Characterization of the interaction between the transcription factors human polyamine modulated factor (PMF-1) and NF-E2-related factor 2 (Nrf-2) in the transcriptional regulation of the spermidine/spermine N^1-acetyltransferase (SSAT) gene

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Polyamines and polyamine analogues have been demonstrated to modulate the transcription of various genes. Spermidine/spermine N^1-acetyltransferase (SSAT) is transcriptionally regulated through the interaction of at least two *trans*-acting transcription factors, NF-E2-related factor 2 (Nrf-2) and PMF-1 (polyamine modulated factor-1). Nrf-2 has previously been shown to regulate transcription of other genes through interactions between its C-terminal leucine zipper and the leucine-zipper region of other members of the small Maf protein family (the term 'Maf' is derived from **m**usculo**a**poneurotic-**f**ibrosarcoma virus). Here it is demonstrated that the interaction between Nrf-2 and PMF-1 is mediated through the binding of the leucine-zipper region of Nrf-2 and a C-terminal coiled-coil region of PMF-1 that does not contain a leucine zipper. Mutations that interrupt either the leucine zipper of Nrf-2 or the coiled-coil region of PMF-1 are demonstrated to alter the ability of these factors to interact, thus their ability to regulate the transcription of the SSAT gene is lost.

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

INTRODUCTION

Polyamines have been proposed to play a role in the regulation of gene expression at the levels of transcription and translation [1–5]. The mechanisms by which the polyamines can alter transcription have only recently been elucidated. We have previously identified transcription factors that regulate the transcription of the rate-limiting step in polyamine catabolism, spermidine/spermine N^1-acetyltransferase (SSAT) [6,7]. Nrf-2 (NF-E2-related factor 2), a transcription factor related to the globin gene transcription factor NF-E2, was found to bind constitutively to the polyamine-responsive element (PRE) in the promoter region of the SSAT gene. Nrf-2 normally binds to its native cognate sequence only when heterodimerized with a member of the small Maf protein family of transcriptional co-factors. Small Maf proteins do not contain a transcriptional activating domain; however, they facilitate the binding of Nrf-2 once heterodimerized. A leucine zipper in both proteins mediates the association of Maf proteins with Nrf-2 [8–11]. Our initial results indicated that the Maf family proteins were not the proteins involved in the regulation of the SSAT gene. However, a previously unidentified protein partner to Nrf-2, polyamine modulated factor-1 (PMF-1) was identified. PMF-1 does not appear to be related to the Maf family, but has been demonstrated to be responsible for the polyamine-induced transcriptional activation of Nrf-2-mediated SSAT transcription [7]. Our initial sequence analysis indicated that PMF-1 possesses a leucine-zipper-like region with the amino acid sequence **I**^{41}**A**QL**Q**T**S**I**R**EEI**S**D**I**K**E**EG**N**L**E**AVL**N**A**L**^{68} and our yeast two-hybrid analysis demonstrated that this 165-amino-acid, 20 kDa protein, PMF-1, binds to the leucine-zipper domain of Nrf-2. These results suggested that the association between Nrf-2 and PMF-1 was also mediated by the interaction of two leucine-zipper domains. However, the required structural motifs of the binding were not known. Therefore it was the goal of the present study to verify what regions of PMF-1 and Nrf-2 are necessary for their interaction and the subsequent transcriptional activation of SSAT and potentially other polyamine-responsive genes.

EXPERIMENTAL

Chemicals and reagents

The Matchmaker® yeast two-hybrid system and yeast culture media were purchased from Clontech (Palo Alto, CA, U.S.A.). QuikChange® Site-Directed Mutagenesis Kit was purchased from Stratagene (La Jolla, CA, U.S.A.). Restriction and DNA-modifying enzymes were purchased from Life Technologies, Inc. (Rockville, MD, U.S.A.), New England Biolabs Inc. (Beverly, MA, U.S.A.) and Sigma (St. Louis, MO, U.S.A.). Life Technologies, Inc. synthesized all oligonucleotides used in the experiments. Other chemicals were purchased from Sigma, Roche Molecular Biochemicals (Indianapolis, IN, U.S.A.) and J. T. Baker Inc. (Philipsburg, NJ, U.S.A.).

