Cloning and Characterization of Human Polyamine-modulated Factor-1, a Transcriptional Cofactor That Regulates the Transcription of the Spermidine/Spermine \(N^1\)-Acetyltransferase Gene

(Received for publication, April 9, 1999, and in revised form, May 11, 1999)

Yanlin Wang, Wendy Devereux, Tracy Murray Stewart, and Robert A. Casero, Jr.‡

*From the Johns Hopkins Oncology Center Research Laboratories, Baltimore, Maryland 21231

The increased transcription and ultimate superinduction of the spermidine/spermine \(N^1\)-acetyltransferase (SSAT) gene has been associated with the antineoplastic activity of several new antitumor polyamine analogues. In sensitive tumor cell types, the transcriptional induction appears to be regulated by the constitutive association of the transcription factor Nrf-2 with the recently discovered polyamine-responsive element. Using the yeast two-hybrid system, a new transcriptional cofactor, polyamine-modulated factor-1 (PMF-1), has been identified as a partner protein of Nrf-2 that, in combination with Nrf-2, regulates the polyamine analogue-induced transcription of SSAT. The human PMF-1 gene, located on chromosome 1 near the 1q12/1q21 border, yields an mRNA transcript of \(\sim 1.2\) kilobases that codes for a 165-amino acid protein with a predicted molecular mass of \(\sim 20\) kDa. The PMF-1 mRNA appears to increase in response to analogue exposure only in analogue-responsive cells. In addition to the transcriptional regulation of SSAT, PMF-1 or similar factors should be considered in the regulation of other polyamine-dependent genes.

The absolute requirement for polyamines in the growth of eukaryotic cells has led to the targeting of their metabolic pathway as a means of antineoplastic intervention (1). Several newly synthesized polyamine analogues designed to alter regulation of polyamine metabolism are currently under investigation for their antitumor activity (2–5). Some of these analogues appear to exert their cytotoxic effects in association with the superinduction of spermidine/spermine \(N^1\)-acetyltransferase (SSAT),\(^1\) the rate-limiting step of polyamine catabolism (6–11). The initial induction of this enzyme occurs at the level of increased transcription in response to analogue treatment (12–14). We have recently identified a cis-acting polyamine-responsive element (PRE) and a trans-acting protein, the transcription factor Nrf-2, to be involved in the regulation of SSAT gene transcription (14). However, the Nrf-2 transcription factor appears to be constitutively expressed only in those tumor cell types capable of highly expressing SSAT. Furthermore, the binding of Nrf-2 to the PRE does not change in response to treatment with either the analogues or the natural polyamines as measured by electrophoretic mobility shift assays (14).

The results of these recent studies suggest at least two possibilities: 1) PRE-bound Nrf-2 is altered by analogue treatment, leading to transcriptional activation, or 2) an additional factor that is induced by analogue exposure leads to transcriptional activation of the SSAT gene. In this study, we have used the yeast two-hybrid system to identify and clone a newly identified human protein, polyamine-modulated factor-1 (PMF-1), that interacts with the leucine zipper region of Nrf-2 and activates SSAT transcription. Additionally, we demonstrate that this gene is rapidly induced in response to analogue treatment in a polyamine analogue-sensitive lung tumor cell line, strongly suggesting that PMF-1 mediates SSAT transcriptional induction by acting in cooperation with Nrf-2. These results also implicate the possibility that this or other transcriptional cofactors are important in the response of other genes regulated by the natural polyamines or their analogues and that PMF-1 may serve as a paradigm for understanding the increased requirement of polyamines in the transformed phenotype (15–18).

