Cloning and characterization of the mouse polyamine-modulated factor-1 (mPMF-1) gene: an alternatively spliced homologue of the human transcription factor

Yanlin WANG*, Wendy DEVEREUX*, Patrick M. WOSTER† and Robert A. CASERO, JR*¹

*The Johns Hopkins Oncology Center, Bunting Blaustein Cancer Research Building, Room 551, 1650 Orleans Street, Baltimore, MD 21231, U.S.A., and †Department of Pharmaceutical Sciences, 539 Shaper Hall Wayne State University, Detroit, MI 48202, U.S.A.

The natural polyamines and their analogues have been implicated in transcriptional regulation of specific genes. Human polyamine-modulated factor-1 (hPMF-1) was the first polyamine-responsive transcription factor identified. Here the mouse homologue of the hPMF-1 gene is described. Interestingly, the mouse gene (mPMF-1) codes for two alternatively spliced mRNAs. Both of the mouse splice variants, mPMF-1S and mPMF-1L, possess C-terminal coiled-coil domains nearly identical to that found in hPMF-1 and are highly homologous with the human protein. The C-terminal coiled-coil structure is necessary for transcriptional activation. However, the shorter protein, mPMF-1S, does not contain an N-terminal coiled-coil region as do both hPMF-1 and the longer mPMF-1L. mPMF-1L mRNA codes for a protein

of 202 amino acids, 37 amino acids longer than the human protein. By contrast, mPMF-1S codes for only 133 amino acids, as a result of two exons being omitted compared with mPMF-1L. Both mouse transcription factors can interact with Nrf-2 (nuclear factor-E2-related factor 2), the normal partner of hPMF-1, substantiating the importance of the C-terminal coiled-coil region responsible for this interaction. Finally, the expression of mPMF-1 is induced when mouse M1 myeloid leukaemia cells are exposed to polyamine analogues, suggesting control similar to that observed for the hPMF-1.

Key words: coiled-coil, leucine zipper, transcription.

INTRODUCTION

The eukaryotic polyamines putrescine, spermidine and spermine are naturally occurring cationic alkylamines that are absolutely required for cell growth and differentiation [1]. Their tightly regulated metabolic pathway bespeaks the importance of these organic cations. Increased activity of the first rate-limiting step in polyamine biosynthesis, ornithine decarboxylase, frequently accompanies cellular transformation, suggesting that polyamines are not only important for normal cell growth but, when strict regulation of the pathway breaks down, they may also have a part in the neoplastic transformation process [2–4]. Because of their importance in cell growth and in the carcinogenic process polyamine metabolism has become a target for anti-neoplastic intervention [5]. Although the requirement of polyamines for cell growth has long been established, only recently have precise molecular roles been attributed to them. One such role that we and others have demonstrated is that intracellular polyamines have a significant effect in modulating specific gene expression [6–9]. The depletion of polyamines by inhibiting ornithine decarboxylase results in a specific decrease in the transcription of various early growth-related genes including *c-myc*, *c-fos* and *c-jun* [6–8]. Conversely, the exposure of several tumour types to the natural polyamines or anti-tumour polyamine analogues leads to a rapid increase in the expression of the rate-limiting step in polyamine catabolism, spermidine/spermine *N*¹-acetyltransferase (SSAT). Initially, this increase in expression of SSAT occurs at the level of transcription [10,11]. Using the SSAT gene as a model, we recently identified a polyamine-responsive element

(PRE), and the *trans*-acting protein factors that are involved in the transcriptional activation of this gene [12–14]. Here [12] the mouse homologue of one of these factors, the human polyamine-modulated factor-1 (hPMF-1) transcription factor, is described. Importantly, the cloning of the mouse PMF-1 (mPMF-1) revealed previously undetected alternatively spliced PMF-1 mRNAs. The resulting two proteins have significantly different predicted tertiary structures and may consequently have different functional roles.

