5′ −829 through 5′ −570) showed significant transactivation following the addition of forskolin, until the deletions were extended to position −435 (5′ −435) and beyond (Fig. 5). It is likely that the high basal activity of this construct (5′ −435) may have obscured the forskolin response. However, since deletion mutants 5′ −409 and 5′ −398, which also have a high basal activity, failed to respond to forskolin (Fig. 5), the present results suggest that forskolin responsive elements (FRE) must exist upstream of position −435.

Activation of the P_2 Promoter by the Adenylate Cyclase cAMP α Subunit

Another strategy used was to increase cAMP levels by introducing one of the genes in the cAMP synthetic pathway, the adenylate cyclase cAMP α subunit gene. An expression plasmid for this protein, pCMV-Cα, was cotransfected into secondary Schwann cells with pP_2cat and the 5′ deletion mutants controlling the *cat* reporter gene. These results demonstrated that the effect of pCMV-Cα on the P_2 promoter closely mimicked that of forskolin, both in the fold transactivation and in the specific deletion mutants that were significantly responsive (Fig. 6). Thus, it seems likely that forskolin and the catalytic subunit of the adenylate cyclase are effecting the transactivation of the P_2 promoter by the same pathway, namely through the increase in the level of cAMP, since the promoter sequences responsive to both reagents reside in the same 435 bp region.

Effect of C/EBP on P_2 Promoter Activity

Several reports have shown that increases in intracellular cAMP result in the induction of transcription factors such as the cAMP responsive element binding protein (CREB) (Sheng et al., 1991), the cAMP responsive element modulator (CREM) (Foulkes et al., 1991), and the CAAT/enhancer binding protein (C/EBP) (McKnight et al., 1989). Since the P_2 promoter does not contain any consensus homology to the reported CRE motif (TGACGTCA) (Montminy et al., 1986), we decided to test the possibility that forskolin is acting indirectly via the activation of the transcription factor C/EBP. This factor seemed the most likely mediator since, as mentioned before, P_2 shares an extensive homology with the adipocyte FABP (aP2/422) (Narayanan et al., 1991), which has been shown to be transactivated by C/EBP (Christy et al., 1989).

To test if the expression of the mouse myelin P_2 promoter is modulated by C/EBP, we cotransfected

