Structural Analysis of α-Enolase

MAPPING THE FUNCTIONAL DOMAINS INVOLVED IN DOWN-REGULATION OF THE c-myc PROTOONCOGENE*

(Received for publication, July 23, 1999, and in revised form, November 20, 1999)

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Myc-binding protein-1 (MBP-1) is a 37-kDa protein with sequence homology to the 3' portion of the α-enolase gene. α-Enolase is a 48-kDa protein, which plays a critical role in the glycolytic pathway. MBP-1 binds to the c-myc P2 promoter and down-regulates c-myc expression. We have investigated the role of α-enolase in regulation of the c-myc protooncogene. RNase protection assay shows that α-enolase is transcribed into a single RNA species in HeLa cells. A start codon, 400 base pairs downstream of the α-enolase ATG, corresponds to the MBP-1 ATG, suggesting that MBP-1 is an alternative translation initiation product of the α-enolase RNA. Domain mapping was performed using constructs containing truncations of the α-enolase gene. In vitro binding to the c-myc gene was abolished after deletion of the N-terminal portion of α-enolase. In order to determine the relationship between DNA binding activity and transcription inhibition, we performed co-transfection assays in HeLa cells. These studies confirmed that an N-terminal deletion of α-enolase is unable to down-regulate c-myc promoter activity. Our data suggest that α-enolase plays an important role in regulation of c-myc promoter activity in the form of an alternative translation product MBP-1, which is distinct from its role as a glycolytic enzyme.

The c-myc protooncogene is a DNA-binding phosphoprotein that plays an important role in the regulation of cell growth and differentiation (1, 2). Regulation of c-myc gene expression is quite complex and involves several mechanisms, including changes in transcription initiation and elongation, RNA stability and turnover, and translation (3, 4). Overexpression of the c-myc gene is a common characteristic of many malignant cell types (5). The human c-myc protooncogene contains two TATA box sequences separated by about 165 base pairs located near the 5' end of the first exon (6). The transcription of c-myc from P1 and P2 is regulated by a composite of positive and negative elements located both upstream and downstream of the promoters (7–10).

A human cDNA clone encoding MBP-1 was detected by screening a HeLa cell cDNA library. The Myc-binding protein-1 (MBP-1) is a 37-kDa human c-myc promoter-binding protein that binds in a region +123 to +153 relative to the c-myc P2 promoter (11). MBP-1 is a negative regulator of c-myc expression and binds in the minor groove of the c-myc P2 promoter simultaneously with the TATA-binding protein (12). Consistent with its negative regulation of c-myc and as a potential tumor suppressor protein, transfection of human breast carcinoma cells with MBP-1 cDNA results in inhibition of tumor formation in nude mice (13). Exogenous expression of MBP-1 has been suggested to play an important role in the regulation of human immunodeficiency virus-1 replication in infected cells (14). Careful sequence reanalysis of MBP-1 has shown that it has extensive homology to the sequence of the 3' portion of the α-enolase gene (11, 15).

Enolase is the glycolytic enzyme that catalyzes the formation of phosphoenolpyruvate from 2-phosphoglycerate, the second of the two high energy intermediates that generate ATP in glycolysis (16). The MBP-1 cDNA shares sequence homology with the α-enolase cDNA, which encodes a 1.8-kb mRNA and a polypeptide of about 48 kDa. The high degree of sequence homology is confined to the 1.4-kb 3’ region of α-enolase and the full-length 1.4-kb MBP-1 and suggests that α-enolase and MBP-1 are both products of the α-enolase gene. The presence of an ATG start codon followed by the Kozak sequence suggests that MBP-1 may be the product of alternate translation initiation from an in frame internal translation initiation site 400 bp downstream on the α-enolase cDNA (Fig. 1A).

Western blot analysis using an antibody specific to non-neuronal enolase from human brain (Biogenesis) has identified both 48- and 37-kDa proteins in HeLa nuclear extracts (Fig. 1B). The cellular localization of α-enolase is known to be predominantly cytosolic. The function of MBP-1 as a down-regulator of c-myc gene expression suggests that it would be localized in the nucleus. HeLa cell extract made using Promega reporter lysis buffer (prepared as described under “Experimental Procedures”) is primarily cytosolic and does not show the presence of MBP-1.