EXPERIMENTAL

Chemicals and reagents

The radionucleotides [α -³²P]dCTP and [³⁵S]methionine were supplied by Amersham Pharmacia Biotech (Piscataway, NJ, U.S.A.). TnT-coupled transcription/translation rabbit reticulocyte lysate system was purchased from Promega (Madison, WI, U.S.A.). The TA cloning kit was purchased from Invitrogen (Carlsbad, CA, U.S.A.). Trizol total RNA reagent was from Life Technologies (Rockville, MD, U.S.A.). Restriction and DNA-modifying enzymes were purchased from New England Biolabs (Beverly, MA, U.S.A.), Life Technologies and Sigma (St. Louis, MO, U.S.A.). Life Technologies synthesized all oligomers used in the experiments. The yeast two-hybrid system was purchased from Clontech Laboratories (Palo Alto, CA, U.S.A.). DNA sequencing was performed with a Perkin-Elmer ABI automated DNA sequencer. Other chemicals came from Sigma, Roche Molecular Biochemicals (Indianapolis, IN, U.S.A.), Bio-Rad

Abbreviations used: ES, embryonal stem; PMF-1, polyamine-modulated factor-1; hPMF-1, human PMF-1; mPMF-1, mouse PMF-1; Nrf-2, nuclear factor-E2-related factor 2; SSAT, spermidine/spermine *N*¹-acetyltransferase; BENSpm, *N*¹,*N*¹-bis(ethyl)norspermine; CPENSpm, *N*¹-cyclopropylmethyl-*N*¹-ethyl-norspermine; EST, expressed sequence tag.

¹ To whom correspondence should be addressed (e-mail rcasero@jhmi.edu).

The nucleotide sequences reported here have been submitted to the GenBank Nucleotide Sequence Database under the accession numbers AF348509, AF348510, AF348511 and AF348512.

Primers for cloning MPMF-1L and MPMF-1S cDNA

P1. 5'-TTGGCGGTTCATTTCAACATGGC

P2. 5'-TTTTTTTTTGTGTGGGAAAGGAAG

Primers for cloning mouse PMF-1 genome fragment D

P3. 5'-CAAACACTTCCACCAGTTGAACCC

P4. 5'-AGCTTCTAGTTCCCTCTCTTT

Primers for cloning mouse PMF-1 genome fragment E

P5. 5'-AAAGAGGAGGGGAACCTAGAAGCT

P6. 5'-AGCGTCGGCCAGTTCCTGGTTCTT

Primers for cloning mouse PMF-1 genome fragment F

P7. 5'-AAGAACCAGGAAGTGGCCGACGCT

P8. 5'-AAGTTATTACGGATGTGGGGAG

Primers for cloning mouse PMF-1 genome fragment A

P9. 5'-GCCATGTTGAAATTGAACCGCCAA

P10. 5'-AGGTGAGCCGAGATAGCGAGGCTG

P11. 5'-CAGGAGTTTACCCTGGGGATGGT

P12. 5'-CGCCATAGTAGACACTTTCCTCCA

Primers for cloning mouse PMF-1 genome fragment C

P13. 5'-CGCAGAGGATCTATGACAAGTTTG

P14. 5'-GGGTTCAACTGGTGGAAAGTGTG

Primers for cloning mouse PMF-1 genome fragment B

P15. 5'-ATAGAAGAAGGCAGAGAGCGCGGA

P16. 5'-GGGGCTGTCTCCAAGGCTGGTGG

Primers for constructing pcDNA3.1/MPMF-1L and pcDNA3.1/MPMF-1S

P17 5'-ATTAAGGATCCACATGGCTGAGGTGAGCCGAG

BamHI

P18 5'-AAATTGAGCTCGGGACTCCATTACTGCCTGG

SacI**Figure 1** Primer sequences used for PCR cloning

(Hercules, CA, U.S.A.) and J. T. Baker (Phillipsburg, NJ, U.S.A.).