Construction of plasmids

For construction of pAS2.1/Nrf-2-LZ, the wild-type bait plasmid used in yeast two-hybrid, a 704 bp Nrf-2 cDNA fragment from PCR reaction using primer pair P1–P2 (Figure 1) was digested with the restriction endonucleases *Sal*I and *Xma*I and directionally inserted in-frame into the pAS2.1 vector. To construct pACT2/PMF-1N, plasmid pACT2/PMF-1 was digested with *Bam*HI. The small DNA fragment that contains the 277 bp 5′ end of PMF-1 cDNA was recovered and cloned into pACT2

Abbreviations used: PMF-1, polyamine modulated factor-1; Nrf-2, NF-E2-related factor 2; SSAT, spermidine/spermine N^1-acetyltransferase; PRE, polyamine-responsive element; SD-Trp, SD-Trp-Leu, SD-Trp-Leu-His synthetic dextrose medium lacking tryptophan, lacking tryptophan and leucine and lacking tryptophan, leucine and histidine respectively.

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Primers for cloning the C-terminal portion of Nrf-2
P1. 5′-ACATCCGTGTCGACCAGAAACTAGCCCAAATGGTGTCC
SalI
P2. 5′-GTCGTAGACCCGGGCTTGGAGGCTCATCTCACAAGAGA
XmaI
Primers for constructing mutation pACT2/PMF-1Mu
P3 5′-GCAGACATCTAGCCGGGAGGAAATCTCTGACAGCAAAGAGGAGGG
P4 5′-CCCTCCTCTTTGCTGTCAGAGATTTCCTCCCGGCTAGATGTCTGC

Primers for constructing mutation pACT2/PMF-1CMu1
P5 5′-GGCGCCATGTGCCGAAACAGGAGGCCGAGAACCCGCAGCTGGCAG
P6 5′-CTGCCAGCTGCGGGTTCTCGGCCTCCTGTTTCGGCACATGGCGCC

Primers for constructing mutation pACT2/PMF-1CMu2
P7 5′-GAGGAGCTGCAGCCACAGGTCCAGGCCCAGCCGCAGGCCTGGCAG
P8 5′-CTGCCAGGCCTGCGGCTGGGCCTGGACCTGTGGCTGCAGCTCCTC

Primers for constructing mutation pAS2.1/Nrf2-LZMu1
P9 5′-TTTAGATCATGTGAAAGATGAAAAAGAAAAAGTGCTCAAAGAAAAAG
P10 5′-CTTTTTCTTTGAGCACTTTTTCTTTTTCATCTTTCACATGATCTAAA

Primers for constructing mutation pAS2.1/Nrf2-LZMu3 and pAS2.1/Nrf2-LZMu4
P11 5′-TTTAGATCATTTGCCAGATGAAAAAGAAAAATTGCCCAAAGAAAAAG
P12 5′-CTTTTTCTTTGGGCAATTTTTCTTTTTCATCTGGCAAATGATCTAAA

Figure 1 Primers used as indicated in the Experimental section

vector digested with the same enzyme and dephosphorylated using calf intestine alkaline phosphatase. For construction of pACT2/PMF-1C, the larger fragment from the *Bam*HI digestion of pACT2/PMF-1, which contains the 637 bp 3′ end of PMF-1 cDNA and the open pACT2 vector sequence, was circularized by T4 ligase. The plasmids containing mutations, pACT2/PMF-1Mu, pACT2/PMF-1CMu1, pACT2/PMF-1CMu2, pAS2.1/Nrf-2-LZMu1, pAS2.1/Nrf-2-LZMu3 and pAS2.1/Nrf-2-LZMu4 were constructed as described below. Plasmid pACT2/PMF-1 was obtained by screening a cDNA library constructed from the human non-small cell lung cancer line NCI H157 for use in the yeast two-hybrid technique as previously reported [7].