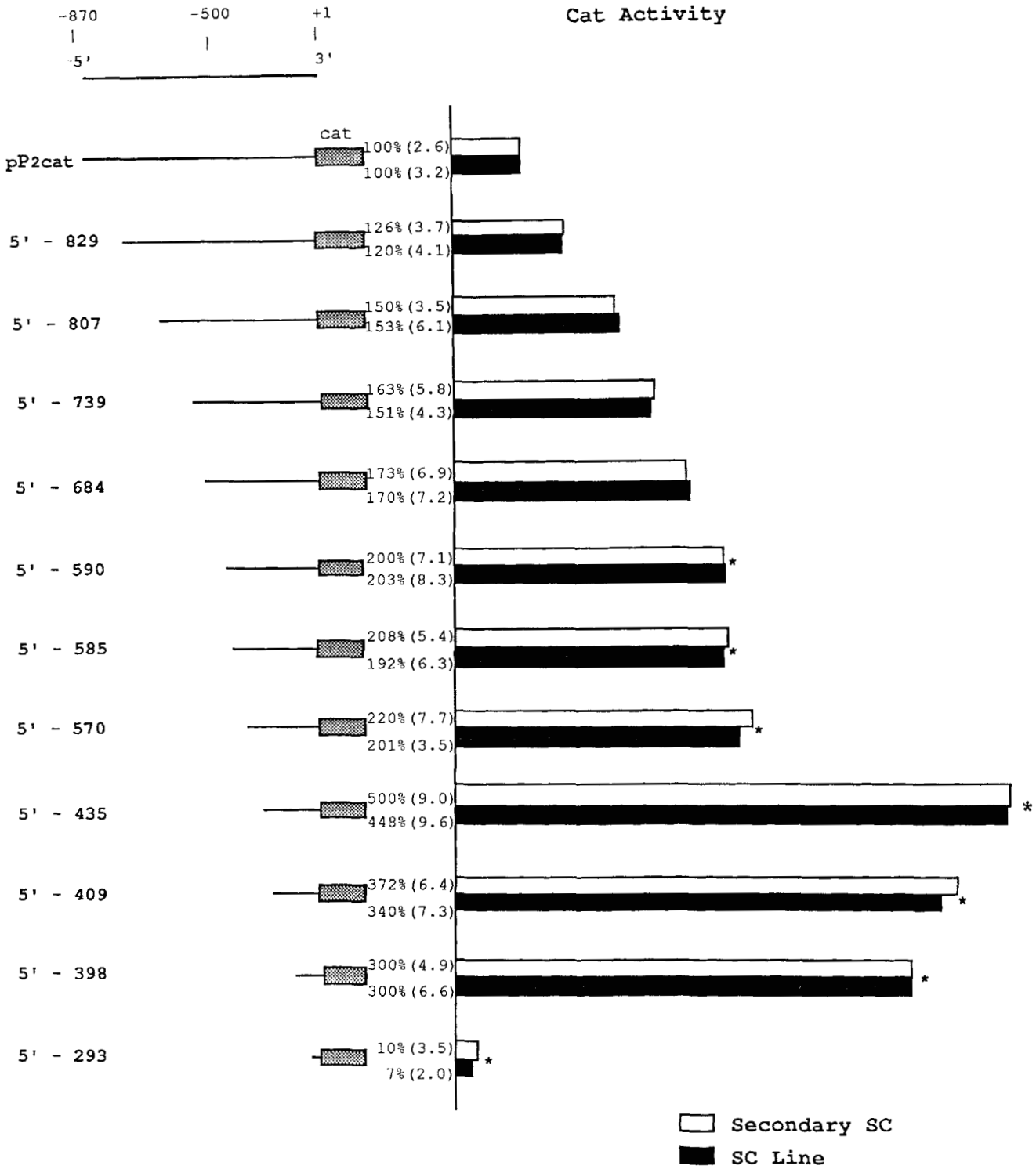


Fig. 4. 5′ deletion analysis of the P_2 promoter region reveals positive and negative regulatory elements. Secondary Schwann cells (open bars) and the MT_4H1 cell line (solid bars) were transiently transfected with 5 μg of pP_2cat and 5′ deletion clones. To account for transfection efficiencies, 2 μg of the β-galactosidase gene driven by the RSV promoter was cotransfected. For each construct, Cat activity was calculated as the percentage of the value obtained for pP_2cat (= 100%), normalized for transfection efficiency by 100 units of β-galactosidase. Results are the average of 5 separate measurements for each construct and the standard error of measurement (± SEM) for each construct is shown in parentheses. Constructs significantly different (determined by ANOVA) from pP_2cat are indicated by an asterisk ($P \leq 0.05$). The RNA start site of the P_2 promoter is indicated as +1.