EXPERIMENTAL PROCEDURES

Chemicals and Reagents—\(N^1\),\(N^3\)-Bis(ethyl)norspermine (BENSpm) was kindly provided by Parke-Davis. 2-Difluoromethylornithine was obtained as a gift from the Marion-Merrell-Dow Research Institute (Cincinnati, OH). Radiumucleotides, \([\alpha-\text{32P}]\text{dCTP}\), and \([\alpha-\text{35S}]\text{methionine}\) were supplied by Amersham Pharmacia Biotech. A human placental retroviral cDNA library, the Matchmaker yeast two-hybrid system, yeast culture media, and a human multiple-tissue Northern blot system were purchased from CLONTECH (Palo Alto, CA). A human bacterial artificial chromosomal (BAC) DNA library was obtained from Research Genetics (Huntsville, AL). Lipofectin reagent was purchased from Life Technologies, Inc. The luciferase assay system, TnT coupled transcription/translation reticulocyte lysate systems, and the cDNA synthesis system were purchased from Promega (Madison, WI), and the Gal-\(\alpha\)-X: chemiluminescent reporter gene assay system was purchased from ICN Pharmaceuticals (Cosa Mesa, CA). Restriction and DNA-modifying enzymes were purchased from Life Technologies, Inc., New England Biolabs Inc. (Beverly, MA), and Sigma. Oligo(dT)-cellulose was purchased from Roche Molecular Biochemicals. The TA cloning kit was purchased from Invitrogen (Carlsbad, CA). All oligonucleotides used in the experiments were synthesized by Life Technologies, Inc. Other chemicals were purchased from Sigma, Roche Molecular Biochemicals and J. T. Baker Inc.

Plasmid Construction and Yeast Two-hybrid Screening—For construction of plasmid pCR2.1/Nrf-2, reverse transcription-PCR was performed using the primer pair P1–P2 (see Fig. 1). A 2017-bp Nrf-2 cDNA (+27 to +2043 bp) fragment was derived from H157 mRNA. The PCR

This paper is available on line at http://www.jbc.org

Vol. 274, No. 31, Issue of July 30, pp. 22095–22101, 1999
Printed in U.S.A.

© 1999 by The American Society for Biochemistry and Molecular Biology, Inc.
product was then ligated into the pCR2.1 vector. To construct plasmid pcDNA3.1/Nrf-2, the Nrf-2 cDNA fragment was cut from pcDNA3.1/Nrf-2 by KpnI-EcoRV and inserted into the pcDNA3.1(−) vector digested with the same enzymes. To construct plasmid pcDNA3.1/PMP-1, the PMP-1 cDNA was cut from pcCR2.1/PMP-1 with EcoRI-XhoI and cloned into the pcDNA3.1(−) vector digested with the same restriction enzymes. The pBKH-93 plasmid was constructed as previously reported (14). To make pBKH-93(ΔPRE), the complementary oligonucleotides P3 and P4 (see Fig. 1) were annealed, and the protruding ends were filled with Taq DNA polymerase. The resulting 30-bp double-stranded DNA fragment containing two PRE elements in the same direction (GACCCTAT-GAAGCGTGCTCTAAACC) was cloned into the pCR2.1 vector. The PRE-containing fragment was then excised by KpnI-XhoI digestion and inserted into pBKH-93 in the same restriction sites.

Yeast two-hybrid screening was performed with the Matchmaker two-hybrid system. To construct the bait plasmids, the coding region of the Nrf-2 cDNA (+39 to +2043 bp) and the leucine zipper domain (+1338 to +2043 bp) of Nrf-2 protein were produced by PCR with pCR2.1/Nrf-2 as the template. The primers used to amplify full-length Nrf-2 (P5 and P7) or the Nrf-2 leucine zipper domain (P6 and P7) were designed to contain an XbaI or SalI restriction site in the 5′-end, respectively (see Fig. 1, underlined sequences). The PCR products were digested with XbaI-SalI and then subcloned in frame into the same restriction sites in the DNA-binding domain of Gal4 in the pAS2.1 vector. To construct the pACT-2/H157 cDNA library that expressed proteins fused to the Gal4 transactivation domain in the pACT-2 vector, which had been digested with EcoRI and dephosphorylated with calf intestine alkaline phosphatase. The pACT-2/H157 DNA library was then screened by the ‘HIS3 Jump-Start’ procedure according to the protocol from the manufacturer.