Site-directed mutagenesis

Site-directed mutation was performed with the QuikChange Site-Directed Mutagenesis Kit from Stratagene according to the protocol supplied by the manufacturer. All mutations were verified by DNA sequencing using a Perkin–Elmer ABI Automated DNA sequencer.

Yeast two-hybrid analysis

Yeast two-hybrid analysis was performed using the Matchmaker two-hybrid system in a 'HIS3 jump-start' procedure. *Saccharomyces cerevisiae* Y190 cells were first transformed with the bait plasmid (pAS2.1/Nrf-2-LZ, pAS2.1/Nrf-2-LZMu1, pAS2.1/Nrf-2-LZMu3 or pAS2.1/Nrf-2-LZMu4) and selected on synthetic dextrose medium lacking tryptophan (SD-Trp). The transformants selected by the SD-Trp medium were subsequently transformed with the prey plasmid (pACT2/PMF-1, pACT2/PMF-1C, pACT2/PMF-1N, pACT2/PMF-1Mu, pACT2/PMF-1CMu1 or pACT2/PMF-1CMu2) and selected by medium lacking tryptophan and leucine (SD-Trp-Leu). The clones co-transformed with the 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.

Prediction of coiled-coil structure of Nrf-2, PMF-1 and their mutants

The primary protein sequence of each of the proteins and their mutant constructs was analysed for their predicted coiled-coil content using the methods of Lupas [12] and Lupas et al. [13]. A program using this protocol can be found at the following URL:

http://dot.imgen.bcm.tmc.edu.:9331/seq-search/struc-predict.html

RESULTS

The pseudo-leucine-zipper motif in PMF-1 is not responsible for the association of PMF-1 with Nrf-2

Our initial results suggested that the leucine-zipper-like motif in the PMF-1 protein might be responsible for the binding of PMF-1 to Nrf-2 and subsequent transcriptional activation of SSAT.

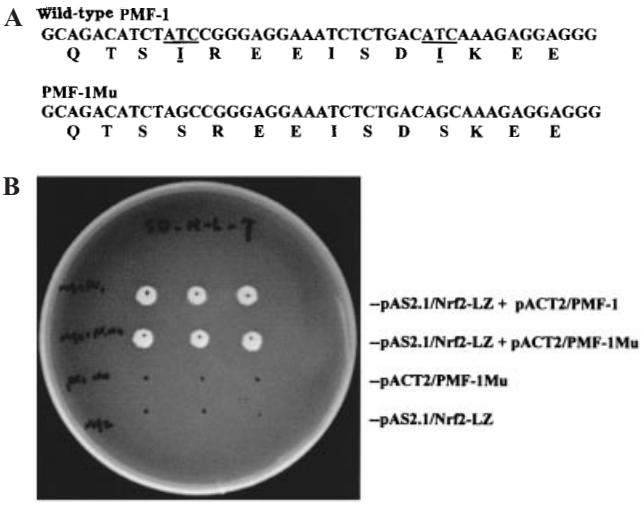

Figure 2 Leucine-zipper motif of PMF-1 is not responsible for Nrf-2–PMF-1 interaction

(**A**) Two complementary oligomers (P3 and P4) were synthesized and used in the PCR reaction with wild-type pACT2/PMF-1 as the template to obtain the construct pACT2/PMF-1Mu, in which two members of PMF-1 leucine-zipper motif (Ile^{48} and Ile^{55}) were mutated to serine. (**B**) *S. cerevisiae* Y190 cells were transformed with pAS2.1/Nrf-2-LZ or pACT2/PMF-1Mu alone, or co-transformed with pAS2.1/Nrf-2-LZ and pACT2/PMF-1 or pAS2.1/Nrf-2-LZ and pACT2/PMF-1Mu. The transformants were then selected on SD-Trp-Leu-His medium with 30 mM 3-amino-1,2,4-triazole for 4 days. Two kinds of co-transformed clones can grow on the selection medium, indicating that both wild-type and leucine-zipper-mutated PMF-1 can interact with Nrf-2.