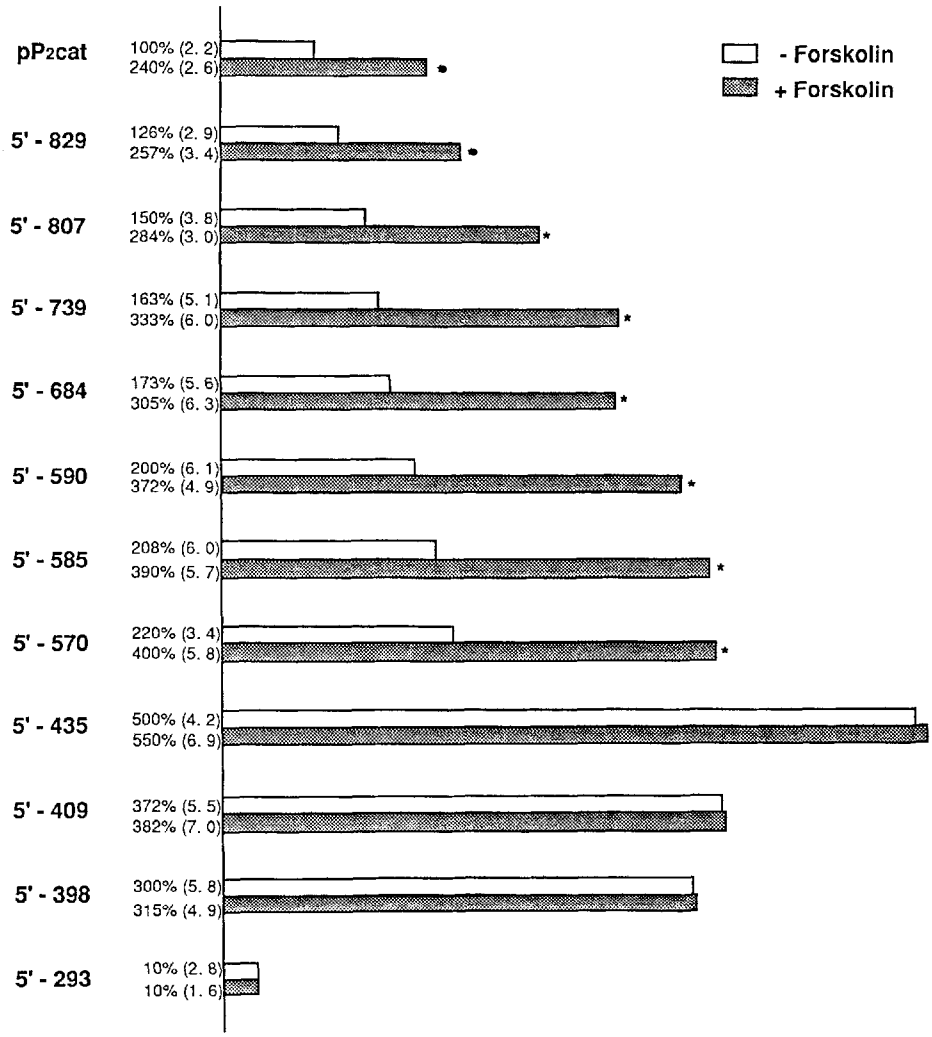


Fig. 5. Deletion analysis of the P_2 promoter region reveals the FRE. Secondary Schwann cells were transiently transfected with 5 μg of pP_2cat and 5′ deletion clones. Twenty-four hours following transfection, forskolin was added to the medium to a final concentration of 4 μM, and Cat activity was determined on the following day. For each construct, Cat activity in the presence (filled bars) and absence (open bars) of forskolin was calculated as indicated in Figure 3. The standard error of measurement (± SEM) for each construct is shown in parentheses. Deletion constructs significantly different (determined by ANOVA) following the addition of forskolin are indicated by an asterisk ($P \leq 0.01$).

pP_2cat along with a C/EBP expression vector, pMSV-C/EBP. Figure 7 shows that there was a 3–4-fold activation of the full length P_2 promoter (pP_2cat) and deletion mutants 5′ −829 through 5′ −570, as assayed by Cat expression. This is comparable to that found with forskolin treatment and cotransfection of pCMV-Cα. Thus, these results clearly indicate that the genetic elements required for transactivation of the P_2 promoter by all three agents reside in the 435 bp upstream of the transcriptional start site.

DISCUSSION

Our primary aim in this study was to identify the sequences of the mouse myelin P_2 promoter that are responsible for the regulated expression of this gene. By using transfection experiments, we were able to show that the isolated 1 kb of the gene is able to drive the *cat* reporter gene in cultured secondary Schwann cells and in a Schwann cell line. By progressively deleting from the 3′ end of the promoter, we were able to show that deletion of the TATA box resulted in complete inactivation of the promoter. These data indicate that the cloned mouse P_2 promoter contains a functional RNA start site and basal responsive elements that control transcription. Systematic deletion of the 5′ regulatory region of the P_2 promoter indicated that there were positive and negative regulatory elements that control the transcriptional activity of P_2.

