Direct Regulation of Myelin Protein Zero Expression by the Egr2 Transactivator*

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During myelination of the peripheral nervous system, the myelin protein zero (Mpz) gene is induced to produce the most abundant protein component (P₀) of mature myelin. Although the basal embryonic expression of Mpz in Schwann cells has been attributed to regulation by Sox10, the molecular mechanism for the profound up-regulation of this gene during myelination has not been established. In this study, we have identified a highly conserved element within the first intron of the Mpz gene, which contains binding sites for the early growth response factor 2 (Egr2/Krox20) transcription factor, a critical regulator of peripheral nerve myelination. Egr2 can trans-activate the intron element, and the induction is blocked by two known repressors of Egr2 activity. Using chromatin immunoprecipitation assays, we find that Egr2 binds in vivo to the intron element, but not to the Mpz promoter. Known inducers of Mpz expression such as forskolin and insulin-like growth factor-1 also activate the element in an Egr2-dependent manner. In addition, we found that Egr2 can act synergistically with Sox10 to activate this intron element, suggesting a model in which cooperative interactions between Egr2 and Sox10 mediate a large increase in Mpz expression to the high levels found in myelinating Schwann cells.

The myelin sheath is a lipid rich, multilamellar structure that is essential for normal nerve development as well as rapid conduction of nerve impulses. Perturbations of peripheral nerve myelination by Schwann cells can cause debilitating neuropathies such as Charcot-Marie-Tooth disease and Dejerine-Sottas syndrome (1, 2). Generation of myelin involves the coordinate synthesis of many membrane proteins, some of which are expressed primarily in Schwann cells. The most abundant of these proteins is the P₀ glycoprotein, encoded by the Myelin protein zero (Mpz) gene, which accounts for nearly 50% of the protein content of peripheral myelin (3), and is necessary for the formation of compact myelin (4). In addition, MPZ mutations have been identified in a significant number of peripheral neuropathies (2, 5), confirming its central role in peripheral nerve myelination.

Initial studies of Mpz expression suggested that it was exclusively expressed in myelinating Schwann cells, but subsequent analysis revealed that Mpz is also expressed during embryonic development of Schwann cells from the neural crest, albeit at a much lower basal level (6–8). The Sox10 transcription factor is required for the basal embryonic expression of Mpz, and indeed Sox10 binding sites have been localized in the Mpz promoter (9, 10). However, the mechanism by which axonal contact initiates the massive increase of Mpz expression at the onset of myelination has not been elucidated.

Regulation of Mpz expression has been attributed to a variety of transcriptional regulators including not only Sox10 but also Pax3, and ZBP99, Sp1, and NF-Y, which interact with the proximal 1.1-kb promoter of Mpz (9–12). However, several lines of evidence indicate that the zinc finger transcription factor, early growth response (Egr2)/Krox20, plays a central role in induction of Mpz during myelination. First, Egr2 is induced in Schwann cells at the onset of myelination (13, 14), and the expression profiles of Egr2 and Mpz are closely matched during myelination and nerve injury (15–17). Second, transgenic mice bearing a targeted disruption of Egr2 show arrested peripheral nerve myelination (13, 18), and a markedly reduced expression of Mpz and other myelin genes. Finally, ectopic expression of Egr2 in Schwann cell cultures induces the endogenous Mpz gene (as well as other myelin-associated genes) in the absence of axonal contact (19, 20).

Despite this evidence, little is known regarding the mechanism by which Mpz is induced during myelination. Some transfection studies have shown modest inductions of Mpz promoter activity as a result of Egr2 expression (10, 12, 19), but other reports have seen no measurable effect of Egr2 on the Mpz promoter (9). Interestingly, Egr2 has been shown to cooperatively activate the Mpz promoter along with the Sox10 transcription factor (10), but the cooperative activation was not observed in other experiments (9). Based on these data and the lack of demonstrable Egr2 binding to the promoter (11), it was proposed that any activation of the Mpz promoter by Egr2 is indirect (9, 12).