The presence of MBP-1 in nuclear extracts corroborates with its role in down-regulation of c-myc promoter activity. α-Enolase constructs are able to down-regulate c-myc promoter activity, albeit to a lower extent than MBP-1. On the other hand, preliminary experiments indicate that MBP-1 does not have enolase enzyme activity. Here we have studied the structure-function relationship of α-enolase as a negative regulator of c-myc activity using DNA binding studies and transfection pair(s); PCR, polymerase chain reaction; CMV, cytomegalovirus; DOTAP/DOPE, 1,2 dioleyl glycero 3-phosphoethanolamine/3-trimethyl ammonium propane.

2. A. Subramanian, J. O. Trent, and D. M. Miller, unpublished data.

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‡ The abbreviations used are: MBP, Myc-binding protein; EMSA, electrophoretic mobility shift assay; bp, base pair(s); kb, kilobase
assays. Our results suggest that the c-myc down-regulating activity of α-enolase lies in the N-terminal region of the protein also present in the alternative translation initiation product MBP-1.

**EXPERIMENTAL PROCEDURES**

**RNase Protection Assay**—The α-enolase fragment from 181 to 600, containing the MBP-1 start site, was PCR-amplified and subcloned into the pBluescript II SK vector (Stratagene). In order to make an antisense RNA probe, the vector containing the 420-base pair α-enolase fragment was linearized with XhoI and in vitro transcribed with T3 RNA polymerase and [α-32P]CTP, using a Maxiscript T3 kit (Ambion). Labeled probe was purified on 5% acrylamide, 8 m urea denaturing gel and eluted overnight at 37 °C. RNase protection assay was carried out using the RPA II kit (Ambion). The antisense cRNA probe (1 μg) was hybridized overnight at 37 °C with increasing concentrations of HeLa nuclear extracts: α-enolase protein (lane 1), HeLa cell extract (lane 2), and HeLa nuclear extract (lane 3) were assayed by immunoblotting with an α-enolase antibody. Positions of the molecular mass standards are indicated. HeLa extracts assayed contained 5 μg of protein.

**Plasmid Construction**—The N-terminal truncations of α-enolase were generated by PCR using upstream primers that contained the start codon. Downstream primers containing the stop codon were used to PCR-amplify the C-terminal deletions. The PCR products were then cloned directly into the PCR 2.1 vector using the TA cloning kit (Invitrogen). From PCR 2.1, they were excised and cloned directionally into the pBluescript (Stratagene) and pCITE vectors. The full-length α-enolase cDNA was cloned under control of the CMV promoter. The full-length α-enolase cDNA was also cloned into the pCITE vector under control of the T7 promoter and isopropyl-1-thio-β-D-galactopyranoside induced as described previously (11). The induced proteins were separated on a 12.5% SDS-polyacrylamide gel and analyzed by Coomassie staining. The 45-bp double-stranded oligonucleotide (GGAGG-GATCGCGCTGATATAAAAGCCGGTTTTCGGGGCTTTATC) corresponding to the P2 promoter region of c-myc was 32P-labeled and used as probe. The underlined G in the above sequence was mutated to T, and this mutant c-myc probe was used as unlabeled competitor. 15 μg of the extracts prepared from the induced cultures were incubated with the probe (2 ng, 106 cpm) in the EMSA buffer (10 mM HEPES, pH 7.9, 100 mM KCl, 1 mM dithiothreitol, 0.05 mM EDTA, 2.5 mM MgCl2 and 8% glycerol) in the presence of 2 μg of poly(dI-dC)poly(dI-dC) on ice for 30 min. Unlabeled competitor oligonucleotide or 2 μl of antibody (1 μg/μl) were incubated with the protein for 30 min on ice before addition of the labeled oligonucleotide. The resulting complexes were then separated on a native 5% polyacrylamide gel at room temperature in 1× Tris borate-EDTA at 10 V/cm. After electrophoresis, gel retardation was visualized by autoradiography.

**Cell Line**—The HeLa human cervical carcinoma cell line was stably transfected with the luciferase reporter gene under control of the c-myc P2 promoter. These stably transfected cells were called MYC1 cells. All transient transfection assays were performed in MYC1 cells.