Cloning of mPMF-1 cDNAs and genomic DNA

Reverse transcriptase PCR was used to clone mPMF-1 cDNAs. Simply, oligo(dT) and avian myeloblastosis virus reverse transcriptase were used to prime the first strand of cDNA from 2 µg of total RNA derived from a mouse embryonal stem (ES) cell line or mouse liver. The mPMF-1 cDNAs were amplified from the first strand of cDNA by PCR and then cloned into the pCR2.1 vector (Invitrogen; pCR2.1/MPMF-1L and pCR2.1/MPMF-1S). The 3' region of mPMF-1 genomic DNA was obtained by PCR using normal mouse chromosomal DNA as template. Inverse PCR was used to clone the 5' region.

Plasmid construction

For construction of pcDNA3.1/MPMF-1L and pcDNA3.1/MPMF-1S, the mPMF-1L or mPMF-1S cDNA fragments (for the long and short splice variants of mPMF-1) were cut from pCR2.1/MPMF-1L or pCR2.1/MPMF-1S by *Bam*HI/*Xho*I digestion and then inserted into the pcDNA3.1(+) vector (Invitrogen) into the same restriction sites. To make pACT2/MPMF-1L and pACT2/MPMF-1S, the mPMF-1L or PMF-1S cDNAs were amplified by PCR using primer pair P17 and P18 (Figure 1). PCR products were digested with *Sac*I and *Bam*HI and then they were cloned in-frame into pACT2 vector (Clontech) treated with the same restriction enzymes. The bait plasmid pAS2.1/Nrf2-LZ was constructed as previously reported [12,13].

```

TTGGCGGTTCAATTTCAACATGGCTGAGGTGAGCCGAGATAGCGAGGCTG
      M A E V S R D S E A
CGGAAAGGGGGCTGAGGGCTCCTCTCCGGAAGCTGTGCCAGGGGACGCG
A E R G P E G S S P E A V P G D A
ACCATCCCCAGGGTGAACTCCTGGAGCCATAGTAGACACTTTCCTCCA
T I P R V K L L D A I V D T F L Q
GAAGCTAGTCGCCGACAGGAGCTACGAGAGGTTACCACCTGCTACAAAC
K L V A D R S Y E R F T T C Y K
ACTTCCACCAAGTTGAACCTGAGGTGACGCGAGGATCTATGACAAGTTT
H F H Q L N P E V T Q R I Y D K F
GTGGCTCAGTTGCAGACATCCATCCGCGAGGAAATCTCAGAAATCAAAGA
V A Q L Q T S I R E E I S E I K E
GGAGGGGAACCTAGAAGCTGTCTGAACTCCCTGGATAAGATCATAGAAG
E G N L E A V L N S L D K I I E
AAGGCAGAGAGCGCGGAGAGCCAGCTGGCGACCCAGTGAATCCGAGAG
E G R E R G E P A W R P S G I P E
AAAGACCTGTGTAGTGTCTATGGCCACCTACTTCTGAAGCAACAGGATAC
K D L C S V M A P Y F L K Q Q D T
CCTGTGTCATCAAGTACGGAAACAGGAAGCCAAGAACCAGGAAGTGGCCG
L C H Q V R K Q E A K N Q E L A
ACGCTGTCTCTGGCCGGCGCAGGCAGGTGGAGAGCTGCAGCAGCAGGTT
D A V L A G R R Q V E E L Q Q Q V
CGGGCCCTCCAGCAACATGGCAGGCTCTACACAGAGAGCAGAGGAGCT
R A L Q Q T W Q A L H R E Q R E L
GCTGTGCTGCTGAGGGCGCTGAGTGAACAGCTGCTGGGCTCAGCCCTC
L S V L R A P E
TAGAGCGCTGTGAGGCAGTTTCCAGGCAGTAATGGAGTCCCCACCACC
GCTTAGTGTCTGCTGGGGTCACTGCCTTTCAGATTCTGCCGCCATCTT
CTGCTGCAGGATTGGGACTCCCACTTGGCATCAGACACCAGGCTTTGT
CTTCTGGAGTTGCAGCCAGCGCTGCCCATGCCCTTCTGATCCTCCCCCT
CTCCACCGCCCTCCCTGGATAGGATTGGGCGAGGTTTCAGTCTCCACT
GATTGCCGCTTGTCTCCTGACTGATTGCTGCCCTGCTGGCTAGCCCA
AGGGCCCTGCCATGTTCTCCCCACATCCGTAAATAAACTTCCTTTCCCA
ACAAAAA

```

Figure 2 Coding sequences of the two mPMF-1 splice variants

Nucleotide and amino acid sequences for the mPMF-1L cDNA are shown. The underlined region of the mPMF-1L amino acid sequence represents the region deleted from mPMF-1S.