Saccharomyces cerevisiae Y190 cells were first transformed with bait plasmids and selected on synthetic dextrose medium lacking tryptophan (SD-Trp). The transformants grown on the SD-Trp medium were subsequently transformed with the pACT-2/H157 cDNA library and selected on medium lacking tryptophan and leucine (SD-Trp-Leu). The clones co-transformed with the bait and library were collected and replated onto medium lacking tryptophan, leucine, and histidine (SD-Trp-Leu-His) with 30 μg 3-amino-1,2,4-triazole to inhibit the leaking growth of Y190 cells. The clones selected in this step were further assayed for their β-galactosidase activity. The pACT-2 library plasmids were purified from individual positive clones and amplified in Escherichia coli. Sequencing of the cDNA insert in the positive clones was performed with a Perkin-Elmer ABI Automated DNA Sequencer.

Cloning PMF-1 cDNA from the Human Placental Retroviral cDNA Library—The P10 primer, representing the 5′-lplb vector sequence upstream from the multiple cloning site, and the P11 primer, corresponding to sequence in the 3′-end of PMF-1 cDNA (997–1020 bp), were used to clone the PMF-1 cDNA from a human placental retroviral cDNA library into the pFL vector (CLONTech) by PCR. This PCR product was then subcloned into the pCR2.1 vector (pCR-2/PMP-1).

Human BAC Library Screening—A human BAC library was screened to obtain the PMF-1 genomic sequence using the PCR protocol supplied by the manufacturer. Three pairs of primers used in the PCR screening were designed according to PMF-1 cDNA sequence (P12-P13, P14-P15, and P16-P17) (see Fig. 1). P-6, the positive BAC library clone, was digested with HindIII restriction enzyme and subcloned into the pBluescript SK vector. The P-6 sublibrary was then screened for the clones containing PMF-1 genomic fragments (clones A, B, and E) by the colony lift procedure and subjected to Southern blot analysis with random primer-labeled (19, 20) PMF-1 cDNA. Another three PMF-1 genomic DNA fragments (clones C, D, and E) were obtained by PCR techniques with the P-6 plasmid as the template. The DNA inserts in the positive clones were sequenced on the ABI Automated DNA Sequencer.

Chromosomal Localization of PMF-1—The radiation hybrid screening technique with the Stanford G3 RH panel (Research Genetics) was used to determine the chromosomal location of PMF-1. As per the supplier's recommendation, the primers used in this study were those that were unique to human chromosome 23 (see Fig. 1). Fluorescent in situ hybridization analysis was performed as previously reported (21) using the labeled 5-kb B and 10-kb E fragments (see Fig. 4A) as probes.

In Vitro Transcription and Translation—In vitro transcription and translation were performed with the TnT coupled transcription/translation system using [α-35S]methionine according to the manufacturer's protocol. Purified plasmid pcDNA3.1/PMP-1 was used as the template. The labeled translation products were separated by 15% SDS-polyacrylamide gel electrophoresis and exposed to Kodak X-Omat film.

RNA Isolation and Northern blot analysis—Total cellular RNA from H157 and H82 cells was extracted using the acid phenol-guanidine isothiocyanate method (22). Poly(A)+ RNA was isolated using oligo(dT)-cellulose chromatography following the manufacturer's directions. The total RNA from H157 or H82 cells was separated on a denaturing 1.5% agarose gel containing 6% formaldehyde, transferred to GeneScreen membrane (NEN Life Science Products), and hybridized with a random primer-labeled PMF-1 cDNA as a probe. Blots were washed and reprobed with a 28 S ribosomal cDNA probe as a loading control. The human multiple-tissue Northern blot system was used to examine the expression of PMF-1 in various human tissues according to the manufacturer's protocol.

Transient Transfection Assays—For transient transfection, 2 × 10⁵ H157 cells were seeded in a 35-mm diameter culture dish and cultured in RPMI 1640 medium containing 5 μg 2-difluoromethylornithine for 48 h to reduce endogenous polyamines and background transcription, as previously reported (14). The Lipofectin-mediated transfection was performed using 1.5 μg of the reporter constructs described under “Results” and 0.4 μg of control plasmid pSVβ-galactosidase according to the manufacturer's protocol. After a 5-h incubation, the DNA-Lipofectin complex-containing medium was replaced by RPMI 1640 medium containing 5 μg 2-difluoromethylornithine. Forty-eight h after transfection, the cells were exposed to 10 μM BENSpm for 2 h. The cells were harvested, quick-frozen, and subsequently prepared for luciferase activity measurements as per the instructions of the manufacturer. To account for variations in transfection efficiency, the luciferase activity was normalized to the β-galactosidase activity.