**Transfection**—MYC1 cells were plated at an initial density of 5 × 104 cells/well of a 24-well plate in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. Immediately prior to trans-
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Expression of the α-Enolase Transcript—Although we have hypothesized that MBP-1 and α-enolase are alternate translation products of a single α-enolase mRNA, it is important to document this fact. RNase protection assay was used to analyze expression of the α-enolase transcript. A cRNA antisense probe of 420 nucleotides corresponding to nucleotides 181–600 of α-enolase, containing the MBP-1 start site, was synthesized as described under “Experimental Procedures.” The probe was annealed to 5 μg of total RNA from HeLa cells and analyzed for levels of expression of message from the transcribed constructs. The resulting protected fragment protected by the 1.8-kb full-length α-enolase cDNA labeled with [32P]dCTP using Ready-To-Go DNA labeling beads (Amersham Pharmacia Biotech). Hybridizations were performed at 42 °C overnight in 5 ml of formamide ready-to-go DNA labeling beads (Amersham Pharmacia Biotech). Hybridization products of a single α-enolase RNA. Lanes 6 and 7 show the 1.2-kb RNA fragment protected by the in vitro transcribed α-enolase RNA. Lanes 8–11 show RNase protection of the probe after hybridization with indicated amounts of HeLa cell RNA.

Site-directed Mutagenesis of MBP-1 ATG on α-Enolase DNA—In order to confirm that MBP-1 is a product of translation initiation from an internal ATG on the α-enolase cDNA, site-directed mutagenesis was performed (Fig. 3A). The ATG codon for methionine at position 97 of α-enolase was transcribed into the ATC codon for isoleucine (Enomut1). This mutation failed to abolish translation of the MBP-1 protein from the α-enolase cDNA. This may have been due to the presence of another in frame ATG at position 377 of α-enolase, six bases upstream of the first ATG, coding for methionine 94, which could have been used for translation. After mutating methionine 94 to isoleucine (Enomut2), a 37-kDa MBP-1 protein band was still visible. Site-directed mutagenesis at both positions was performed on the same template DNA and the resulting α-enolase cDNA (Enomut3) translated into a single protein of 48 kDa (Fig. 3B).

In order to determine if the α-enolase protein could down-regulate c-myc promoter activity in the absence of MBP-1 translation, Enomut3 cloned under control of the CMV promoter was used in transient transfection assays as described under “Experimental Procedures.” Extracts from the MYC1-transfected cells were analyzed by Western blot, and similar levels of protein were observed (Fig. 3C). Luciferase assay results show that, although full-length α-enolase down-regulates c-myc promoter activity by about 40%, Enomut3, which does not generate MBP-1 on translation, is able to repress it by less than 20% (Fig. 4). This indicates that the c-myc down-regulating activity of α-enolase lies in the alternative translation product MBP-1.

Construction and Expression of α-Enolase Deletion Mu-
We have demonstrated that α-enolase has c-myc downstream regulation activity, although less significant than MBP-1. In order to map the functional domains of regulation activity, although less significant than MBP-1. In vitro translated proteins to the 50-base pair labeled c-myc promoter were transfected into HeLa cells and expression assayed by immunoblotting with α-enolase antibody. The α-enolase and MBP-1 bands are indicated. Lane 1, pure human α-enolase positive control; lane 2, pBKCMV vector control showing endogenous α-enolase expression; lane 3, CMVα-enolase; lane 4, CMVMBP-1; lane 5, CMV promoter controlled ATG → ATC mutations of methionines at position 94 and 97 of α-enolase (CMVαEnomut3).

**Binding of α-Enolase and Its Deletion Mutants to the c-myc P2 Promoter**—It has been shown previously that MBP-1 binds to the c-myc P2 promoter (11). EMSA was performed with a 50-base pair labeled c-myc probe (see “Experimental procedures”) and lysates from induced BL21(DE3) cells expressing α-enolase and its deletion mutants. The in vitro translated full-length and truncated α-enolase proteins were initially used for gel shift analysis. However, addition of any of the in vitro translated proteins to the 50-base pair labeled c-myc oligonucleotide caused a shift to the same extent in every lane. We soon realized that endogenous enolase from Rabbit reticulocyte lysate (in which the in vitro translation is carried out) interfered with the EMSA. In order to get around the problem of interference from endogenous α-enolase, bacterially expressed proteins were made and used in EMSAs. The crude bacterial extracts, when analyzed by SDS-polyacrylamide gel electrophoresis and Coomassie-stained, indicated the presence of the translation products from full-length α-enolase and its deletion mutants. However, the smaller peptides due to translation from the internal initiation site on the C-terminal deletions, Eno Δ242–434 and Eno Δ373–434, were not observed (data not shown). A specific DNA-protein complex was visualized by autoradiography in all the α-enolase deletion mutants except Eno Δ1–236. The visible DNA-protein complexes were not disrupted upon addition of 100-fold molar excess of a mutant cold competitor (Fig. 6A). The unlabeled oligonucleotide used as competitor has been described previously (11) and has a mutation that prevents binding of MBP-1 to the DNA. A polyclonal α-enolase antibody was able to bind to and supershift the DNA-protein complexes (Fig. 6B). An antibody to human c-myc was unable to supershift the full-length α-enolase-DNA complex, indicating that the supershifts obtained using the α-enolase antibody are specific. Deletion of amino acids 1–236 of α-enolase prevents the protein from binding to the c-myc P2 promoter. These results indicate that the DNA binding region of the α-enolase protein lies between amino acids 96 and 236.

