Transcriptional Repression from the c-myc P2 Promoter by the Zinc Finger Protein ZF87/MAZ*

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Marc W. Izzo, Gordon D. Strachan, Matthew C. Stubbs, and David J. Hall:

From the Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

ZF87/MAZ is a zinc finger-containing transcription factor that was cloned based on its ability to bind to a site within the c-myc P2 promoter. However, its role in the control of c-myc transcription has not yet been well established. Here we have analyzed the effect of ZF87/MAZ overexpression on transcription from the murine c-myc P2 promoter. It was found that when overexpressed in COS cells, ZF87/MAZ significantly represses transcription from P2. The repression is mediated through the ME1a2 element, located at position −86 relative to the P2 transcriptional start site, and is not mediated through either the E2F or the ME1a1 sites. ZF87/MAZ functions as a true transcriptional repressor since it can repress transcription independently of the c-myc promoter, as part of a fusion with the GAL4 protein. The repressive domain within ZF87/MAZ is located in the amino-terminal half of the protein, a region rich in proline and alanine residues. ZF87/MAZ therefore shares features (i.e. a Pro/Ala-rich region) with those of known transcriptional repressor proteins.

The c-myc proto-oncogene is known as a critical factor in the control of both cell proliferation (1–5) and apoptosis (6). In particular, c-myc is known as a cooperating oncogene and is thought to be an important contributing factor to neoplastic transformation and the generation of many cancers (1–5). That c-myc plays such a key role in regulating cell proliferation and cell death is consistent with the observations that its expression is tightly controlled in normal cells. Expression of both c-myc mRNA and protein is extremely low in quiescent cells, and it increases in proliferating cells (1–5). Since c-myc plays an important role in regulating cell proliferation, much effort has gone into understanding how its expression is modulated.

c-myc gene expression is controlled at least in part at the level of transcription initiation, and extensive research has gone into identifying the transcriptional control regions within the c-myc promoter. This has been complicated by the fact that c-myc has multiple promoters, which are termed P0, P1, P2, and P3 (4, 5). Yet in many normal and transformed cells, a majority of transcripts initiate at the P2 promoter (4, 5). A large number of cis-acting sequences exist both 5′ and 3′ to the P2 transcription start site (5, 7). These sequences, although some quite distant from P2, regulate transcription initiation from P2 and include binding sites for the FUSE factor (8, 9), Fos/Jun (10, 11), Cut (12), YY1 (13), v-Abl (14, 15), Blimp-1 (16), NF-κB/Rel (17), CTCF (18), and CF1 (19). The expression of some of these factors are cell- and tissue-type specific. Additionally, some of these factors, such as Fos/Jun, Blimp-1, and CTCF, function to repress the transcription of c-myc.

cis-Acting sequences more proximal to the P2 promoter are comprised of an initiator sequence at the start site, a well defined TATA sequence at position −29, and three elements termed ME1a1, E2F, and ME1a2 at positions −46, −64, and −85 relative to P2 (4, 20–23). It has been demonstrated that growth factor induction of c-myc takes place at least in part through the E2F site (24) as does p53-mediated repression (25), SV40 large T antigen (23), and adenovirus E1a-mediated transactivation (26, 27). The p53-mediated repression from P2 can be overcome by co-expression of SV40 T antigen (23).

The ME1a1 and ME1a2 elements have been shown to contribute positively to initiation at the P2 promoter (20–23). To understand better how the ME1a2 site controls transcription from P2, a novel transcription factor was cloned based on its ability to bind this element (28, 29). This factor termed ZF87/MAZ contains six zinc fingers of the Kruppel/TFIIIA type and was also found to bind the ME1a1 site (28, 29). ZF87/MAZ also contains glutamine-, alanine-, and proline-rich regions (28, 29) but appears to lack the KRAB-A/B domains present in many zinc finger proteins (30). Evidence indicates that full-length ZF87/MAZ can act as a low level activator of c-myc expression in certain cell lines devoid of p53 or pRb (7). In contrast, a protein-related ZF87/MAZ, termed THZif-1, has been identified in HL60 cells that functions to suppress transcription from the c-myc promoter (31, 32). Given that multiple members of the ZF87/MAZ family now appear to exist (33, 34), the data suggest that the ZF87/MAZ family of proteins may play an important role in controlling c-myc transcription, yet to date little is known of their exact role in c-myc transcription. To gain insight into the function of ZF87/MAZ, we have overexpressed it in COS cells. We find that ZF87/MAZ is a potent repressor of transcription from the c-myc P2 promoter acting through the ME1a2 element.