A significant limitation of the transfection studies is their exclusive focus on the Mpz promoter region. Transgenic experiments using 1.1 kb of the Mpz promoter have displayed low levels of Schwann cell-specific expression (21–23). However, subsequent experiments utilizing a 20-kb fragment containing 6 kb of flanking region along with the complete Mpz gene have been successful in driving significant lacZ expression (23), and epitope-tagged P₀ levels similar to that of endogenous Mpz. Because constructs containing as much as 9 kb of upstream sequence are inadequate for this response (23), these data indicate that elements outside of the promoter region are required for robust expression of Mpz during myelination.

Our studies investigating the mechanism of Mpz activation have identified a highly conserved region within the first intron of Mpz and several experiments indicate that this element is directly regulated by Egr2 during peripheral nerve myelination.
Regulation of Myelin Protein Zero by Egr2

EXPERIMENTAL PROCEDURES

Transgenic Mice—All experiments on mice were performed in strict accordance with experimental protocols approved by the Institutional Animal Care and Use Committee, San Raffaele Scientific Institute and the Italian Ministry of Health, or the University of Wisconsin School of Veterinary Medicine. The Egr2/Krox20 null (13) allele (Krox20-null mice were the gift of Dr. Giovanni Levi, Genova, Italy) was maintained on the DBA2/B6 genetic background to promote survival of Egr2/Krox20-null animals. Genotypes were determined by PCR analysis of genomic DNA prepared from tail samples as described (17).

Quantitative Reverse Transcriptase-PCR Analysis—For Egr2/Krox20 wild type, heterozygous and homozygous-null P7 littermates, total RNA was prepared from pools of 20 sciatic nerves by the cesium gradient method (24). One μg of total RNA from each sample was used to prepare cDNA essentially as described (25). Quantitative reverse transcriptase-PCR was performed by monitoring in real-time the increase in fluorescence of the SYBR GREEN dye as described (26) using the Taqman 7000 Sequence Detection System (Applied Biosystems). Relative amounts of each gene between samples were determined using the Comparative Ct method (27) and normalized to the relative levels of 18 S rRNA. Primer sequences are available upon request.

Intron and Promoter Analysis—The PGL3-Mpz-Int plasmid was generated by cloning a 762-bp SacI-Nhel fragment of the mouse Mpz gene, which corresponds to positions 984 to 1749 downstream of the transcription start site, into the pGL3 vector (Promega, Madison, WI) containing the adenosine E1B TATA element. The pGL3-Mpz—1118/ +1749 was generated by deletion and religation of the SacI-EcoRI fragment; the +984/+1500 corresponds to a BstII-Nhel deletion and religation; and +1118—1500 refers to a SacI/EcoRI deletion and religation of the +984/+1500 construct. Site-directed mutagenesis of the full-length +984/+1749 plasmid was performed by the Megaprim method (28) to alter the indicated Egr2 sites to guanine at positions 2 and 8 of the G-rich strand, and these specific mutations have been previously reported to abrogate Egr2 DNA binding (29). The pGL3-Mpz promoter (−1275/+60) luciferase reporter was generated by subcloning a SacI-HindIII of the 1.3-kb Mpz luciferase reporter described previously (19) into the pGL3 vector. Int+Pro was constructed as a SacI/Nhel insertion upstream of the pGL3-Mpz-PRO vector. Sequence analysis for potential binding sites within the Mpz gene was performed using the rVISTA program (30), and identified Egr2 sites were confirmed by comparison to the previously defined consensus Egr2 binding site (29). The expression construct for Egr2 has been described previously (31). The Sox10 expression construct (provided by Dr. Robin Miskimins) was previously described (32).