In vitro transcription and translation

In vitro transcription and translations were performed with the TnT-coupled transcription/translation rabbit reticulocyte lysate system using [³⁵S]methionine according to the supplied protocol. Purified pcDNA3.1/MPMF-1L, pcDNA3.1/MPMF-1S and empty vector pcDNA3.1 were used as the templates. The labelled translation products were separated by SDS/PAGE (12% gel) and exposed to film.

RNA purification and Northern-blot analysis

Total cellular RNA from the ES cell line or mouse liver tissue were extracted using Trizol total RNA reagent according to the protocol from the manufacturer. Total RNA (10 µg) was separated on a denaturing 1.5% agarose gel containing 6% formaldehyde, transferred to Zetaprobe membrane (Bio-Rad), and hybridized with a random-primer-labelled mPMF-1L cDNA as a probe. Blots were washed and re-probed with an 18 S ribosomal cDNA probe as a loading control. For determination of the effects of polyamine analogue exposure on mPMF-1 expression, mouse myeloid leukaemia M1 cells (obtained from Dr M. J. Fackler, Johns Hopkins University, Baltimore, MD, U.S.A.) were treated with 10 µM N¹,N¹¹-bis(ethyl)norspermine

Figure 3 Sequence homology between human and mouse PMF-1 and predicted structures for the mouse splice variants

(A) The hPMF-1 and mPMF-1L share 79% identity and 89% similarity over the 165 amino acids of the human clone. (B) The longer mPMF-1L cDNA clone possesses nearly identical predicted coiled-coil structures in both the N- and C-terminal regions to those found in the hPMF-1 protein [13]. (C) The shorter splice variant, mPMF-1S, possesses only the C-terminal coiled-coil domain. It should be noted that the ordinate represents the total fraction of predicted coiled-coil structure. Additionally, each prediction uses three different frame sizes, 14 (short-dashed line), 21 (long-dashed line) and 28 (solid line) amino-acid windows.

(BENSpm) or 10 μ M N^1 -cyclopropylmethyl- N^{11} -ethylnorspermine (CPENSpm) for 24 h. RNA from treated and untreated control cells was then extracted and processed as above.

Yeast two-hybrid analysis

Yeast two-hybrid analysis was performed using the Matchmaker two-hybrid system. *Saccharomyces cerevisiae* Y190 cells were first transformed with the bait plasmid pAS2.1/Nrf2-LZ and selected on synthetic dextrose medium lacking tryptophan (SD-Trp). The transformants selected by the SD-Trp medium were subsequently transformed with pACT2/mPMF-1L, pACT2/mPMF-1S or vector pACT2 and selected on medium lacking tryptophan and leucine (SD-Trp-Leu). The clones co-transformed with bait and prey were used for the *lacZ* reporter-gene assay or for plating on to medium lacking tryptophan, leucine and histidine (SD-Trp-Leu-His) with 30 mM 3-amino-1,2,4-triazole for the HIS3 reporter-gene assay.

RESULTS

Cloning of mPMF-1 cDNAs and gene

As the first step in our efforts to clone the mPMF-1 gene, we used the hPMF-1 cDNA sequence to perform a homology search of the mouse expressed sequence tag (EST) sequences in GenBank. A mouse EST sequence (BE308097) was found to be highly homologous to the open reading frame region of the hPMF-1 cDNA. The EST BE308097 sequence was used to further search the same mouse EST library. This search resulted in three additional ESTs that contained more 5' (AW10022) and 3' (AA18600 and AW060657) sequence corresponding to the PMF-1 cDNA. The primers P1 and P2 (Figure 1) were designed according to the mouse EST sequence data and used to clone mPMF-1 cDNA by reverse transcriptase PCR. From total RNA of the mouse ES cell line, two mPMF-1 cDNAs were identified. The longer cDNA, mPMF-1L, consisted of 1014 bp, contained an open reading frame of 609 bp and was predicted to encode a protein of 202 amino acids with a calculated