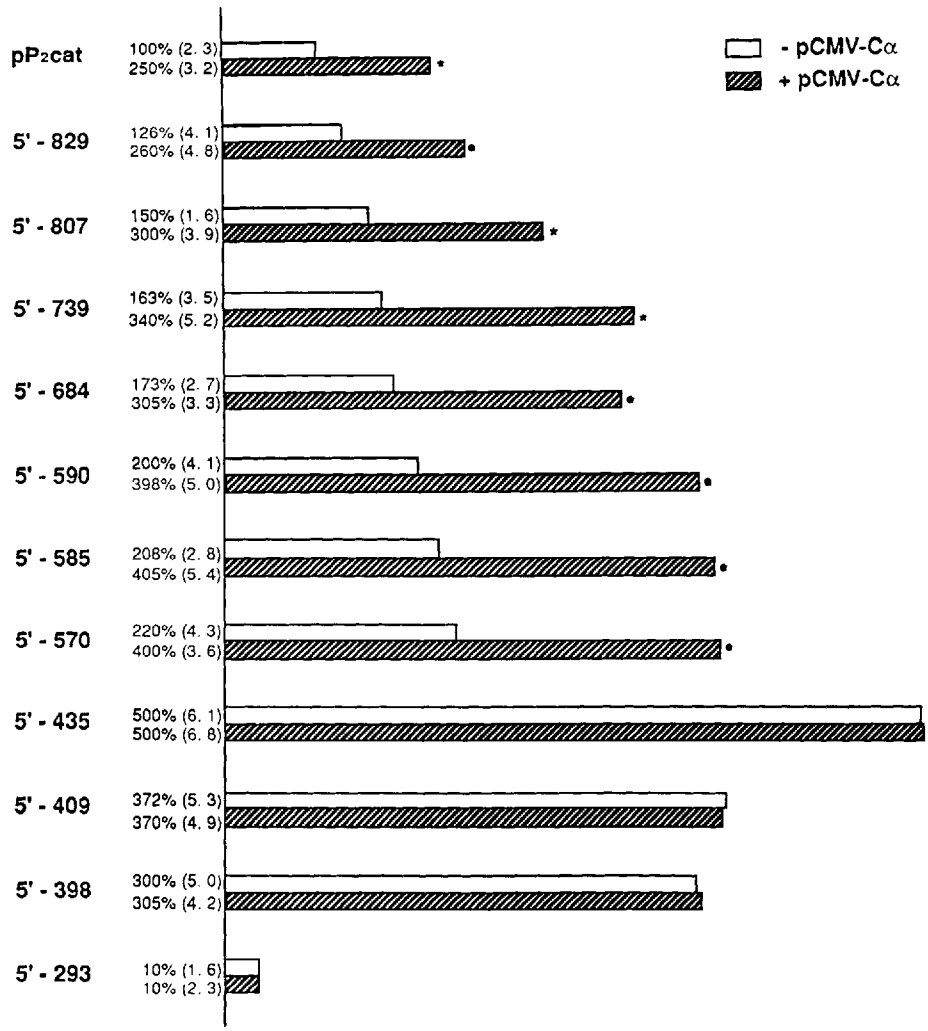


Fig. 6. Activation of the P_2 promoter following cotransfection of the adenylate cyclase cAMP α subunit expression plasmid. Secondary Schwann cells were cotransfected with 5 μg of pP_2cat and 5′ deletion clones along with 2 μg of pCMV-Cα. For each construct, Cat activity in the presence (hatched bars) and absence (open bars) of pCMV-Cα was calculated as indicated in Figure 3. The standard error of measurement (± SEM) for each construct is shown in parentheses. Constructs significantly different (determined by ANOVA) following the cotransfection of pCMV-Cα are indicated by an asterisk ($P \leq 0.01$).

When we progressively deleted the P_2 promoter from position −870 to position −435 (Fig. 4), Cat activity increased by 2–5-fold. As seen in Figure 8, this segment contains a TC tract of 217 bp, which may be responsible for the repression of the P_2 gene. It is very likely that the presence of this TC-rich region may play an important regulatory and evolutionary role in the expression of myelin P_2. Recently, the human P_2 gene has been cloned (Narayanan, unpublished data) and, on examination of the sequences, the TC region was absent. As noted earlier, the amount of P_2 protein expressed in PNS myelin varies among species. A relatively larger amount of this protein is found in humans (14%) than in rodents (1–2%) (Greenfield et al., 1980, 1982), and since the human P_2 lacks a TC-rich region it seems to suggest that such a region may indeed have a repressor function. Negative *cis*-regulatory elements (silencers) have been characterized (Winoto and Baltimore, 1989) and have been postulated to control the rate of gene transcription through repression. This 217 bp TC region does not conform to the reported Z-DNA structure of alternating purine-pyrimidine sequences (dTG dCA), which have been previously shown as negative transcriptional regulators (Naylor and Clark, 1990).