Cell Culture Conditions and Transient Transfection Assays—The S16 rat Schwann cell line (33) was maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% bovine growth serum (Hyclone). HeLa cells were maintained in minimum essential medium supplemented with 10% fetal bovine serum. Primary rat Schwann cells were cultured as described (17). Transfection assays were performed with LT-1 transfection reagent (Mirus) according to the manufacturer’s protocol, using 250 ng of the luciferase reporter, 100 ng of cytomegalovirus-driven lacZ reporter, and the indicated amounts of the expression plasmids. Bluescript plasmid (Stratagene, La Jolla, CA) was added as required to make a total of 1 μg of DNA per transfection. The transfected cells were cultured 48 h before harvesting to measure luciferase activity. The average luciferase activity of duplicate samples was normalized to the β-galactosidase activity from the transfected lacZ reporter. For the Schwann cell stimulations, the cells were washed once in serum-free medium and cultured in N2 medium for 24 h. The cells were treated as specified with 2 μM forskolin (Sigma) and 150 ng/ml insulin-like growth factor-1 (MP Biomedical) for 24 h. For expression of Ddx20, primary rat Schwann cells were infected as described (19) using a recombinant adenovirus prepared using the AdEasy system (34) to express the minimal fragment of Ddx20 (amino acids 612—825) required for repression of Egr2 (35). Schwann cells were infected in N2 medium for 24 h, and then exchanged with N2 medium containing 2 μM forskolin and 150 ng/ml IGF-1 for another 24 h.

RESULTS

Egr2 has been previously shown to up-regulate endogenous Mpz expression in Schwann cells (19, 20), and Egr2-deficient mice fail to up-regulate Mpz (13, 18). However, the failure to identify Egr2 binding sites in the Mpz promoter has suggested that Egr2 activation of Mpz is indirectly mediated by other factors (9, 11, 12). The regulation of Mpz has also been previously associated with a number of transcriptional regulators including Sox10, Pax3, ZBP-99, Sp1, and NF-Y (9—12). To test their potential involvement in the induction of Mpz, we evaluated whether some of these factors were altered in sciatic nerves in the absence of Egr2. Whereas mRNA expression of Mpz was reduced 50-fold in Egr2-deficient mice compared with wild type littermates at postnatal day 7 (Table 1), mRNA levels of Mpz activators, Sox10 and ZBP-99, were relatively unchanged in the absence of Egr2. Because Pax3 is a repressor of MPZ expression (10), the 60% reduction in the Egr2...
Regulation of Myelin Protein Zero by Egr2

knock-out also does not account for lower Mpz levels. Furthermore, none of these transcriptional regulators are coregulated with either Mpz or Egr2 in expression profiling studies of peripheral nerve injury and development (15, 16). Therefore, we conclude that regulation of Mpz by Egr2 is not mediated by these trans-acting factors.

Transgenic experiments have demonstrated that as much as 9 kb of the promoter region of the Mpz gene is not sufficient to drive high level expression (23), and have suggested that the other elements of the Mpz locus are required for the massive up-regulation normally observed. To identify potential regulatory elements within the Mpz gene, we compared the genomic sequences of the murine and human Mpz loci for highly conserved noncoding sequences, and identified a 400-bp region located within the first intron that is 76% conserved between mouse and human (Fig. 1A).

The Mpz Intron Element Activates Transcription in an Egr2-dependent Manner—A segment of the first Mpz intron (+984/+1749) containing the conserved region was cloned upstream of a luciferase reporter gene with a minimal promoter (Int +984/+1749). To test whether this reporter would respond to Egr2, primary rat Schwann cells were transfected with the reporter and increasing concentrations of an Egr2 expression plasmid. Egr2 induces this reporter construct in a concentration-dependent manner. At the maximal levels of expression plasmid used, luciferase activity was induced 20-fold over its basal activity by Egr2 in rat Schwann cells (Fig. 1B). A construct containing the minimal promoter alone shows no induction in response to Egr2 (not shown).

In contrast, a reporter construct containing 1.3 kb of the mouse Mpz promoter (Pro −1275/+60) showed a maximal activation of only 4.2-fold in response to Egr2, consistent with previous results (10, 12, 19). As we defined an Egr2-responsive element within the first intron of Mpz, we next wanted to probe whether Egr2 could cooperate with elements located in the proximal promoter. To test this, we inserted the intron element upstream of the Mpz promoter. Fusion of the 1.3-kb promoter to the first intron fragment augments Egr2-mediated activation to 23-fold in Schwann cells (Fig. 1B). Therefore, addition of the Mpz intron element is sufficient to confer Egr2 responsiveness on the Mpz promoter.