**Down-regulation of c-myc Promoter Activity by α-Enolase and Its Deletion Mutants**—MBP-1 has been shown to down-regulate c-myc promoter activity (11). HeLa cells stably transfected with the luciferase reporter gene under control of the c-myc promoter (MYC1 cells) were transiently transfected with control (empty) pBKCMV expression vector was assigned a value of 100.

**Fig. 3.** A, the drawing shows the locations of the separate site-directed mutations introduced into the α-enolase cDNA. Mutations were verified by nucleotide sequencing of the mutated regions. B, in vitro translation of α-enolase RNA. In vitro transcribed RNAs from MBP-1 cDNA and from the wild type and point-mutated α-enolase cDNAs were translated in a rabbit reticulocyte lysate system, and the products were analyzed as described in “Experimental Procedures”. Lane 1, negative control; lane 2, wild type α-enolase generating α-enolase and MBP-1 proteins; lane 3, MBP-1; lane 4, ATG (coding methionine 97) → ATC mutant; lane 5, ATG (coding methionine 94) → ATC mutant; lane 6, ATG (coding methionine 94 and 97) → ATC mutants. MBP-1 translation is abolished. C, Western blot analysis. The MBP-1 cDNA and wild type and point-mutated α-enolase cDNAs under control of the CMV promoter were transfected into HeLa cells and expression assayed by immunoblotting with α-enolase antibody. The α-enolase and MBP-1 bands are indicated. Lane 1, pure human α-enolase positive control; lane 2, pBKCMV vector control showing endogenous α-enolase expression; lane 3, CMVα-enolase; lane 4, CMVMBP-1; lane 5, CMV promoter controlled ATG → ATC mutations of methionines at position 94 and 97 of α-enolase (CMVαEnomut3).

**Fig. 4.** Regulation of c-myc promoter activity in MYC1 cells. The effect of MBP-1 and of wild type and point-mutated α-enolase on c-myc promoter activity was measured as luciferase activity in transfected MYC1 cells (see “Experimental Procedures”). To eliminate the influence of transfection efficiency, all data from the luciferase assays were normalized against β-galactosidase activity and presented as the mean ± S.D. from at least three independent experiments, each performed in triplicate. The activity of the c-myc promoter when co-transfected with control (empty) pBKCMV expression vector was assigned a value of 100.

**Fig. 5.** A, the drawing shows the locations of the separate site-directed mutations introduced into the α-enolase cDNA. Mutations were verified by nucleotide sequencing of the mutated regions. B, in vitro transcription and translation (Fig. 5B). The C-terminal truncations Eno Δ242–434 and Eno Δ373–434 also generated smaller peptides of 15 and 26 kDa, respectively, due to translation from the internal initiation site. The 15-kDa band is not visible in Fig. 5B, as it ran with the dye front on the gel.
Northern and Western blot analysis confirmed expression of the mutated forms of α-enolase in the transfected cells (Fig. 7, A and B). Similar levels of RNA were expressed from α-enolase and its truncations in the MYC1 cells. Levels of protein expression from α-enolase and its deletion mutants were quantified by densitometry and found to be comparable. The shorter peptide of 26 kDa translated from the internal initiation site of the C-terminal deletions EnoD373–434 is also generated upon transfection into MYC1 cells and can be seen in Fig. 7B. The 15-kDa peptide generated by translation initiation from the internal ATG on EnoD242–434, which ran along with the dye front on the SDS gel, is not shown in Fig. 7B. However, the amount of protein translated from the internal ATG is far less than that from the first ATG of these constructs. Hence, the effect observed on c-myc promoter activity in transient transfection assays is thought to be predominantly due to the larger proteins.