Another observation found upon further 5′ deletion of the P_2 gene was a 142 bp region (position −435 to

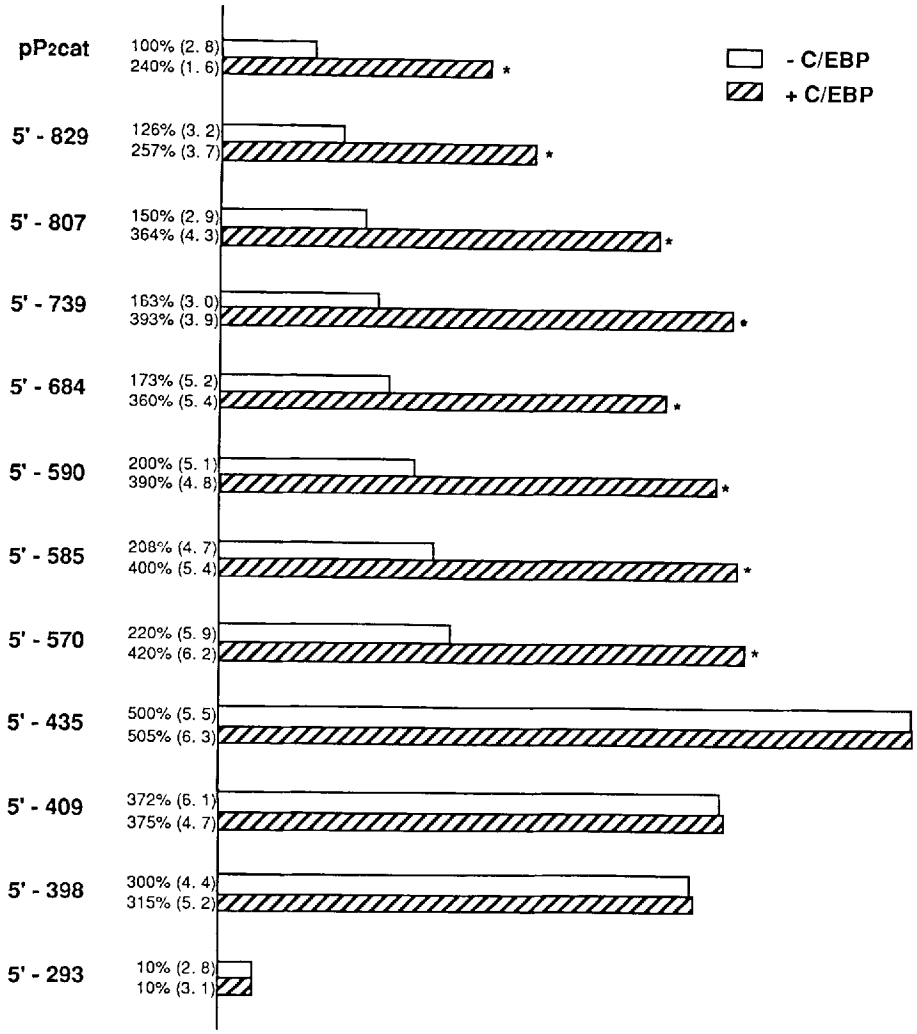
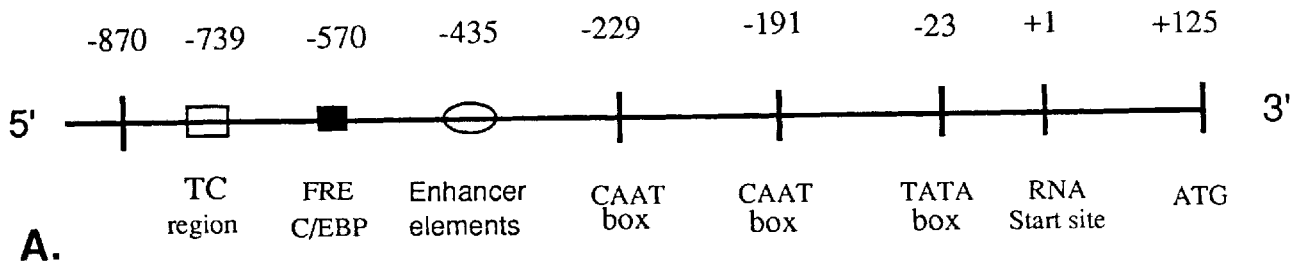


Fig. 7. Activation of the P_2 promoter following cotransfection of a C/EBP expression plasmid. Secondary Schwann cells were cotransfected with 5 μg of pP_2cat and 5′ deletion clones along with 2 μg of pMSV-C/EBP. For each construct, Cat activity in the presence (hatched bars) and absence (open bars) of pMSV-C/EBP was calculated as indicated in Figure 3. The standard error of measurement (± SEM) for each construct is shown in parentheses. Constructs significantly different (determined by ANOVA) following the cotransfection of pMSV-C/EBP are indicated by an asterisk ($P \leq 0.01$).