MATERIALS AND METHODS

Cell Culture, Transfections, and Apoptosis—COS and CV1 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% bovine calf serum or fetal calf serum. All transfection experiments were initiated on 50% confluent monolayer cultures. Plasmids (a total of 40 μg) were transfected by the calcium phosphate procedure (35). The cells were glycerol-shocked 5–6 h after DNA addition.

The ZF87/MAZ cDNA construct was originally isolated by screening a Agt11 expression library (renatured protein immobilized on filters) with a 32P-radio labeled ME1a2 element (28). The cDNA was then cloned into Bluescript KS just 3′ to a FLAG epitope tag sequence. The FLAG epitope tag was linked in frame to the open reading frame of ZF87/MAZ. A monoclonal antibody (M2, Eastman Kodak Co. and IBI) directed toward this epitope was used in the immunoblotting immunofluorescence and gel-shift experiments. For expression studies the tagged ZF87/MAZ gene was cloned into the pcDNA3 plasmid. All of the
c-myc promoter:CAT constructs used were as described previously (23, 25).

Extracts were generated by multiple freeze-thaw cycles approximately 48 h after the glycerol shock and then CAT (chloramphenicol acetyltransferase) and β-galactosidase activity was assayed from the soluble extracts. Equal amounts of protein were assayed for CAT activity by thin layer chromatography, and β-galactosidase activity was measured by a color reaction (35). All transfections were performed multiple times. A plasmid containing the Rous sarcoma virus (RSV)-long terminal repeat controlling expression of the β-galactosidase gene (RSV-β-gal) was included in all transfections (5 μg) of the CAT constructs so that the CAT activity could be normalized for differences in transfection efficiency.

To determine if ZF87/MAZ induced apoptosis, COS cells were plated out onto glass coverslips and then transfected with the ZF87/MAZ expressing plasmid or a vector control. At 48 h after the glycerol shock, the coverslips were processed for fluorescent microscopy, using M2 as a primary antibody, followed by DAPI staining of the DNA. The altered nuclear morphology of an apoptotic cell, as assessed by DAPI staining, is represented by nuclear blebbing, DNA fragmentation, and DNA condensation (36).

Cells to be processed for flow cytometry were rinsed twice in chilled PBS and then trypsinized and resuspended in 10 ml of Dulbecco’s modified Eagle’s medium plus 10% serum. The cells were then pelleted and resuspended in 70% ethanol. The cells were kept on ice for 10 min, pelleted, and then treated with RNase A (1.8 μg) for 30 min at room temperature. Propidium iodide (Sigma) was added to a final concentration of 2 μg/ml for an additional 15 min at room temperature. Cell cycle analysis was then performed on a Coulter Profile 2 Flow Cytometer.

**Generation of Extracts**—Nuclear and cytosolic extracts for immunobLOTS and gel-shift assays were generated by lysing the cells on ice in 0.1% Nonidet P-40, 10 mM Tris (pH 7.9), 10 mM MgCl2, 15 mM NaCl, and 0.4 μM dithiothreitol, and 0.4 mM phenylmethylsulfonyl fluoride. The reactions were then electrophoresed in a low ionic strength (7 mM Tris (pH 7.9), 3.3 mM sodium acetate, 1 mM EDTA) 4% polyacrylamide gel. The gel was dried and exposed to x-ray film, and the protein-DNA complexes were visualized by autoradiography.

Electrophoretic mobility shift assays were performed essentially as described (23). Briefly, 0.5 μg of a 32P-end-labeled, double-stranded oligonucleotide was incubated with nuclear extracts in the presence of 1.5 μg of sheared and denatured salmon sperm DNA as a nonspecific competitor. The final buffer conditions for protein binding to the radiolabeled DNA were 16 mM Hepes (pH 7.9), 16% glycerol, 80 mM KCl, 0.16 mM EDTA, 0.4 mM diithiothreitol, and 0.4 mM phenylmethylsulfonyl fluoride. The reactions were then electrophoresed in a low ionic strength (7 mM Tris (pH 7.9), 3.3 mM sodium acetate, 1 mM EDTA) 4% polyacrylamide gel. The gel was dried and exposed to x-ray film, and the protein-DNA complexes were visualized by autoradiography.