The effect of α-enolase and its deletion mutants on c-myc promoter activity were measured as luciferase activities in transfected MYC1 cells. The results indicate that Eno1–236 does not down-regulate c-myc promoter activity as efficiently as full-length α-enolase (Fig. 8). The MBP-1 protein down-regulates c-myc promoter activity by 65%. These results correspond with the EMSA results and show that the DNA binding and c-myc down-regulating activity of α-enolase lies between amino acids 96 and 236.

**DISCUSSION**

The role of α-enolase as a glycolytic enzyme has been very well characterized. The α-enolase gene is transcribed into a single RNA species, as proven by the RNase protection assay. Here we show that at least two proteins arise from the alternative usage of translation initiation sites present on the α-enolase mRNA. MBP-1, which negatively regulates c-myc promoter activity, was initially identified from a human cervical carcinoma cell expression library. Previous work (13) has demonstrated that this alternate translation product of the α-enolase gene acts as a tumor suppressor when transfected into human breast carcinoma cells, largely preventing anchorage-independent growth and the growth of tumors in nude mice.

We examined the MBP-1 and α-enolase cDNA coding sequences and observed complete sequence homology between the MBP-1 sequence and the 1.4-kb 3'-region of α-enolase. A potential translation initiation site at codon 97 of α-enolase was observed, and the sequence surrounding this ATG triplet exhibits an overall sequence homology to the Kozak consensus cassette (18).

In a construct in which the ATG at codon 97 on the α-enolase cDNA was mutated to ATC (Enomut1), MBP-1 translation was not abolished. Another in-frame ATG at codon 94 was observed and, after mutation of this ATG to generate Enomut2, α-enolase and MBP-1 continued to be translated. However, when both the ATGs were mutated (Enomut3), α-enolase was the sole product of translation. Because Enomut3 gives rise to just the 48-kDa α-enolase, we can exclude the possibility that MBP-1 arises from proteolytic cleavage of the complete protein. Western blot analysis of pure human α-enolase shows the presence of a single band of ~48 kDa. Even after incubation of the pure human α-enolase protein in a transcription/translation system, no smaller fragment the size of MBP-1 could be observed by Western blotting (data not shown). Full-length α-enolase cDNA after *in vitro* transcription/translation gives rise to
both $\alpha$-enolase and MBP-1 protein bands. The ratio of these two proteins remains constant when checked on a gel after storage for a considerable period of time. These results further confirm that MBP-1 is not a product of proteolytic cleavage of $\alpha$-enolase.

The single $\alpha$-enolase mRNA is alternatively translated from methionine 94 or 97 to yield MBP-1. Our data do not allow us to distinguish whether MBP-1 is translated from the codon for methionine at position 94 or 97 on the $\alpha$-enolase mRNA.

Of the two isoforms of $\alpha$-enolase, MBP-1 better down-regulates c-myc promoter activity. In transient transfection assays in HeLa cells, $\alpha$-enolase is unable to down-regulate activity of the c-myc promoter efficiently after mutation of the internal translation initiation site to prevent translation of MBP-1. The $\sim 20\%$ down-regulation of c-myc promoter activity observed...
after transfection with Enomut3 is due to the binding of the full-length a-enolase to the c-myc P2 promoter. These results suggest that the a-enolase gene is bifunctional, encoding two proteins, one of which has a role in glycolysis and the other in regulation of c-myc expression.

Evidence to suggest that a-enolase may have functions other than as a glycolytic enzyme has been generated earlier in yeast, other vertebrates, and mammalian cells (19, 20). These include either a direct function or indirect role in processes such as thermal tolerance, growth control, and hypoxia tolerance (21). A structural role in the lens of some species has been exhibited by a-enolase (22). It also functions as a cell surface receptor for plasminogen, resulting in enhanced plasminogen activation and localization of the proteolytic activity of plasmin on cell surfaces (23). The presence of a-enolase on the surface of pathogenic streptococci has recently been demonstrated (24). The streptococcal surface enolase is thought to play an important role in the disease process and in post-streptococcal autoimmune diseases.