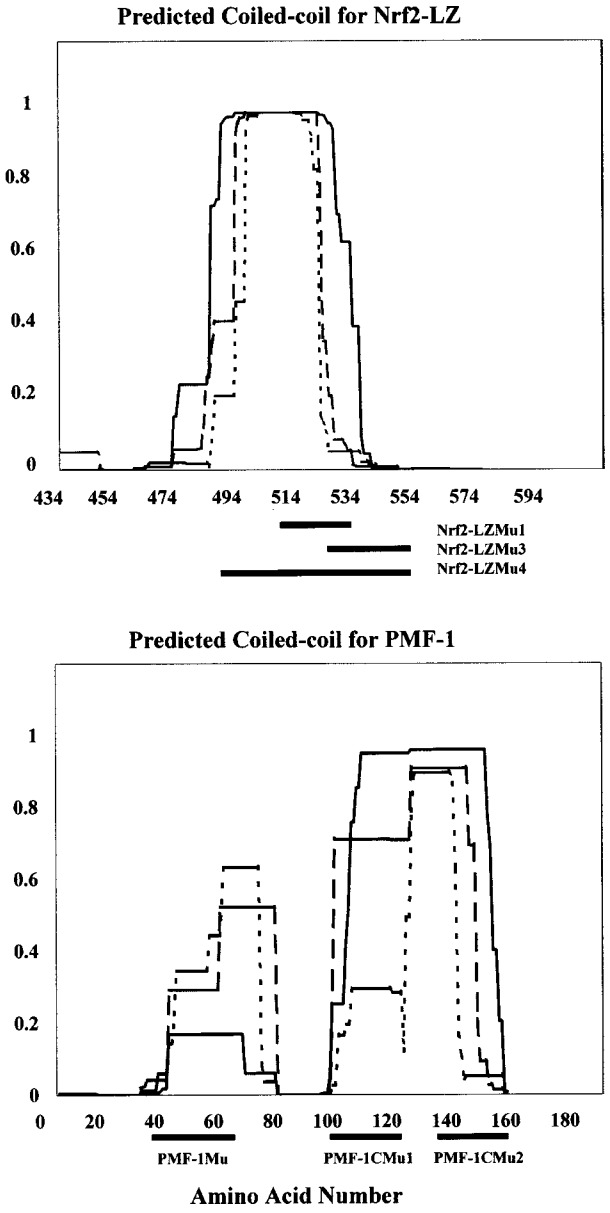

Figure 3 Predicted coiled-coil structure of Nrf-2 and PMF-1

The coiled-coil structure predictions of the wild type genes are depicted in this Figure. It should be noted that each prediction uses three different frame sizes, an 14 (- - - -), 21 (– – – –) and 28 (—) amino-acid window to predict the coiled-coil structure (see the website given in the text and [12,13]). The **bold** lines beneath the abscissae represent the area of the structure that is affected by the designated mutations. It is important to note that, for the yeast two-hybrid system used here, only clones coding for the leucine-zipper region of Nrf-2 (amino acids 434–589) were used.