−293) that seemed to regulate positively P_2 transcription. When this region was deleted, a 30-fold drop in promoter activity was seen, suggestive of an enhancer region. The relatively lower expression of the full length P_2 promoter suggests that the silencer elements may block the activity of the downstream enhancer elements thereby reducing the basal expression. We are currently attempting to determine if this region is a bona fide enhancer and is able to enhance the rate of transcription of heterologous promoters in a position and orientation independent manner.

Several reports have indicated that the adenylyl cyclase-cAMP second messenger pathway may regulate the proliferation and development of Schwann cells (Jessen and Mirsky, 1991; Morgan et al., 1991), and activation of this pathway has also been shown to induce myelin specific genes (Lemke and Chao, 1988; Monuki et al., 1989; Gandelman et al., 1989; Shuman et al., 1988). Addition of forskolin and other cAMP analogues to cultured Schwann cells has been shown to result in differentiation and in activation of P_2, P_0, and MBP mRNA expression (Lemke and Chao, 1988; Monuki et al., 1989). The sequences within the MBP and P_0 promoter do not show any consensus CRE, and the 5′ elements that confer cAMP responsiveness have not been mapped. Although the present study does indicate that the region responsive to forskolin treatment lies 435 bp upstream of the transcriptional start site of the P_2 promoter, it remains



A.

5'TGTGACAACCCTTACCCTACGCCACCCCACCACCCAAAAAGTAACAACCTAGCTAGACAAACATGAAGAGAA
CGCTTAAAACTTGTCATAAAGATGCTCCAATGAGTATATGAACAACACAAACTGGGTTTGTTTCTCCTTCTCC
TTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCT
TCTCCTTCTCCTTCTCCTCCTTTTTCTCTTCTCCTTCTCCTCTTCCTCCTCCTTCTCCTTCTTTTTCTCCTCC
TTTTTCTCCTCCTCCTCCTCTTCTTCCACCTCCTCCTCCTCCTCCTCTTCCTTCTTCTTGAGGGAGGGAGTTG
TAAGGAAGGGAAGCAGACCTGGGAAGACTGAAAGTGAGTGTGATTAGGGTGGAATTATATGAAATTCCCAAat
aacaataaaaattaatgcaaaacaagaactcttaatcgtgctgagtcagaattaaaagtcagtaagatacctа
cacacatgcaaaacagtgtgcgtgtgcacacacatccacactcacacacagacactcagacacacaCACACAC
ACACACACACACACACTCATATAATATATAGAAGGGCTTAAGCATGCCTATcaatGTAAGATGAACCAAAA
ATATCTTAAAGGTTCAAAcaatACTTTCAGTTGTTTTTGTGGTTTTGGAAACACAAGGACATTGTGTAGAGGA
TTATTTCACCCATGGATAGGCAGTGTACGACTTAAGAGCCATTCATCACTCAGCCTGGTCGCCGCCTTTCCTC
ATTGGGTCTCAGGGTGAGTCACATGCTTTGGGTTTCtttgaaaaGAGCTGTGCAAGATCTCAGCATC 3'

B.

Fig. 8. *cis*-Acting elements of the mouse myelin P_2 promoter. **A:** Schematic summary of the basal elements within the 5' flanking region of the mouse P_2 promoter. The 217 bp TC silencer (open box) and the 142 bp enhancer (oval) regions are indicated. The filled box displays the FRE and C/EBP inducive elements. **B:** Nucleotide sequence of the mouse myelin P_2 promoter. Sequences shown are from position −870 to +1 (Narayanan et al., 1991). The transcription start site is indicated at position +1 (block letter "C" underlined). The TATA box (in bold italics underlined) and the two CAAT boxes (italics underlined) are displayed. The 142 positive regulatory region (in lowercase italics) and the negative regulatory 217 bp TC region (in bold block letters) are also indicated. The FRE and C/EBP responsive regions are displayed as the underlined sequences.