The double-stranded oligonucleotide representing the ME1a2 element used in the gel-shift assays is shown in Oligonucleotide 1.

**OLIGONUCLEOTIDE 1**

| GATCCGCTCTGGACGCGGCTGCG | CGAGGACGTTCGCGCAGACGTCG | OLIGONUCLEOTIDE 1 |

The underlined region denotes the sequence that is protected in DNase I footprinting experiments (21, 22). The sequences in bold italics are those that were mutated in mutant 1 and mutant 2 (GG to TT).

**Western Blots**—Extracts were electrophoresed by SDS-PAGE. The proteins were electrophoretically transferred onto nitrocellulose, and then transferred to TBST buffer (1% BSA, 20 mM Tris, 150 mM NaCl, 0.05% Tween 20), blocked with 2.5% bovine serum albumin in TBST for 30 min at room temperature, and then incubated with either the M2 anti-FLAG monoclonal antibody (10 μg/ml) or polyclonal antiserum generated against ZF87/MAZ (1:5,000 dilution). The blots were washed three times with TBST and then incubated with a 1:7500 dilution of secondary antibody (goat anti-mouse or goat anti-rabbit Vector Laboratories) followed by alkaline phosphatase for 30 min at room temperature in TBST. The blots were washed three times with TBST and then stained using the Protoblot system from Promega.

**Immunofluorescence**—For indirect immunofluorescence, cells were plated on 10-cm tissue culture dishes containing glass coverslips and transfected with the ZF87/MAZ expression plasmid. The cells were washed once in PBS and the cells fixed with 4% paraformaldehyde in PBS for 20 min followed by an additional rinse in PBS. To permeabilize the cells, the coverslips were then treated with PBS plus 0.2% Triton X-100 for 15 min followed by three 5-min washes in PBS plus 0.2% gelatin (28). The M2 monoclonal antibody (VWR Scientific/Kodak/IBI) was diluted in PBS plus 0.2% gelatin. 50 μl of diluted antibody was added to a dish; coverslips containing the fixed and permeabilized cells were placed cell side down on the drop of diluted antibody and incubated overnight at 37 °C. The cells were washed twice with PBS plus 0.2% gelatin. Fluorescein-conjugated goat anti-mouse IgG (Vector Laboratories) was diluted to 30 μg/ml in PBS plus 0.2% gelatin. 50 μl of diluted antibody was placed in a dish and the coverslips placed cell side down and incubated for 30 min at 37 °C. The coverslips were washed in PBS plus 0.2% gelatin (10 min), PBS plus 0.2% gelatin plus 0.05% Tween 20 (10 min), and finally in PBS (10 min). The coverslips were rinsed in deionized water, dried, mounted, and analyzed by fluorescence microscopy.

**RESULTS**

**Overexpression of ZF87/MAZ in COS Cells**—To begin functional studies on ZF87/MAZ, the protein was epitope-tagged (FLAG) at the very amino terminus by engineering the tag at the very 5′ end of the cDNA. This tagged construct was cloned into the pcDNA3 expression vector, suitable for high level of expression in eukaryotic cells. The M2 monoclonal antibody (Kodak/IBI), directed to the epitope tag, was then used to detect the ectopically expressed protein. To initiate functional studies on ZF87/MAZ, the pcDNA3-ZF87/MAZ vector was transiently transfected into COS cells. At 48 h post-transfection the cells were harvested, and nuclear and cytosolic extracts were generated. These extracts were electrophoresed by SDS-PAGE, and the gel was immunoblotted. Separate blots were incubated with either the M2 monoclonal antibody or ZF87/MAZ specific antiserum. As seen in Fig. 1, A and B, ectopically expressed ZF87/MAZ is detected by both antibodies. The protein is present almost exclusively in the nuclear extracts. The protein band under the ectopically expressed ZF87/MAZ in Fig. 1A is a nonspecific band detected by the secondary antibody.