To test this hypothesis directly, yeast two-hybrid analysis in combination with site-directed mutagenesis were used. Using primers p3 and p4 (Figure 1) the mutant chimaeric pACT2/PMF-1Mu was generated by PCR, resulting in a construct containing two Ile-to-Ser mutations (Figure 2A). If this region of the PMF-1 protein was responsible for association with Nfr-2, no activation of the reporter genes should occur. However, upon co-transformation of yeast with pAS2.1/Nrf-2-LZ and pACT.2/PMF-1Mu, followed by selection and reporter-activity analysis, the double mutant was as effective as the wild-type construct in

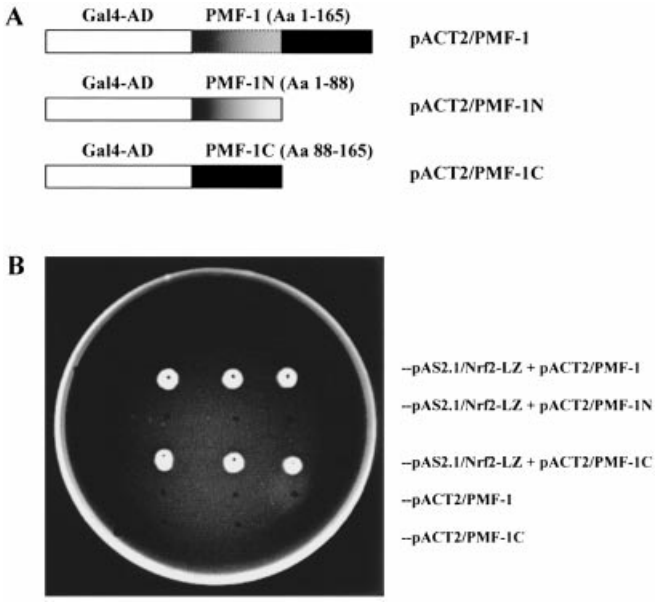

Figure 4 C-terminal portion of PMF-1 involvement in Nrf-2–PMF-1 interaction

(**A**) Two fusion plasmids were constructed in which PMF-1 N-terminal (amino acids 1–88) or C-terminal (amino acids 88–165) portions were fused in-frame to Gal4 (β-galactosidase reporter gene) DNA activating domain. (**B**) *S. cerevisiae* Y190 cells were transformed alone or co-transformed as indicated in the Figure. The transformants were then selected on SD-Trp-Leu-His medium with 30 mM 3-amino-1,2,4-triazole for 4 days. Clones co-transformed with pAS2.1/Nrf-2-LZ and pACT2/PMF-1C can grow as well as clones co-transformed with pAS2.1/Nrf-2-LZ and pACT2/PMF-1 on the selection medium, indicating that the C-terminal portion of PMF-1 contains the Nrf-2-binding region.

allowing the yeast to grow in selection medium, as well as in activating both the *lacZ* and HIS3 reporter genes (Figure 2b). These results suggest that the leucine-zipper-like region of PMF-1 is not responsible for the PMF-1–Nrf-2 interaction. It is important to note that the mutant PMF-1 clone was not self-activating in the yeast system.

The C-terminal region of PMF-1 contains the Nrf-2-binding domain

To determine what region of PMF-1 is responsible for Nrf-2 binding, the yeast two-hybrid system was again employed. Both the Nrf-2 protein and the PMF-1 proteins were found to contain coiled-coil regions that could allow protein–protein interaction (Figure 3). PMF-1 contains two coiled-coil regions, one in the N-terminus ranging from amino acids 40 to 80 and a larger one in the C-terminal region containing amino acids 100–165. Initially, two chimaeric activation plasmids were constructed. The first contains the PMF-1 amino acids 1–88 (pACT2/PMF-1N) and the second contains amino acids 88–165 (pACT2/PMF-1C) (Figure 4A). Only the pACT2/PMF-1C construct was capable of inducing the transcription of the reporter genes (Figure 4B) when co-transformed in yeast with pAS2.1/Nrf-2-LZ. Again it is important to note that this construct was not self-activating.