unclear whether raising intracellular cAMP levels directly results in the activation of the P_2 promoter. The resulting increase in myelin genes including P_2 could be due to 1) activation of transcription factors such as CREB, CREM, or C/EBP, or, alternatively, 2) repression of factors that negatively regulate myelin genes such as c-Jun and SCIP (suppressed cAMP inducible POU) (Monuki et al., 1989, 1990). Both c-Jun and SCIP have been shown to be lower in myelinating Schwann cells and have been found to be elevated in vivo following nerve transection (Hengerer et al., 1990; Monuki et al., 1990). Our results regarding the role of transcription factors that transactivate the P_2 promoter indicate that C/EBP may play a role in inducing the expression of P_2. Although the elements responsive to forskolin, pCMV-Cα, and C/EBP map within the same 435 bp region upstream of the transcriptional start site, it remains unclear whether the transactivation of P_2 by these three treatments is mediated via the same pathway. Since it is likely that the effects of both forskolin and pCMV-Cα are mediated by increasing the level of C/EBP, we are currently testing whether C/EBP binds directly to the P_2 promoter and thereby regulates its expression.

ACKNOWLEDGMENTS

This work was supported by a grant from NIH (RO1 NS 29710-01).

REFERENCES

Agrawal HC, Hartman BK (1980): Proteolipid protein and other proteins of myelin. In Bradshaw RA, Schneider DM (eds): "Pro-

teins of the Nervous System." New York: Raven Press, pp 145–169.

Campagnoni AT (1988): Molecular biology of myelin proteins from the central nervous system. J Neurochem 51:1–14.

Christy RJ, Yang VW, Ntambi JM, Geiman DE, Landschulz WH, Friedman AD, Nakabeppu Y, Kelly TJ, Lane MD (1989): Differentiation-induced gene expression in 3T3-L1 preadipocytes: CCAAT/enhancer binding protein interacts with and activates the promoter of two adipocyte-specific genes. Genes Dev 3:1323–1335.

deFerra F, Engh H, Hudson L, Kamholz J, Puckett C, Molineaux S, Lazzarini RA (1985): Alternative splicing accounts for the four forms of myelin basic protein. Cell 43:721–727.

Foulkes NS, Borrelli E, Sassone-Corsi P (1991): CREM gene: Use of alternative DNA-binding domains generates multiple antagonists of cAMP-induced transcription. Cell 64:739–749.

Gandelman K-Y, Pfeiffer SE, Carson JH (1989): Cyclic AMP regulation of P_0 glycoprotein and myelin basic protein gene expression in semi-differentiated peripheral neurinoma cell line D6P2T. Development 106:389–398.

Gorman CM, Moffat LF, Howard BH (1982): Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol Cell Biol 2:1044–1051.

Greenfield S, Brostoff S, Eylar EH, Morell P (1973): Protein composition of myelin of the peripheral nervous system. J Neurochem 20:1207–1216.

Greenfield S, Brostoff SW, Hogan EL (1980): Characterization of the basic proteins from rodent peripheral nervous system myelin. J Neurochem 34:453.

Greenfield S, Weise MJ, Gantt G, Hogan EL, Brostoff S (1982): Basic proteins of rodent peripheral nerve myelin: Immunochemical identification of the 21.5K, 18.5K, 17K, 14K, and P2 proteins. J Neurochem 39:1278–1282.

Hengerer B, Lindholm D, Heumann R, Ruther U, Wagner EF, Thoenen H (1990): Lesion-induced increase in nerve growth factor mRNA is mediated by *c-fos*. Proc Natl Acad Sci USA 87: 3899–3903.

Jessen KR, Mirsky R (1991): Schwann cell precursors and their development. Glia 4:185–194.

Jones TA, Bergfors T, Sedzik J, Unge T (1988): The three-dimensional structure of P_2 myelin protein. EMBO J 7:1597–1604.

Lemke G (1988): Unwrapping the genes of myelin. Neuron 1:535–543.

Lemke G, Axel R (1985): Isolation and sequence of a cDNA encoding the major structural protein of peripheral myelin. Cell 40:501–508.