Figure 4 Exon structure of the mPMF-1 gene

Note that exons II and III are not included in the mPMF-1S splice variant.

molecular mass of 23.1 kDa. mPMF-1L had 37 more amino acids in its N-terminus (Figure 2) than the hPMF-1 [12]. The shorter cDNA, mPMF-1S, contained 808 bp with an open reading frame that encoded a protein of 133 amino acids with a predicted molecular mass of 15.0 kDa, 32 amino acids shorter than the human counterpart (Figure 2). Identical results demonstrating two mPMF-1 RNAs were obtained when mRNA from normal adult mouse liver was used as the starting material (results not shown).

To facilitate the cloning of the mPMF-1 gene, primer pairs were designed (see Figure 1) according to the mPMF-1L cDNA sequence determined above and used to amplify the genomic DNA fragments D (599 bp; primers P3 and P4), E (3236 bp; primers P5 and P6), F (1794 bp; primers P7 and P8) by PCR. Fragments A (499 bp; primers P9–P12), B (1098 bp, primers P15 and P16) and C (2251 bp, primers P13 and P14) were cloned by inverse PCR using the indicated primers.

mPMF-1L codes for a protein that, over the 165 amino acids that share homology with the hPMF-1 protein, is 79% identical with and 89% similar to the human protein (Figure 3A). It should be noted that the same two coiled-coil motifs identified in the hPMF-1 were predicted from the mPMF-1L sequence [13,15–17]. The N-terminal coiled-coil domain spans amino acids 75–120 and the C-terminal coiled-coil domain spans amino acids 140–200 (Figure 3B). mPMF-1S contains only the C-terminal coiled-coil domain, which has been demonstrated to be required for the nuclear factor-E2-related factor 2 (Nrf-2)–PMF-1 interaction in the human cell lines (Figure 3C).

The overall length of the assembled mPMF-1 genomic sequence from the above fragments is more than 8 kb. These fragments span the entire coding region of mPMF-1 and define the five exons and four introns (Figure 4). The sequences in the exon/intron junctions and the sizes of the exons and introns are defined in Table 1. All of the splice junctions in the mPMF-1 gene conform to the consensus sequences established for a splice donor (5'-GTRAGT-3') and splicing acceptor (5'-Y_nNYAG-3').

From the mouse genomic construct, mPMF-1L was identified to represent a full-length transcript and mPMF-1S results from an apparent differential splicing pattern that deletes exons 2 and 3, connecting exons 1, 4 and 5.

***In vitro* transcription and translation of mPMF-1 isoforms and interactions with the human Nrf-2 transcription factor**

In vitro transcription and translation of mPMF-1L and mPMF-1S cDNAs were performed with the TnT-coupled rabbit reticulocyte lysate system. The translation of each cDNA produced a major band with an apparent molecular mass agreeing with the individual predicted open reading frame (Figure 5A).

To determine whether mPMF-1S and/or mPMF-1L function in a manner similar to their human counterpart in their ability to partner with the Nrf-2 transcription factor [13], yeast two-hybrid analysis was performed. In this study, the C-terminal portion of human Nrf-2 that contains the leucine-zipper domain of Nrf-2 was fused to the Gal4 DNA-binding domain (pAS2.1/Nrf-2-LZ) and mPMF-1L and mPMF-1S were each fused with Gal4-activation domain (pACT2/mPMF-1L and pACT2/mPMF-1S). When co-transforming the *S. cerevisiae* Y190 cells with the bait plasmid pAS2.1/Nrf-2-LZ and the prey plasmid pACT2/mPMF-1L or pACT2/mPMF-1S, each mPMF-1 isoform demonstrated the ability to activate the HIS3 (Figure 5B) and *lacZ* (results not shown) reporter genes in the two-hybrid system. These results verify the protein–protein interaction between the Nrf-2 and each of the mPMF-1 isoforms.