Our results demonstrate that MBP-1 is a product of internal translation initiation from the a-enolase gene. Internal initiation has been described for other genes such as those for C/EBP α and β (25), Myc (26), GATA-1 (27), CREM α/β (28), N-Oct-3 (29), and Oct-4 (30), and appears to be an efficient and rapid means to modulate their activity. Moreover, in most of the reported cases, this mechanism is evolutionarily preserved in rodents and humans. It has been observed that the in-frame internal ATGs at codons 94 and 97 of a-enolase are conserved across the human, rat, mouse, chicken, duck, and frog a-enolase sequence (31–34). The two GATA-1 isoforms share identical binding activity but differ in their transactivation potential and in their expression in developing mouse embryos. The 30-kDa protein generated by alternative translation initiation of C/EBPα (42 kDa) lacks antimitotic activity (35). Although there are numerous examples of alternative translation products, the broad disparity of function between a-enolase and MBP-1 appears to be unique.

Our preliminary observations indicate that MBP-1 lacks a-enolase enzyme activity. Although the cellular localization for the a-enolase protein has been thought to be predominantly cytosolic, the presence of MBP-1 is observed only in the nuclear extract from HeLa cells. The functional significance of this may lie in the negative regulation of expression of the c-myc protooncogene by the MBP-1 isoform of a-enolase. This may represent a mechanism for negative feedback regulation of c-myc. It has been shown that Myc overexpression up-regulates liver carbohydrate metabolism 3–5-fold (36, 37). Furthermore, Myc overexpression is thought to counteract diabetic hyperglycemia by inducing hepatic glucose uptake and utilization and therefore blocking gluconeogenesis. Although there is no direct evidence of the regulation of a-enolase promoter function by Myc, the plausibility of up-regulation of a-enolase by the overall carbohydrate hypermetabolic state cannot be ruled out.

Up-regulated expression of glycolytic enzymes (pyruvate kinase, phosphofructokinase and glucokinase) as well as up-regulated glycolysis has been shown to occur as a consequence of Myc overexpression. This is thought to be due to the presence of two imperfect CACGTG motifs (5 out of 6 bases match) in the carbohydrate response element of the pyruvate kinase gene (38). Two perfect Myc-Max binding motifs (CACGTG) are also present in the promoter of the a-enolase gene (39). These findings along with our observation of the bifunctional role of a-enolase as a glycolytic enzyme and regulator of c-myc expression present a model that places c-myc and a-enolase at the intersection of energy metabolism and growth control.

We have used deletion mutants of a-enolase to characterize its functional domains. The finding that MBP-1 (Enoα1–96) binds to the c-myc P2 promoter, but Eno1–236 does not, indicates that the amino acids between 96 and 236 of a-enolase are essential for DNA interaction. Among the a-enolase deletion mutants, MBP-1 was the most efficient down-regulator of c-myc promoter activity. Our results from the transient transfection assays corroborate those from the DNA binding studies, since Eno1–236 was unable to down-regulate c-myc promoter activity. Both the C-terminal deletions of a-enolase (EnoΔ242–434 and EnoΔ373–434) contained amino acids 96–236 and were able to down-regulate expression of the c-myc promoter by at least 40% of its activity. The region between amino acids 96 and 236 is present in the MBP-1 isoform of a-enolase, which has been shown to down-regulate c-myc expression by 65%.

This result is consistent with the previously published work of Ghosh et al. (40), which demonstrated transcriptional repression activity in the N-terminal portion of MBP-1. Our data show however, that DNA binding activity correlates nicely with ability to inhibit transcription of c-myc. As seen by mutating methionine 94 and 97, the c-myc down-regulating activity of a-enolase is lost, by abolishing translation of the MBP-1 isoform. Thus, it is possible that the bifunctional role of a-enolase could be modulated by the varying ratio of the two isoforms.

The existence of two a-enolase isoforms with distinct functions presents a unique example of a gene encoding proteins with roles in metabolism and cell proliferation. Our data suggest that, while the a-enolase isoform functions as the glycolytic enzyme, the N-terminal region of the MBP-1 isoform is important in binding to and down-regulating expression of the c-myc gene. The MBP-1 isoform has been shown to inhibit anchorage-independent cell growth and tumor growth in nude mice (13). The manner in which the coding capacity of the a-enolase mRNA for the two protein isoforms is regulated has not been ascertained. Internal ribosome entry constitutes a novel mechanism of gene expression regulation. This has been shown in the case of FGF-2, whose CUG-initiated isoforms are translationally activated in response to stress (41). Whether this kind of initiation from the presence of an internal ribosome entry site occurs in the case of MBP-1 remains to be determined.

REFERENCES