To map the Mpz control element in intron 1, several deletion constructs were tested for their response to Egr2. In Schwann cells, deletion of bases +984/+1118 or +1500/+1749 had no substantial effect on transactivation by Egr2 (Fig. 2). Deletion of +984/+1500 reduced the Egr2-mediated activation of this reporter to basal levels. A fragment containing +1118/+1500 retained most of the activation observed in the full-length construct. These results suggest that the conserved region from +1118/+1500 contains the Egr2-responsive elements.

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**TABLE 1**

Quantitation of Mpz expression and putative regulators in Egr2 knock-out mice

| Egr2 (+/+) | Egr2 (+/−) | Egr2 (−/−) |
|------------|------------|------------|
| MPZ        | 1.00 ± 0.01| 0.60 ± 0.05| 0.02 ± 0.001|
| Sox10      | 1.00 ± 0.02| 1.01 ± 0.08| 1.11 ± 0.121|
| Pax3       | 1.00 ± 0.08| 0.38 ± 0.08| 0.40 ± 0.122|
| ZBP-99     | 1.00 ± 0.02| 0.63 ± 0.02| 0.77 ± 0.103|

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**FIGURE 1.** Identification of a conserved region within the Mpz first intron that is activated by Egr2. A, the plot shows percent identity of the mouse and human Mpz loci. The six exons of the Mpz gene as well as −4 kb of upstream sequence are shown. B, rat Schwann cells were cotransfected with luciferase reporter constructs containing the conserved region of the Mpz first intron (+984/+1749), the 1.3-kb Mpz promoter, or a fusion of the Mpz promoter and intron along with the indicated amounts of an Egr2 expression vector. -Fold induction is calculated relative to the luciferase activity of the reporter alone. Mean ± S.E. represent three independent experiments.
Regulation of Myelin Protein Zero by Egr2

This corresponds to the most highly conserved portion of the first intron of the \textit{Mpz} gene.

\textit{Egr2 Binds to the First Intron of Mpz in Vivo}—To determine whether Egr2 specifically binds to this region of \textit{Mpz} in vivo, we carried out a chromatin immunoprecipitation assay (ChIP), which employs formaldehyde to covalently cross-link DNA with associated proteins. We first employed this assay with the S16 Schwann cell line, which exhibits high levels of \textit{Mpz} expression comparable with those found in myelinating Schwann cells (36). After sonication, cross-linked chromatin was immunoprecipitated with either an anti-Egr2 or an IgG control antibody. Following washing and reversal of cross-links, primer pairs located along the \textit{Mpz} locus were used to quantify the ChIP-enriched DNA by quantitative PCR. Significant Egr2 binding was detected at the first intron (IN1) with 7-fold enrichment in the S16 cell line compared with the control immunoprecipitate. A modest <2-fold enrichment was observed in the other locations tested, including the \textit{Mpz} promoter (Fig. 3A), suggesting that Egr2 interacts predominantly with the intron region.

To test whether Egr2 also bound preferentially to the \textit{Mpz} intron 1 during myelination, we adapted the ChIP assay to detect binding of Egr2 in \textit{vivo}. Freshly harvested rat sciatic nerves at postnatal day 9 were homogenized and incubated in 1% formaldehyde and then processed as described above. P9 was chosen because \textit{Mpz} is highly induced by this time point (37, 38). We observed similar results with the sciatic nerve as the S16 cell line, i.e. an enrichment of Egr2 binding at the \textit{Mpz} first intron (Fig. 3B). The VISTA homology search did identify other potential Egr2 binding sites in the \textit{Mpz} locus, and in fact, several of the primer sets used in our ChIP analysis were positioned over these potential sites (IN2 and PRO). However, the \textit{in vivo} ChIP assay did not detect significant Egr2 binding in these regions. These data indicate that Egr2 directly regulates the \textit{Mpz} gene, and that the conserved region of the \textit{Mpz} first intron is a primary target for Egr2-mediated regulation.
intron is the primary target of Egr2 binding in myelinating Schwann cells.