Since the protein was present in the nuclear fraction, we next determined if the protein was actually targeted to the nucleus. COS cells cultured on glass coverslips were next transiently transfected with the ZF87/MAZ expression plasmid. The cells were fixed at 48 h post-transfection and were processed for indirect immunofluorescence using M2 as the primary antibody and a fluorescein-conjugated secondary antibody. As seen in Fig. 2A, fluorescence microscopy reveals that the protein was targeted almost completely to the nucleus.

To test whether ZF87/MAZ overexpression had an adverse effect on the COS cells, apoptosis was measured. Cells grown on coverslips were transfected with the ZF87/MAZ expression vector and then processed for indirect immunofluorescence as described above at 48 h post-transfection. Following the addition of the second fluorescein-conjugated antibody, the DNA in
the cells was stained with the DNA dye DAPI. Apoptosis was measured in the transfected and untransfected cells by determining the percentage of fluorescent-positive cells undergoing nuclear blebbing, DNA fragmentation, and condensation (as in Ref. 36). As seen in Fig. 2B, ZF87/MAZ overexpression does not induce any significant apoptosis in these cells, compared with the untransfected control population.

**c-myc P2 Promoter Analysis in COS Cells**—Since the transfection data indicated that ZF87/MAZ could be transiently expressed to detectable levels by immunofluorescence and immunoblotting in COS cells, it was next important to determine the functional consequences of this expression, by focusing on the c-myc promoter. Transfection experiments were therefore initiated, using c-myc promoter-CAT constructs. The c-myc promoter has been analyzed in detail in a variety of cell lines, using deletion constructs with varying promoter sequences (20–23, 25, 37). However, transcription from the P2 promoter has not been analyzed in detail with these constructs in COS cells. The promoter constructs to be utilized are outlined in Fig. 3A. These show the various combinations of P2 promoter elements, utilizing the c-myc P2 transcriptional start site in all constructs and extending to residue +176 (relative to P2) within the first exon. These promoter constructs were individually transfected into COS cells along with an RSV-LacZ construct. Extracts generated at 48 h post-transfection were used to assay for CAT activity. It is clear from the data in Fig. 3B that optimal transcription from the promoter is dependent primarily on the ME1a1 and ME1a2 elements. Note that the −77CAT construct contains E2F, ME1a1, and TATA, whereas the −61CAT construct contains only the ME1a1 and TATA elements. Elimination of the ME1a2 element results in the greatest drop in promoter activity. Although the E2F element does not appear to affect greatly the overall level of transcription in COS cells, it has been shown to control c-myc transcription in nontransformed cells (24). The data indicate that the ME1a2 and ME1a1 elements contribute the most to transcription from P2 in COS cells. Dependence of P2 promoter strength on these elements in COS cells therefore appears nearly iden-
tical to that seen in NIH3T3 fibroblasts, HeLa cells, and U87mg glioblastoma cells (23, 25, 37).

Transcription from the c-myc P2 Promoter Is Repressed by ZF87/MAZ—The constructs in Fig. 3A were next used as a starting point in cotransfection experiments in COS cells along with the ZF87/MAZ expression plasmid. As a control for transfection efficiency an RSV-LacZ plasmid (5 μg) was cotransfected. Thirty micrograms of plasmid were transfected by the CaPO₄ method, followed by a glycerol shock 5–6 h later. Extracts were generated 44 h later and assayed for CAT and β-galactosidase activity. CAT activity (using [¹⁴C]chloramphenicol) was measured by thin layer chromatography followed by autoradiography. The autoradiograms from multiple experiments were scanned and the levels normalized for any differences in β-galactosidase activity. The relative level of transcription is given as a percent of the maximum (set arbitrarily at 100% for the −1367CAT construct). The results are presented as an average of multiple experiments ± S.D.