Results

Identification of PMF-1 as a Co-transcription Partner of Nrf-2—The Nrf-2 transcription factor was found to be associated with the cell type-specific expression of SSAT in response to the antitumor polyamine analogues by binding to the newly identified PRE (14). However, Nrf-2 was found to bind constitutively to the PRE, strongly suggesting an association with another protein that mediates the analogue-induced expression of SSAT. To identify proteins that can interact with Nrf-2 in the H157 cell line, the yeast two-hybrid screening technique was used. In initial experiments, two pAS2.1 constructs were used as bait: full-length Nrf-2 (amino acids 1–589) and the leucine zipper domain of Nrf-2 (amino acids 434–589). Each construct was fused to the Gal4 DNA-binding domain. However, in testing, the full-length Nrf-2 fusion plasmid (pAS2.1/Nrf-2) strongly activated the lacZ and HIS3 reporter genes in the host Y190 cells in the absence of the Gal4 transcriptional activation domain (data not shown). Consequently, the bait plasmid containing only the leucine zipper domain of Nrf-2 (pAS2.1/Nrf-2-LZ) was used for further experimentation. An H157 cDNA library that expressed proteins fused to the Gal4 transcriptional activation domain was then constructed and screened by the HIS3 Jump-Start procedure (see “Experimental Procedures”). Thirty-three clones from ~10⁶ yeast transformants were identified that could activate the reporter genes. Inserts of the positive clones were amplified by PCR with Matchmaker 5′ (P8) and 3′ (P9) (Fig. 1) binding domain insert screening amplimers and digested with restriction enzyme AluI. Gel electrophoresis/ethidium bromide staining results demonstrated an insert of ~1338 bp (data not shown), which was designated as PMF-1. To confirm the interaction between the Nrf-2 leucine zipper domain and PMF-1, Y190 yeast cells were transformed with pAS2.1/Nrf-2-LZ alone, pACT-2/PMP-1 alone, or pAS2.1/Nrf-2-LZ and pACT-2/PMP-1 together. Transformants were then selected on SD−His−Leu−Trp medium containing 30 μM 3-amino-1,2,4-triazole. Only clones co-transformed with pAS2.1/Nrf-2-LZ and pACT-2/PMP-1 grew on the
selection medium, demonstrating transcriptional activation of
the reporter gene (Fig. 2). Sequencing the insert in the positive
clone (pACT-2/PMF-1) revealed a 958-bp cDNA that coded a
novel Nrf-2 partner. To confirm the PMF-1 sequence, the cDNA
was PCR-cloned using the retroviral cDNA library derived
from human placenta. Using this method, a transcript of 1057
bp was identified. This clone contains 99 more bases in the
5'-end as compared with the cDNA obtained from the yeast
two-hybrid assay. This message size correlates well with the
mRNA length estimated by Northern blotting (see below).

PMF-1 cDNA contains an open reading frame of 495 bp and
is predicted to encode for a protein of 165 amino acids with a
calculated molecular mass of 19.2 kDa (Fig. 3). The sequence
surrounding the translational initiation codon (5'-GACAC-ATG
3') is a nearly perfect consensus with the Kozak trans-
lational consensus sequence (23–25), with 9 out of 10 matches
for the proposed optimal context (5'-GCC(A/G)CCATGG-3').
The polyadenylation signal sequence (AATAAA) is found at nucle-
otide 1025, 13 bp upstream from the poly(A) tail. A leucine
zipper-like structure with IXX6IXLXXIXLX6L was found in the
N-terminal region and is a possible domain responsible for the
protein-protein interaction between Nrf-2 and PMF-1.