The resolution of the ChIP assay does not permit precise localization of specific binding sites within the first intron of Mpz. Therefore, a regulatory VISTA analysis, which identifies conserved transcription factor binding sites in aligned genomic sequence (30), was used to search the Egr2-responsive fragment from 1186 to 1336. By mutating the luciferase reporter constructs for the Mpz intron fragment (+984/+1749) were generated in the three Egr2 binding sites. Rat Schwann cells were transiently transfected with these mutant luciferase reporter constructs in the presence of 50 ng of Egr2 expression plasmid. Fold induction by Egr2 was calculated relative to the level of the wild type luciferase reporter alone. Mean ± S.E. represent three independent assays.

The Mpz First Intron Responds to Inducers of Mpz Expression in an Egr2-dependent Manner—Endogenous Mpz levels in Schwann cells are highly induced by pharmacological agents that raise cAMP levels, such as forskolin (39, 40), and the induction is further potentiated by IGF-I (41–43). To determine whether the intron element mediates the response to these inducers of Mpz expression, Schwann cells were transfected with several reporters and treated with forskolin and IGF-I. The intron-containing reporter was induced 2.3-fold in response to forskolin, and 4-fold in response to forskolin and IGF-I (Fig. 5, top). As a control, a construct containing the TATA element alone showed no significant response to the stimulations. As previously reported (44–46), the Mpz promoter was also modestly induced in response to these agents (Fig. 5, bottom). We next wanted to test whether combining the intron and promoter would augment the response to these inductive signals. Therefore, we used the luciferase construct containing the intron element with both the wild type and mutant Egr2 binding sites fused upstream of the Mpz promoter. The conserved region when inserted upstream of the Mpz promoter confers a 6-fold increase in forskolin/IGF-I-induced transcription from the Mpz promoter (Fig. 5, bottom). To assess whether the induction of this reporter was dependent on Egr2, we treated the reporter containing the mutated binding sites and observed a response similar to the Mpz promoter alone (Fig. 5, bottom). These results indicate that activation by forskolin/IGF-I through the conserved Mpz intron element is Egr2-dependent.

**Ddx20 and Nab Proteins Inhibit Egr2-mediated Activation of the Mpz First Intron**—We also tested whether the Mpz intron element could be directly modulated by known corepressors of Egr2. Therefore, we utilized two proteins that have been previously shown to repress the activity of Egr2: Ddx20 (35) and Nab2 (47). Egr2 activated the intron luciferase reporter (+984/+1749) 25-fold, and Nab2 repressed this induction in a concentration-dependent manner (Fig. 6A). We also used a neuropathy-associated Egr2 mutant (I268N) that was previously shown to prevent association with Nab proteins (48). This mutant is more active than wild type Egr2 and was no longer responsive to Nab2 repression (Fig. 6A).

The Ddx20 corepressor also repressed Egr2 activation of the intron element in a dose-dependent manner (Fig. 6B). We next tested whether the presence of Ddx20 would block the endogenous activation of Mpz in response to forskolin and IGF-I signaling. Schwann cells were infected with an adenovirus expressing Ddx20 or GFP, and stimulated with forskolin and IGF-I. Treatment with a GFP control vector had no effect on the induced Mpz levels (Fig. 6C). However, addition of increasing amounts of Ddx20 significantly diminished the endogenous up-regulation of Mpz (Fig. 6C), consistent with the results from the luciferase reporter assay studies showing Egr2-dependent effects on Mpz induction in response to these agents (Fig. 5B).

**FIGURE 4.** Identification of Egr2-responsive sites within the first intron of the Mpz gene. A, alignment of conserved Egr2 binding sites (boxed in gray) in mouse, human, and rat sequences from Mpz +1186 to +1336. B, mutations in the luciferase reporter constructs for the Mpz intron fragment (+984/+1749) were generated in the three Egr2 binding sites. Rat Schwann cells were transiently transfected with these mutant luciferase reporter constructs in the presence of 50 ng of Egr2 expression plasmid. Fold induction by Egr2 was calculated relative to the level of the wild type luciferase reporter alone. Mean ± S.E. represent three independent assays.