**Transcription from the c-myc P2 Promoter Is Repressed by ZF87/MAZ**—The constructs in Fig. 3A were next used as a starting point in cotransfection experiments in COS cells along with the ZF87/MAZ expression plasmid. First, the largest c-myc promoter fragment (−1367CAT) was cotransfected along with increasing amounts of the ZF87/MAZ expression plasmid. As seen below in Fig. 4A, ZF87/MAZ shows a dose-dependent repressive effect on transcription from this promoter construct. At the highest concentration used, a 10-fold repressive effect was evident. Optimal repression was seen at a 2:1 ratio of reporter to ZF87/MAZ expression plasmid. As controls, the ZF87/MAZ expression plasmid at the high concentration had no effect on transcription from either the Rous sarcoma virus-long terminal repeat or on the herpesvirus thymidine kinase promoter (Fig. 4B). An RSV-LacZ construct was used in all transfections to normalize for any differences in transfection efficiency, as described above since it is not affected by ZF87/MAZ (Fig. 4C). As a control for these experiments, CV1 cells, the parental cell line of COS but lacking SV40 T antigen, were transfected with the −1367CAT and the −140CAT constructs.
in the presence and absence of the ZF87/MAZ expression plasmid. As seen in Fig. 4D, ZF87/MAZ significantly down-regulates transcription from these promoter constructs in CV1 cells. This indicates that the down-regulation in COS cells is not dependent on T antigen.

Experiments were next performed to identify the site(s) within the P2 promoter that were affected by ZF87/MAZ. Co-transfections were therefore performed on the deletion constructs outlined in Fig. 3A, namely -140CAT, -77CAT, -61CAT, -48CAT (containing only the TATA element and the P2 start site), and -24CAT (containing only the P2 start site). Since the high concentration of the ZF87/MAZ expression plasmid led to the greatest repression of expression from the -1367CAT construct in COS cells, this ratio of reporter to ZF87/MAZ expression plasmid was used to test whether repression could occur on smaller promoter fragments. Significant repression (>5-fold) occurred to the -140CAT construct by ZF87/MAZ as shown in Fig. 5, which would indicate that ZF87/MAZ is acting through elements in the P2 promoter. However, as also shown in Fig. 5, the other constructs, which all lack the ME1a2 site, were not repressed by ZF87/MAZ. In fact the -77CAT construct is activated by ZF87/MAZ. Although ZF87/MAZ is known to bind the ME1a1 element (28), the mechanisms responsible for this activation will be discussed below. However, the results would indicate that the repressive effect of ZF87/MAZ on P2 is primarily due to its action on the ME1a2 site and not on the E2F or ME1a1 sites. Also, since no repression was seen using a construct containing
only the TATA box (−48CAT), it appears that ZF87/MAZ is not acting directly on the basal transcription machinery.

To analyze more thoroughly the effect of ZF87/MAZ on the P2 promoter, additional promoter CAT constructs were used in the cotransfection assays. These constructs include ME1a2-E2FCAT, ME1a2-ME1a1CAT, ME1a2CAT, and 3E2FCAT. As shown in Fig. 6, transcription from each promoter construct, except the 3E2FCAT, is inhibited by ZF87/MAZ. The data in total are consistent with a role of ZF87/MAZ inhibiting transcription from the P2 promoter through the ME1a2 site.

Constructs were next generated that contained the basal TATA box with the P2 start site (−248CAT). Cloned 5′ to the TATA box were two wild type ME1a2 elements or two sets of mutant elements. In mutant 1, two Gs within the first set of Gs were mutated to Ts. In mutant 2, the two Gs within the second set of Gs were mutated to Ts. The mutant sequences are shown in Fig. 7A. Previous DNase I footprinting analysis indicates that the central Gs are bound by protein (22). Following transfection of these constructs, it is clear that two wild type ME1a2 elements provide strong promoter activity, nearly equal to the level of the −140CAT construct (Fig. 7B). This is similar with the results of transfections of this construct into other cell types (23, 25). Mutant 1 shows significantly reduced transcriptional activity, and mutant 2 behaves nearly to wild type levels.

This indicates that the central Gs are essential for optimal activity in COS cells. Consistent with these data, ZF87/MAZ has a potent effect on down-regulating activity from the wild type ME1a2 element and mutant 2. However, it appears that mutant 1 is not affected by ZF87/MAZ. Thus, the central Gs in the sequence AA\_

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\text{GGGCAGGGC}
\]

appear to mediate the effect of ZF87/MAZ on the c-myc P2 promoter.

Increased Protein Binding Occurs to the ME1a2 Element in the ZF87/MAZ-transfected Cell Extracts—Since it appeared that ZF87/MAZ is acting through the ME1a2 element, this sequence was radiolabeled and used in a gel-shift assay with extracts from the transfected cells. This would allow a determination of any alteration in binding activity on this element by ectopically expressed ZF87/MAZ. As seen in the Fig. 8, at least three protein complexes are formed on the ME1a2 element. The data in total are consistent with a role of ZF87/MAZ-inhibiting transcription from the P2 promoter through the ME1a2 site.