Genomic Structure of PMF-1—
To obtain the PMF-1 gene, the
human BAC library was screened by PCR techniques. Screen-
ing at three consecutive levels of the library (BAC super pool
library, BAC plate pool library, and BAC single clone library)
resulted in the discovery of a positive clone, P-6, which was
used as a template for all three primer pairs (Fig. 1). To
facilitate plasmid preparation and sequencing, the DNA insert
in the P-6 clone was digested with HindIII and subcloned into
the pBluescript SK vector. The P-6 subclone library was then
screened by colony lift assay and Southern blot analysis with
the PMF-1 cDNA probe. Three positive clones (A, B, and E)
were found to contain the PMF-1 genomic fragments. The iden-
tification of DNA sequences of clones A, B, and E allowed for
the design of new primers (Fig. 1) for cloning DNA sequences
between A and B (fragment AB) and between B and E (frag-
ments C and D) using the PCR technique with the P-6 plasmid
as the template.

The overall length of the assembled human PMF-1 genomic
sequence from the above clones is 28 kb. These clones span
the entire cDNA region of PMF-1 and define the five complete
exons and four introns (Fig. 4A). Clone A also defines 11.2 kb
upstream from the transcriptional start site. The sequences in
the exon/intron junctions and the sizes of the introns and exons
are shown in Table I. All of the splice junctions in the PMF-1
gene conform to consensus sequence established for splice do-
nor (5'-GT(A/G)AGT-3') and splice acceptor (5'-((Py)nN(C/
T)AG-3') sites (26). Exon 1 contains 111 bp of 5'-untranslated
sequence and the first 41 bp of coding sequence. Exon 5 con-
tains the sequence coding for the last 17 amino acids of PMF-1,
the stop codon (TGA), and a 434-bp 3'-untranslated sequence of
the PMF-1 cDNA.
It should be noted that extensive searching of the GenBank™ data base indicates that the PMF-1 cDNA and genomic sequences are unique. No previous expressed sequence tags, cDNAs, or genomic clones of this region have been reported.

Putative Transcription Factor-binding Sites in the PMF-1 Promoter Region—The TESS program was used to identify putative binding sites (Fig. 4B) for known transcription factors in the PMF-1 promoter region 2 kb immediately upstream from the transcriptional start site.3 It should be noted that one potential PRE-binding site (14) was identified in this region (−1450 to −1442 bp). This site has 8 out of 9 base pairs identical to the previously described PRE (14) and oriented in the antisense direction. Although several putative transcription factor-binding sites have been identified, further experimentation will be necessary to determine whether any of the identified sites have any actual function. It should be noted that, similar to SSAT (21), the transcription of PMF-1 is driven by a TATA-less promoter.

Chromosomal Location of PMF-1—The radiation hybrid screening technique and the Stanford G3 RH panel were used to localize the human PMF-1 gene. Primers P22 and P23, spanning from within exon 4 into intron 4, were used, and the results indicate that the PMF-1 gene is on the long arm of chromosome 1 at the 1q12 locus. Since this region represents the large pericentric heterochromatin of 1q and the resolution of the markers determined from the radiation hybrid screening was not sufficient to precisely place PMF-1, fluorescent in situ hybridization analysis was performed to confirm the location of PMF-1. The results of 22 G-banded metaphase chromosome pairs indicated PMF-1 to be at the 1q12/1q21 border (14 of 22 signals were at the 1q12/1q21 border).

In Vitro Transcription and Translation of PMF-1 cDNA—In vitro transcription and translation of full-length PMF-1 cDNA

---

3 TESS can be found at the following URL: http://dot.imgen.bcm.tmc.edu:9331/ under "Gene Features Search."
produced a major band with an apparent molecular mass of ~20 kDa, which agrees with the predicted open reading frame (Fig. 5).

**Expression of PMF-1 mRNA in Human Tissues**—The expression of PMF-1 mRNA in a variety of normal human tissues was evaluated by Northern blot analyses. A 1.2-kb mRNA transcript of PMF-1 was observed and appears to be expressed almost ubiquitously, although at different levels, in multiple tissues. The heart and skeletal muscle were among the highest in PMF-1 expression, with significant levels expressed in the kidney and liver (Fig. 6).