Expression of mPMF-1 in the ES cell line and liver tissue and induction by polyamine analogues

The expression of the mPMF-1 mRNA in the ES cell line and mouse liver tissue were evaluated by Northern-blot analysis. An mRNA band of ≈ 1 –1.2 kb was observed and appears to have been expressed at much higher levels in the ES cell line than in the liver tissue (Figure 6A).

hPMF-1 expression increases in response to exposure of human tumour cells to various polyamine analogues [12]. Therefore, to determine whether mouse tumour cells responded in a similar fashion, M1 mouse myeloid leukaemia cells were exposed to 10 μ M BENSp_m or 10 μ M CPENSp_m for 24 h. The results (Figure 6B) clearly indicate that each analogue was capable of inducing mPMF-1 ≥ 2 -fold. These results are similar to that observed for hPMF-1 [12]. It should be noted that it was not possible from the Northern-blot analysis to determine the prevalence of either mPMF-1L or mPMF-1S, because the resolution of the agarose gel was not sufficient to differentiate between the two.

Table 1 Exon/intron organization of the mPMF-1 gene

Exon sequences are in upper-case letters and intron sequences are in lower-case letters.

Exon no.	Exon size (bp)	Sequence at exon/intron junction		Intron size (bp)	Codons interrupted
		5' splice donor	3' splice acceptor		
1	190	AGG AG gtgagt ...	... tgacag C TAC	> 3000	Ser ⁵¹
2	106	ATC CGC gtgagt ...	... gctcag GAG GAA	475	Arg ⁸⁶ –Glu ⁸⁷
3	101	GCC TG gtgaga ...	... ccccg G CGA	3026	Trp ¹²⁰
4	196	TGG CAG gcaagt ...	... ttccag GCT CTA	1335	Gln ¹⁸⁵ –Ala ¹⁸⁶
5	428

Figure 5 Functional analysis of mPMF-1 splice variants

(A) mPMF-1 proteins produced in TnT reactions. Lane 1, empty vector control; lane 2, mPMF-1L protein; lane 3, mPMF-1S protein. (B) Both mPMF-1S and mPMF-1L are capable of pairing with Nrf-2 and activating transcription as determined in the yeast two-hybrid assay. *S. cerevisiae* Y190 cells were transformed alone or co-transformed as indicated in the Figure. The transformants were selected on SD-Trp-Leu-His medium with 3-amino-1,2,4-triazole. Clones co-transformed with pAS2.1Nrf-2/LZ and either pACT2mPMF-1L or pACT2/MPMF-1S were able to grow on the selection medium, indicating that both the long and short versions of mPMF-1 are capable of interacting with the leucine-zipper region of Nrf-2.

Figure 6 Expression of mPMF-1

(A) Northern blots demonstrating the expression of mPMF-1 in ES cells and mouse liver. Duplicate lanes are included for each. Note that the expression in the ES cells appears to be significantly higher than in the adult mouse liver. (B) Induction of mPMF-1 in mouse M1 cells. M1 cells were treated for 24 h with 10 μ M CPENSpm (lane 2) or 10 μ M BENSpm (lane 3). Lane 1 represents the untreated control cells. Based on PhosphorImager analysis, CPENSpm was found to induce mPMF-1 by 2-fold and BENSpm induced mPMF-1 by 2.6-fold in M1 cells. It should be noted that the resolution of the agarose gels depicted here is insufficient to differentiate between mPMF-1L and mPMF-1S.