FIG. 5. ZF87/MAZ-mediated repression from the c-myc P2 promoter is localized to the ME1a2 site. COS cells were transfected with the indicated c-myc promoter-CAT deletion constructs (30 μg, outlined in Fig. 3) with or without the ZF87/MAZ expression plasmid (15 μg). Shown is the level of transcription, given as a percent of CAT activity relative to the level seen in the absence of ZF87/MAZ (set at 100% for the −140CAT construct). The results are presented as an average of multiple experiments ± S.D. The transfection and assay for CAT activity is as described in Fig. 3. In all cases the amount of DNA transfected was kept constant by the addition of the pcDNA3 plasmid.

FIG. 6. ZF87/MAZ-mediated repression from the c-myc P2 promoter is dependent on the ME1a2 site. COS cells were transfected with the indicated c-myc promoter-CAT constructs (30 μg, outlined in Fig. 3) with or without the ZF87/MAZ expression plasmid (15 μg). Shown is the level of transcription, given as a percent of CAT activity relative to the level seen in the absence of ZF87/MAZ (set at 100% for the −140CAT construct). The results are presented as an average of multiple experiments ± S.D. The transfection and assay for CAT activity is as described in Fig. 3. In all cases the amount of DNA transfected was kept constant by the addition of the pcDNA3 plasmid.
sity to the level seen in the control extract. This would indicate that the ectopically expressed ZF87/MAZ protein is functionally capable of binding the ME1a2 element within the promoter, and it forms multiple protein complexes with the DNA. The other band in the gel shift in Fig. 8 that is not affected by ZF87/MAZ overexpression is likely a distinct protein complex formed on the ME1a2 sequence.

**FIG. 8.** Ectopically expressed ZF87/MAZ binds the ME1a2 site, forming multiple protein-DNA complexes. Nuclear extracts (8 µg) from cells transfected with pcDNA3 alone (control extracts) or extracts from cells transfected with the ZF87/MAZ expression plasmid were used in a gel-shift assay with a 32P-labeled ME1a2 element as a probe. The arrows point to the bands showing increased intensity following expression of ectopic ZF87/MAZ. The asterisk identifies the protein complex unaffected by ZF87/MAZ. Where indicated, 0.1 µg of M2 monoclonal antibody (directed to the epitope tag) or a double-stranded oligonucleotide representing the ME1a2 element (100 ng) as a specific competitor was added to the binding reactions.

**DISCUSSION**

Here we show that the ZF87/MAZ transcription factor represses transcription from the c-myc P2 promoter, when transiently overexpressed in COS cells. Using a variety of promoter
ZF87/MAZ contains a potent transcriptional repressor domain that localizes to the amino terminus. ZF87/MAZ was cloned in frame with the DNA binding domain of GAL4 in the eukaryotic expression vector pG4. A. Cotransfections were performed in COS cells using pG4-ZF87/MAZ (20 µg) and the target promoter pGAL4-TKCAT (25 µg). Additional, the amino-terminal domain (NTD, residues 1–246) and the carboxyl-terminal domain (CTD, residues 246–493) were also cloned in frame with GAL4 in pG4. Cotransfections in COS cells were also with these expression constructs (20 µg) and pGAL4-TKCAT (25 µg). Shown is the level of transcription, given as a percent of CAT activity relative to the level seen for pG4 alone (set at 100%). The results are presented as an average of multiple experiments ± S.D. B, nuclear extracts from cells transfected with the indicated GAL4 fusions were electrophoresed by SDS-PAGE, immunoblotted, and incubated with an anti-ZF87/MAZ antibody. Shown is the developed blot. The arrows indicate the bands corresponding to the fusion proteins.

A

![A](image)

B

![B](image)

Transcriptional Repression from the c-myc P2 Promoter

In addition to its ability to activate and repress transcription, ZF87/MAZ is able to repress transcription from the c-myc promoter in COS cells independent of its ability to bind the c-myc promoter. The repressor domain is localized to the amino half of the protein, which is rich in proline and alanine residues. This is consistent with the fact that proline and alanine-rich sequences comprise the repressor domains of the well characterized transcriptional repressor proteins Kruppel, Even Skipped, and Runt (40–42). The role of these domains may be to establish protein-protein interactions. Consistent with this idea, a number of cellular factors were found to associate with ZF87/MAZ overexpression experiments CBP was found to enhance the repressive effect of ZF87/MAZ in a cotransfection assay (data not shown). This would be consistent with the dual role of CBP as both a coactivator and corepressor (49, 50).