**Expression of PMF-1 in Response to Polyamine Analogue Exposure in Human Lung Cancer Cells**—To determine if PMF-1 expression can be induced by treatment with the polyamine analogue BENSpm in a cell type-specific manner, total RNA from the analogue-sensitive H157 and analogue-insensitive H82 cells was analyzed by Northern blot analysis after a 24-h exposure to 10 μM BENSpm. Significant induction of PMF-1 mRNA was detected only in the polyamine analogue-sensitive cell line, H157 (Fig. 7). To further characterize the expression of PMF-1 in H157 cells, a time course analysis of BENSpm exposure was performed. PMF-1 was induced in a biphasic manner, peaking first at ~4 h post-treatment and reaching levels >5-fold after a 24-h exposure to BENSpm (Fig. 8). It should be noted that longer treatment times of H157 cells with BENSpm are precluded since there is significant drug-induced toxicity between 12 and 24 h of exposure (3, 27). It is also important to note that, although the biphasic nature of PMF-1 expression was reproducible in several experiments, the biological significance of this observation is not currently known. Once a PMF-1 antibody is developed, definitive experiments will be performed to determine whether the biphasic nature of message expression is mirrored by protein expression.

**Functional Analysis of PMF-1 by Transient Transfection of H157 Cells**—To determine whether PMF-1 could induce PRE-mediated transcription of the SSAT promoter, two reporter plasmids were constructed. One plasmid, pBKH-93, contains the minimal promoter region of the SSAT gene (~93 to ~1 bp) upstream from the luciferase gene. The second plasmid, pBKH-93/dPRE, contains the minimal promoter region and a 30-bp oligonucleotide containing two PRE consensus sequences (14) (TATGACTAA) cloned into a site upstream from the luciferase gene. The assay was performed in the presence of [35S]methionine with 2 μg of plasmid as template in a 50-μl TnT reaction. The labeled translation products were then separated by 15% SDS-polyacrylamide gel electrophoresis. The templates used in the assays were pcDNA3.1/PMF-1 (lane A) and the pcDNA3.1 vector (lane B). The arrow indicates the position of PMF-1 protein.

**DISCUSSION**

The superinduction of the rate-limiting step in polyamine catabolism, SSAT, has been implicated in the cytotoxic response of some tumor types to treatment with antineoplastic polyamine analogues (6, 9, 29). Although regulated at many levels, the first and necessary step is a modest increase in transcription (12, 30). The recently discovered PRE and associated Nrf-2 transcription factor necessary for the transcriptional induction of SSAT provided an excellent lead for defining other proteins required in the transcriptional regulation of SSAT. That Nrf-2 was found to be constitutively bound to the PRE in sensitive cell types even in the absence of treatment suggested that other factors were involved in the induction response (14). In this work, a transcriptional cofactor has been

---

**TABLE I**

**Exon/intron organization of the human PMF-1 gene**

| Exon | Exon size | Sequence at exon/intron junction | Intron size | Codons interrupted |
|------|-----------|---------------------------------|-------------|-------------------|
| 1    | 152       | GGC AG gtaaag------cgccag C TAC | ~20 kb      | Ser<sup>14</sup>  |
| 2    | 106       | ATC CGG gggagt------ctctca G GAA | 1203 bp     | Arg<sup>69</sup>Glu<sup>50</sup> |
| 3    | 101       | GCC TG gtaga------ctccag G CGC  | 2559 bp     | Trp<sup>83</sup>  |
| 4    | 196       | TGG CAG gtcagt------ttttca GCT CTA | 3066 bp     | Gln<sup>146</sup>Ala<sup>149</sup> |
| 5    | 488       |                                  |             |                   |

*Exon sequences are in uppercase letters, and intron sequences are in lowercase letters.*
identified using the yeast two-hybrid system. This factor, designated PMF-1, binds to the leucine zipper region of Nrf-2, resulting in increased transcription.

More important, PMF-1 demonstrates a cell type-specific response to analogue exposure. Specifically, PMF-1 appears to be induced only in the analogue-responsive cell types. Furthermore, using a luciferase reporter construct system, PMF-1 is capable of inducing PRE-regulated transcription, even in the absence of analogue. These results are consistent with the hypothesis that Nrf-2 is constitutively expressed in H157 cells and that an increase in PMF-1, either as a result of analogue treatment or by direct transfection, is capable of increasing PRE-mediated transcription in human cells. However, it is currently not known whether preexisting PMF-1 protein is important in the activation of SSAT expression or if newly synthesized protein is required. These experiments will require the development of an antibody against the new PMF-1 protein.