**FIGURE 5.** The first intron enhances the Mpz promoter in response to myelination agents. Rat Schwann cells were transiently transfected with: top, a minimal TATA or the Mpz intron (+984/+1749) fused to luciferase, or bottom, the Mpz promoter or a fusion of the intron upstream of the promoter containing either wild type or mutated Egr2 binding sites. At 24 h after transfection, cells were treated with either vehicle (Me2SO) or the specified agents and incubated another 24 h prior to harvest. Results are expressed as relative light units to reflect differences in basal activity of the reporters. Mean ± S.E. represent two independent experiments.
none of the other known activators were significantly deregulated in the Egr2-deficient mice, whereas Mpz levels were reduced 50-fold, we explored whether Egr2 played a direct role in regulating Mpz expression. By comparing the sequence of the Mpz locus in rat, mouse, and human genomes, we identified a highly conserved fragment of the first intron that contains functional Egr2 binding sites and is highly responsive to Egr2.

The Egr2-responsive element within the first intron reconciles a number of contradictory findings with regard to regulation of Mpz. First and foremost, Mpz is one of the most highly regulated genes in both gain of function and loss of function experiments involving Egr2 (13, 18, 19). However, attempts to induce the Mpz promoter with Egr2 have yielded inconsistent results and even studies showing a modest induction have not identified Egr2 binding sites within the promoter (9–12). Our data indicate the primary Egr2-responsive element of the Mpz gene resides within the first intron. In addition, the localization of this element in the first intron provides an explanation for previous transgenic studies suggesting that elements necessary for strong expression reside outside of the Mpz promoter (23).

Overall, our results suggest a model to account for the large induction of Mpz expression during peripheral nerve myelination. Basal embryonic expression of Mpz is regulated by Sox10, which can bind to sites residing within the promoter region (9, 10) as well as the conserved element within the first intron (Fig. 7). Upon induction by axon-derived signals, induction of Egr2 strongly up-regulates this gene during myelination by direct binding to conserved sites within the intron element. These data suggest that Egr2 binding in the intron likely cooperates with Sox10 (or other factors) bound to the promoter and conserved intron element to robustly activate Mpz transcription. Furthermore, this mechanism is probably involved in not only induction but also mainte-

FIGURE 7. Egr2 and Sox10 synergistically activate the conserved intron element of Mpz. A, alignment of Sox10 binding sites from +1220/+1236 and diagram of the Mpz intron indicating the position of the Sox10 sites relative to the Egr2 binding sites. B, HeLa cells were transiently cotransfected with the Mpz intron luciferase reporter (+984/+1749) along with 50 ng of an expression vector for Egr2 and 100 ng of Sox10. -Fold induction is calculated relative to the luciferase activity of the reporter alone. Mean ± S.E. represent two independent assays.
nance of Mpz expression in myelinating Schwann cells, because both EGR2 and Sox10 persist into adulthood (14, 15, 32).

To localize Egr2 binding within the Mpz locus, we used ChIP assays performed on both S16 cells and on rat sciatic nerve. With this technique, we show that Mpz is a direct target gene of Egr2 through its binding in the first intron at the peak period of Mpz expression. These data provide the first in vivo evidence of direct binding of Egr2 to a myelin-associated target gene. Similar ChIP assays performed on sciatic nerve will be a useful technique to assess the roles of not only Egr2 but also other transcription factors as well as chromatin modifications during peripheral nerve myelination. Although we do not observe significant binding of Egr2 to the Mpz promoter, binding of Egr2 to the promoter cannot be completely excluded by our data. However, previous DNase I footprinting as well as modest Egr2 activation of luciferase reporter constructs have suggested that the activation of Mpz expression by Egr2 is indirect (9, 11, 12, 52). Interestingly, it was reported that the Mpz promoter is activated by Egr2 in a melanoma cell line, and the Egr2 response was diminished by mutations in Sox10 binding sites (10). Therefore, one possible explanation for all of these results is that the modest Egr2 response of the Mpz promoter is mediated by protein-protein interactions with Sox10. Such interactions may not be efficiently cross-linked by formaldehyde to the Mpz promoter and therefore would not be detected in the ChIP assay.