That ZF87/MAZ is able to repress transcription from the c-myc promoter is interesting in light of the fact that it has been shown to enhance the expression of other genes. These include insulin (43, 44), the adenovirus major late promoter (46), the serotonin 1a receptor (47), and the CD4 receptor (48). Interestingly, that ZF87/MAZ can activate transcription is in line with the results presented here, where ZF87/MAZ overexpression increases transcription from a construct containing just the E2F and ME1a1 elements. At this time we do not know the basis for this activation, but our preliminary data indicate that due to the absence of the ME1a2 element, ZF87/MAZ indirectly increases the activity of the E2F factor on the c-myc promoter. The Sp1 transcription factor is known to bind the ME1a1 site, and Sp1 and E2F have been shown to interact with each other (51). Therefore, ZF87/MAZ may indirectly affect (increase) this interaction, which is particularly evident in the absence of the ME1a2 site (i.e. due to loss of repression). Current efforts are underway to determine if this is the case.

In addition to its ability to activate and repress transcription initiation, ZF87/MAZ also functions to terminate transcription at the 3′ end of the complement C2 gene (45). It therefore appears that activation and repression of transcription by
ZF87/MAZ are contrasting features of this transcription factor. It is possible that these functions are accomplished by cell/tissue-dependent associated factors that act as coactivators or corepressors. It is also possible that since ZF87/MAZ is a multiprotein family, some members may be involved in repression and others in activation. This may have an important function, for example, in cells undergoing differentiation, where enhancement of differentiation specific genes (e.g. serotonin, CD4, and complement) and suppression of cell cycle genes (e.g. c-myc) are essential for the completion of the differentiation process. Further analysis into the structure and function of ZF87/MAZ will shed light on the role this factor plays in proliferation and differentiation.

REFERENCES
1. Cole, M. (1986) Annu. Rev. Genet. 20, 361–384
2. Weinberg, R. A. (1989) Cancer Res. 49, 3713–3721
3. Spencer, C. A., and Groudine, M. (1991) Adv. Cancer Res. 56, 1–48
4. Marcu, K. B., Bossone, S. A., and Patel, A. J. (1992) Annu. Rev. Biochem. 61, 809–860
5. Ashfield, R., Patel, A. J., and Calame, K. (1995) Curr. Top. Microbiol. Immunol. 226, 1–77
6. Duncan, D. D., Stupakoff, A., Hedrick, S. M., Marcu, K. B., and Siu, G. (1995) Biochem. Biophys. Res. Commun. 226, 47–57
7. Avigan, M. I., Strober, B., and Levens, D. (1990) J. Biol. Chem. 265, 18538–18545
8. Duncan, R., Bazar, L., Michelotti, G., Tomonaga, T., Krutzsch, H., Avigan, M., and Levens, D. (1994) Mol. Cell. Biol. 14, 4251–4257
9. Riggs, K. J., Saleque, S., Wong, K. K., Merrell, K. T., Lee, J. S., Shi, Y., and Genes Dev. 11, 5340–5349
10. Marcu, K. D., Patel, A. J., and Yang, Y. (1997) Adv. Cancer Res. 74, 71–142
11. Hay, N., Takimoto, M., and Bishop, J. M. (1987) Nature 323, 377–388
12. Hiebert, S. W., and Nevins, J. R. (1991) Mol. Cell. Biol. 11, 2643–2653
13. Moberg, K. H., Tyndall, W. A., Pyrc, J., and Hall, D. J. (1993) Biochem. Biophys. Res. Commun. 192, 1204–1209
14. Ashfield, R., Patel, A. J., Brown, H., Campbell, R. D., Marcu, K. B., and Calame, K. (1995) Mol. Cell. Biol. 15, 1379–1386
15. Lennon, S. Y., Rhys-Black, A., Kostic, D., Pajovic, S., Hoovr, C.N., and Azizkhan, J. C. (1996) Mol. Cell. Biol. 16, 1668–1675