Although PMF-1 clearly plays a role in the transcriptional regulation of SSAT, it is possible that it may be responsible for the regulation of other genes, in combination with either Nrf-2 or other potential partners. It may be significant that the highest expression of PMF-1 in normal tissues occurs in tissues that are highly differentiated, including heart, skeletal muscle, kidney, and liver. The possibility that PMF-1 expression is involved in differentiation or is inversely proportional to growth cannot presently be excluded. Again, it is important to emphasize that the relative amounts of PMF-1 protein may be critically important in determining its role in transcriptional regulation for both SSAT and other potential gene targets in response to various stimuli. However, the performance of studies to investigate PMF-1 protein levels and roles will require the development of a PMF-1-specific antibody.

It has been demonstrated that c-Myc transcription is negatively regulated by polyamine depletion in several tumor types along with other growth-related genes (32–35). Recently, it has been demonstrated that the depletion of polyamines by 2-difluoromethylornithine or the alteration of polyamine metabolism through the use of analogues leads to cell cycle arrest and can induce apoptosis through a p53-mediated mechanism.
lated by PMF-1 is underway.

search for other transcription factors whose activity is modu-
modulated by PMF-1 or similar transcriptional cofactors. The
polyamines (15–18, 28, 41–42). These genes may in turn be
transformation potential of increased polyamine biosynthesis
of ornithine decarboxylase as a means of determining the
neoplastic phenotype. Recent studies using the overexpression
may play a role in the regulation of genes associated with the
activities are typically higher in tumors than in surrounding
(36, 37). Since polyamines and polyamine biosynthetic enzyme
activities are typically higher in tumors than in surrounding
normal tissue (38–41), the resulting higher polyamine content
may play a role in the regulation of genes associated with the
neoplastic phenotype. Recent studies using the overexpression
of ornithine decarboxylase as a means of determining the