One interesting finding was the 60% reduction of Pax3 levels in the Egr2 knock-out, suggesting a potential interaction between these two regulators of myelination. Because Pax3 is only expressed in nonmyelinating Schwann cells (53), it is not coexpressed with Egr2 in mature myelinating Schwann cells, although Pax3 and Egr2 may be transiently coexpressed at the onset of myelination (54). Interestingly, Pax3 represses expression of Mbp and Mpz in cultured cells (10, 53). Given these observations, induction of Mpz could theoretically involve active repression of Pax3 expression by Egr2, but we did not find evidence for such a mechanism (e.g., increased expression of Pax3 in the Egr2 knock-out). Pax3 mutant mice exhibit early neural crest defects and embryonic lethality (55). Therefore, characterizing the role of Pax3 in Mpz expression at the onset of myelination will require conditional inactivation of Pax3.

Our results show that the Mpz intron element responds to forskolin/IGF-I signals that act as inducers of Mpz expression (39–41, 43). In addition, the coupling of the conserved portion of the intron with the promoter acts to amplify the response to these stimulants of myelination in culture. One interesting finding is that forskolin induction of the Mpz promoter alone is not further enhanced by IGF-I (Fig. 5). In contrast, the intron element mimics endogenous Mpz expression (41, 43) in that its response to forskolin is further potentiated by addition of IGF-I. Importantly, this enhancement was blocked by mutation of Egr2 binding sites and expression of the Ddx20 corepressor, suggesting that the induction is Egr2-dependent. IGF-I primarily activates the phosphatidylinositol 3-kinase pathway, and recent work has shown that neuregulin-mediated survival of Schwann cells and stimulation of myelination initiation requires induction of phosphatidylinositol 3-kinase activity (43, 56–58). IGF-I also activates other signaling pathways (42, 43), and the specific signaling cascades leading to the activation of Mpz during myelination have yet to be defined. However, our data argue that the potentiation effect of IGF-I on Mpz expression is mediated by Egr2 binding within the intron element.

Recently published work has shown that double knock-out of the NAB1 and NAB2 corepressors prevents peripheral nerve myelination, similar to the phenotype exhibited by disruption of Egr2/Krox20 (59). One surprising finding was that many Egr2 target genes, including Mpz, exhibit reduced expression rather than the elevated expression that would be expected from lack of repression. These results would support a proposed model (60) that NAB proteins are required to act as coactivators of at least some Egr2 target genes. Indeed, transfection experiments have indicated that NAB proteins can potentiate activation of two Egr target promoters (Fas ligand and luteinizing hormone B, Ref. 31). However, there was also up-regulation of some genes in the double NAB knock-out (59), suggesting an alternative possibility that NAB corepressors must repress certain Egr2 target genes for myelination to occur. We tested the newly discovered element in the Mpz intron, and the results do not support NAB coactivation of Mpz expression through this element.

The identification of an Egr2-responsive element in the first intron of Mpz suggests a potential model for amplification of myelination signals not only for Mpz, but other myelin proteins as well. Our findings are consistent with those of Parkinson et al. (20) where Egr2 was proposed to act as an amplifier of periaxin gene expression during myelination. In addition, our data significantly extend previous results by describing the first direct Egr2 response element in one of the most highly expressed genes in peripheral myelin. From other studies on the Krox20 enhancer (61), Oct6SC enhancer (62), the MBP enhancer (63, 64), as well as the antisilencer/enhancer element of PLP in the central nervous system (65), it is increasingly clear that regulation of many myelin genes requires additional elements outside of the proximal promoter region. Further characterization of the Mpz intron element will provide a greater understanding of how Egr2 regulates myelin gene expression.

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Regulation of Myelin Protein Zero by Egr2

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