DISCUSSION

The role of polyamines in the regulation of transcription has been implicated by many studies [6–9,18–20]. However, it has only directly been demonstrated for the transcriptional regulation of human SSAT [12,14]. To facilitate the development of a model to allow the systematic study of the importance of the transcript-

ional regulatory factor, PMF-1, the mPMF-1 gene and cDNA were cloned. Although there is considerable homology with their human counterpart, the cloning of mPMF-1 revealed alternative splice variants. mPMF-1L is 37 amino acids longer than the hPMF-1, whereas mPMF-1S is 32 amino acids shorter than the 165 amino acid hPMF-1. However, both mPMF-1L and mPMF-1S maintain C-terminal coiled-coil secondary structures nearly identical to the coiled-coiled structure of the human protein [13]. By contrast, the shorter mPMF-1S protein does not contain the coiled-coiled domain in the N-terminal region that is shared by the mPMF-1L and hPMF-1. The differences in secondary structure between the two splice variants suggest the potential of functionally distinct roles for each of the proteins. It should be noted though, that both proteins are capable of binding to the leucine-zipper region of human Nrf-2 [21–23] and thereby activate transcription in a manner that is indistinguishable from their human homologue [13]. However, since mPMF-1S does not contain the N-terminal coiled-coil domain, it would not have the ability to interact with putative partners that rely on such a structure.

Currently, no data exist to suggest that the hPMF-1 gene is expressed as multiple splice variants. However, it is entirely possible that in some cell types or tissues the hPMF-1 gene may express multiple splice variants. Studies to determine this possibility are ongoing.

It is important to note that the mouse myeloid leukaemia line, M1, responded to polyamine analogue treatment with an increase in mPMF-1 mRNA in a manner similar to increases in hPMF-1 mRNA observed in various human tumour cells in response to the same analogues. Although not as highly induced as hPMF-1, these results verify that the response to analogue treatment is conserved between mouse and human cells with respect to PMF-1 expression. It should be noted that the M1 basal level of mPMF-1 appears to be higher than that observed in the human non-small-cell lung cancer line, H157, used to determine human responsiveness [13].

hPMF-1 has been demonstrated in human cells to play a direct role in the regulation of SSAT gene transcription. However, it is possible that hPMF-1 and mPMF-1 are involved in the polyamine-modulated regulation of other genes and expression pathways. To facilitate the study of other potential targets, we have now cloned the mouse gene that encodes mPMF-1. Interestingly, the mouse gene has alternative splice variants, which have the potential to interact with different partner proteins, as suggested by their predicted tertiary structures. With the molecular tools now at hand it will be possible to determine the effects of a PMF-1 knockout and to determine the significance of the splice variants.

This work was supported by National Institutes of Health grants CA51085, CA85509 and CA58184.