REFERENCES
1. Marton, L. J., and Pegg, A. E. (1995) Annu. Rev. Pharmacol. 35, 55–91
2. Saab, N. H., West, R. E., Bieszk, N. C., Preuss, C. V., Mank, A. R., Casero,
R. A., and Woster, P. M. (1993) J. Med. Chem. 36, 2998–3004
3. Casero, R. A., Mank, A. R., Saab, N. H., Wu, R., Dyer, W. J., and Woster,
P. M. (1995) Cancer Chemother. Pharmacol. 36, 69–74
4. Bernacki, R. J., Oberman, E. J., Seweryniak, K. E., Atwood, A., Bergeron, R. J.,
and Porter, C. W. (1995) Clin. Cancer Res. 1, 847–857
5. Bergeron, R. J., Feng, Y., Weimar, W. R., McManis, J. S., Dimova, H., Porter,
C., Raisler, B., and Phanstiel, O. (1997) J. Med. Chem. 40, 1475–1494
6. Casero, R. A., Celano, P., Ervin, S. J., Porter, C. W., Bergeron, R. J., and Libby,
P. R. (1989) Cancer Res. 49, 3829–3833
7. Casero, R. A., Mank, A. R., Xiao, L., Smith, J., Bergeron, R. J., and Celano, P.
(1992) Cancer Res. 52, 5359–5363
8. Alhonen, L., Karpipinen, A., Uusi-Oukari, M., Vujicic, S., Korhonen, V.-P.,
Halmekyto, M., Kramer, D. L., Hines, R., Janne, J., and Porter, C. W. (1996)
J. Biol. Chem. 271, 1964–1969
9. Porter, C. W., Ganis, B., Libby, P. R., and Bergeron, R. J. (1991) Cancer Res.
51, 3715–3720
10. Porter, C. W., Bernacki, R. J., Miller, J., and Bergeron, R. J. (1993) Cancer Res.
53, 581–586
11. Ha, H. C., Woster, P. M., Yager, J. D., and Casero, R. A., Jr. (1997) Proc. Natl.
Acad. Sci. U. S. A. 94, 11557–11562
12. Fogel-Petrovic, M., Shappell, N. W., Bergeron, R. J., and Porter, C. W. (1993)
J. Biol. Chem. 268, 19118–19125
13. Xiao, L., and Casero, R. A. (1996) Biochem. J. 323, 691–696
14. Wang, Y., Xiao, L., Thigalingam, A., Nelkin, B. D., and Casero, R. A., Jr.
(1999) J. Biol. Chem. 273, 34623–34630
15. Smith, M. K., Trempus, C. S., and Gilmour, S. K. (1998) Carcinogenesis (Lond.)
19, 1654–1659
16. Peralta, S. A., Gilliard, G., Megosh, L., George, K., and O’Brien, T. G. (1998)
Cancer Res. 58, 5298–5302
17. Auvine, M., Laine, A., Paasinen-Sohns, A., Kangas, A., Kangas, L., Saksela,
O., Andersson, L. C., and Haltia, E. (1997) Cancer Res. 57, 3016–3025
18. Paasinen-Sohns, A., and Haltia, E. (1997) Oncogene 15, 1953–1966
19. Southern, E. M. (1975) J. Mol. Biol. 98, 503–517
20. Feinberg, A. P., and Vogelstein, B. (1984) Anal. Biochem. 137, 266–267
21. Xiao, L., Celano, P., Mank, A. R., Griffin, C., Jabs, E. W., Hawkins, A. L., and
Casero, R. A. (1992) Biochem. Biophys. Res. Commun. 187, 1493–1502
22. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
23. Kozak, M. (1989) J. Cell. Physiol. 108, 229–241
24. Kozak, M. (1991) J. Cell. Physiol. 145, 268, 897–903
25. Kozak, M. (1991) J. Biol. Chem. 266, 19867–19870
26. Mount, S. M. (1982) Nucleic Acids Res. 10, 459–472
27. McCloskey, D. E., Yang, J., Woster, P. M., Davidson, N. E., and Casero, R. A.,
Jr. (1996) Clin. Cancer Res. 2, 441–446
28. Shantz, L. M., and Pegg, A. E. (1998) Cancer Res. 58, 2574–2575
29. Stennicke, H. R., and Halazonetis, T. D. (1998) J. Cell. Physiol. 177, 69–74
30. Itoh, K., Chiba, T., Takahashi, S., Ishii, T., Igashiri, K., Katoh, Y., Oyake, T.,
Hayashi, N., Satoh, K., Hayakawa, Y., and Nishimura, Y. (1999) Biochim. Biophys.
Res. Commun. 266, 313–322
31. Celano, P., Baylin, S. B., and Casero, R. A., Jr. (1995) J. Biol. Chem. 270, 14991–14995
32. Kozak, M. (1991) J. Biol. Chem. 266, 19867–19870
33. Patel, A. R., and Wang, Y. J. (1997) Am. J. Physiol. 273, C1020–C1029
34. Bryans, M., Harley, E., and Gilmour, S. K. (1996) Biochem. Biophys. Res.
Commun. 236, 618–625
35. Tabib, A., and Bachrach, U. (1994) Biochim. Biophys. Res. Commun. 202, 720–727
36. Ray, R. M., Zimmerman, B. A., McCormack, S. A., Patel, T. B., and Johnson,
L. R. (1999) Am. Phys. Soc. 276, C684–C691
37. Kramer, D. L., Vujicic, S., Begelman, P., Alderfer, J., Miller, J. T., Black, J. D.,
Bergeron, R. J., and Porter, C. W. (1999) Cancer Res. 59, 1278–1286
38. Lak, D. G., and Baylin, S. B. (1984) N. Engl. J. Med. 311, 80–83
39. Giardiello, F. M., Hamilton, S. R., Hylind, L. M., Yang, V. W., Tamez, P., and
Casero, R. A. (1992) J. Clin. Oncol. 10, 3016–3025
40. Marton, L. J., and Pegg, A. E. (1995) Annu. Rev. Pharmacol. 35, 55–91
41. Tabib, A., and Bachrach, U. (1998) Int. J. Biochem. Cell Biol. 30, 133–146
42. Kubota, S., Kiyosawa, H., Nomura, Y., Yamada, T., and Seyama, Y. (1997)
J. Natl. Cancer Inst. 89, 567–571