Lemke G, Chao M (1988): Axons regulate Schwann cell expression of the major myelin and NGF receptor genes. Development 102: 499–504.

Lemke G, Lamar E, Patterson J (1988): Isolation and analysis of the gene encoding peripheral myelin protein zero. Neuron 1:73–83.

Macklin WB, Campagnoni CW, Deininger PL, Gardinier MV (1987): Structure and expression of the mouse myelin proteolipid protein gene. J Neurosci Res 18:383–394.

McKnight SL, Lane MD, Gluecksohn-Waelsch S (1989): Is CCAAT/enhancer-binding protein a central regulator of energy metabolism? Genes Dev 3:2021–2024.

Mikoshiba K, Okano H, Tamura T, Ikenaka K (1991): Structure and function of myelin protein genes. Annu Rev Neurosci 14:201–207.

Miller JH (1972): "Experiments in Molecular Genetics." Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Milner RJ, Sutcliffe JG (1983): Gene expression in rat brain. Nucleic Acids Res 11:5497–5520.

Montminy MR, Sevarino KA, Wagner JA, Mandel G, Goodman RH (1986): Identification of a cyclic-AMP-responsive element within the rat somatostatin gene. Proc Natl Acad Sci USA 83:6682–6686.

Monuki ES, Weinmaster G, Kuhn R, Lemke G (1989): SCIP: A glial POU domain gene regulated by cyclic AMP. Neuron 3:783.

Monuki ES, Kuhn R, Weinmaster G, Trapp BD, Lemke G (1990): Expression and activity of the POU transcription factor SCIP. Science 249:1300–1303.

Morgan L, Jesson KR, Mirsky R (1991): The effects of cAMP on differentiation of cultured Schwann cells: Progression from an early phenotype (04^+) to myelin phenotype (P_0^+ $GFAP^-$, $N\text{-}CAM^-$, $NGF\text{-}receptor^-$) depends on growth inhibition. J Cell Biol 112:457–467.

Narayanan V, Barbosa E, Randall R, Tennekoon GI (1988): Characterization of a cloned cDNA encoding rabbit myelin P_2 protein. J Biol Chem 263:8332–8337.

Narayanan V, Kaestner KH, Tennekoon GI (1991): Structure of the mouse myelin P_2 protein gene. J Neurochem 57:75–80.

Nave KA, Lai C, Bloom FE, Milner RJ (1987): Splice site selection in the gene for myelin proteolipid protein (PLP) and primary structure of the DM-20 protein of CNS myelin. Proc Natl Acad Sci USA 84:5665–5669.

Nayor LH, Clark EM (1990): $d(TG)_n \cdot d(CA)_n$ sequences upstream of the rat prolactin gene form Z-DNA and inhibit gene transcription. Nucleic Acids Res 18:1595–1601.

Omlin FX, Webster H, deFerra F, Palkovits CG, Cohen SR (1982): Immunocytochemical localization of basic protein in major dense line regions of central and peripheral myelin. J Cell Biol 95:242–248.

Peden KWC, Charles C, Sanders L, Tennekoon GI (1989): Isolation of rat Schwann cell lines: Use of SV40 T antigen gene regulated by synthetic metallothionein promoters. Exp Cell Res 185:60–72.

Sanger F, Nicklen S, Coulson AR (1977): DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467.

Sheng M, Thompson MA, Greenberg ME (1991): CREB: A Ca^{2+}-regulated transcription factor phosphorylated by calmodulin-dependent kinases. Science 252:1427–1430.

Shuman S, Hardy M, Sobue G, Pleasure D (1988): A cyclic AMP analogue induces synthesis of a myelin specific glycoprotein by cultured Schwann cells. J Neurochem 50:190–194.

Takahashi N, Roach A, Teplow DB, Prusiner SB, Hood L (1985): Cloning and characterization of the myelin basic protein gene from mouse: One gene can encode both 14 kd and 18.5 kd MBPs by alternate use of exons. Cell 42:139–148.

Trapp BD, Dubois-Dalcq M, Quarles RH (1984): Ultrastructural localization of P_2 protein in actively myelinating Schwann cells. J Neurochem 43:944–948.

Winoto A, Baltimore D (1989): Alpha-beta lineage specific expression of the alpha T cell receptor gene by nearby silencers. Cell 59:649–665.