REFERENCES

- Pegg, A. E. (1986) Recent advances in the biochemistry of polyamines in eukaryotes. *Biochem. J.* **234**, 249–262
- Chen, Y., Megosh, L. C., Gilmour, S. K., Sawicki, J. A. and O'Brien, T. G. (2000) K6/ODC transgenic mice as a sensitive model for carcinogen identification. *Toxicol. Lett.* **116**, 27–35
- Megosh, L., Gilmour, S. K., Rosson, D., Soler, A. P., Blessing, M., Sawicki, J. A. and O'Brien, T. G. (1995) Increased frequency of spontaneous skin tumors in transgenic mice which overexpress ornithine decarboxylase. *Cancer Res.* **55**, 4205–4209
- Giardiello, F. M., Hamilton, S. R., Hyland, L. M., Yang, V. W., Tamez, P. and Casero, Jr., R. A. (1997) Ornithine decarboxylase and polyamines in familial adenomatous polyposis. *Cancer Res.* **57**, 199–201
- Marton, L. J. and Pegg, A. E. (1995) Polyamines as targets for therapeutic intervention. *Annu. Rev. Pharmacol. Toxicol.* **35**, 55–91
- Wang, J. Y., McCormack, S. A., Viar, M. J., Wang, H., Tzen, C. Y., Scott, R. E. and Johnson, L. R. (1993) Decreased expression of protooncogenes c-fos, c-myc, and c-jun following polyamine depletion in IEC-6 cells. *Am. J. Physiol.* **265**, G331–G338
- Wang, J. Y. and Johnson, L. R. (1994) Expression of protooncogenes c-fos and c-myc in healing of gastric mucosal stress ulcers. *Am. J. Physiol.* **266**, G878–G886
- Celano, P., Berchtold, C. M., Giardiello, F. M. and Casero, Jr., R. A. (1989) Modulation of growth gene expression by selective alteration of polyamines in human colon carcinoma cells. *Biochem. Biophys. Res. Commun.* **165**, 384–390
- Bryans, M., Harley, E. and Gilmour, S. K. (1996) Elevated cellular polyamine levels enhance promoter activity *in vivo*. *Biochem. Biophys. Res. Commun.* **226**, 618–625
- Fogel-Petrovic, M., Shappell, N. W., Bergeron, R. J. and Porter, C. W. (1993) Polyamine and polyamine analog regulation of spermidine/spermine N¹-acetyltransferase in MALME-3M human melanoma cells. *J. Biol. Chem.* **268**, 19118–19125
- Xiao, L. and Casero, Jr., R. A. (1996) Differential transcription of the human spermidine/spermine N¹-acetyltransferase (SSAT) gene in human lung carcinoma cells. *Biochem. J.* **313**, 691–696
- Wang, Y., Devereux, W., Stewart, T. M. and Casero, Jr., R. A. (1999) Cloning and characterization of human polyamine-modulated factor-1, a transcriptional cofactor that regulates the transcription of the spermidine/spermine N¹-acetyltransferase gene. *J. Biol. Chem.* **274**, 22095–22101
- Wang, Y., Devereux, W., Stewart, T. M. and Casero, Jr., R. A. (2001) Characterization of the interaction between the transcription factors human polyamine modulated factor-1 (PMF-1) and NF-E2-related factor 2 (Nrf-2) in the transcriptional regulation of the spermidine/spermine N¹-acetyltransferase (SSAT) gene. *Biochem. J.* **354**, 45–49
- Wang, Y., Xiao, L., Thiagalingam, A., Nelkin, B. D. and Casero, Jr., R. A. (1998) The identification of a *cis*-element and a *trans*-acting factor involved in the response to polyamines and polyamine analogues in the regulation of the human spermidine/spermine N¹-acetyltransferase gene transcription. *J. Biol. Chem.* **273**, 34623–34630
- Lupas, A., Van Dyke, M. and Stock, J. (1991) Predicting coiled coils from protein sequences. *Science* **252**, 1162–1164
- Lupas, A. (1996) Prediction and analysis of coiled-coil structures. *Methods Enzymol.* **266**, 513–525
- Lupas, A. (1996) Coiled coils: new structures and new functions. *Trends Biochem. Sci.* **21**, 375–382
- Celano, P., Baylin, S. B., Giardiello, F. M., Nelkin, B. D. and Casero, Jr., R. A. (1988) Effect of polyamine depletion on c-myc expression in human colon carcinoma cells. *J. Biol. Chem.* **263**, 5491–5494
- Celano, P., Baylin, S. B. and Casero, Jr., R. A. (1989) Polyamines differentially modulate the transcription of growth-associated genes in human colon carcinoma cells. *J. Biol. Chem.* **264**, 8922–8927
- Wang, J. Y., Wang, H. and Johnson, L. R. (1995) Gastrin stimulates expression of protooncogene c-myc through a process involving polyamines in IEC-6 cells. *Am. J. Physiol.* **269**, C1474–C1481
- Marini, M. G., Chan, K., Casula, L., Kan, Y. W., Cao, A. and Moi, P. (1997) hMAF, a small human transcription factor that heterodimerizes specifically with Nrf1 and Nrf2. *J. Biol. Chem.* **272**, 16490–16497
- Itoh, K., Chiba, T., Takahashi, S., Ishii, T., Igarashi, K., Katoh, Y., Oyake, T., Hayashi, N., Satoh, K. and Hatayama, I. et al. (1997) An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem. Biophys. Res. Commun.* **236**, 313–322
- Ishii, T., Itoh, K., Takahashi, S., Sato, H., Yanagawa, T., Katoh, Y., Bannai, S. and Yamamoto, M. (2000) Transcription factor Nrf2 coordinately regulates a group of oxidative stress-inducible genes in macrophages. *J. Biol. Chem.* **275**, 16023–16029

Received 4 June 2001/9 July 2001; accepted 9 August 2001