To confirm that the C-terminal coiled-coil region of PMF-1 was responsible for the interaction with Nrf-2, two mutants interrupting the coiled-coil structure in two locations were constructed (Figure 5A). The first construct, pACT2/PMF-1C-Mu1 reduces the coiled-coil structure from amino acids 100–160

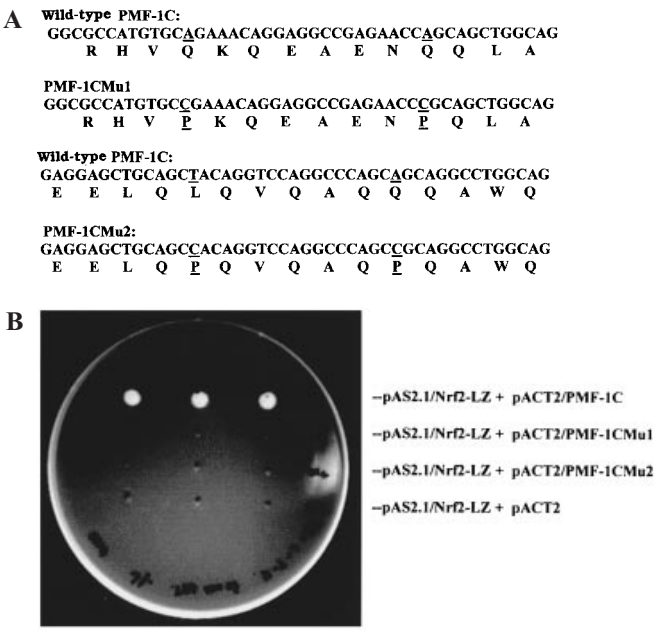

Figure 5 The coiled-coil region in the C-terminal portion of PMF-1 is responsible for Nrf-2–PMF-1 interaction

(**A**) Two pairs of complementary oligomers (P5–P6 and P7–P8) were synthesized and used in the PCR reaction with wild-type pACT2/PMF-1C as the template to obtain two mutated constructs, pACT2/PMF-1CMu1 and pACT2/PMF-1CMu2. In pACT2/PMF-1CMu1, amino acids Gln^{113} and Gln^{120} were mutated into proline and in pACT2/PMF-1Cmu2, Leu^{138} and Gln^{144} were mutated into proline. These mutations result in partial destruction of the C-terminal coiled-coil structure. (**B**) *S. cerevisiae* Y190 cells were co-transformed with the constructs as described in the Figure. The transformants were then selected on SD-Trp-Leu-His medium with 30 mM 3-amino-1,2,4-triazole for 4 days. Except wild-type PMF-1C, none of the mutated fusion proteins can interact with Nrf-2, suggesting the necessity of a C-terminal coiled-coil region in Nrf-2 binding.

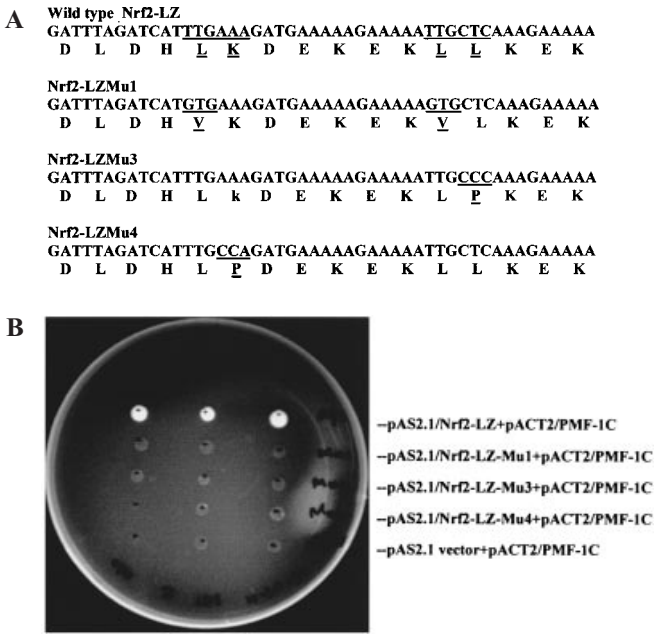

Figure 6 The leucine-zipper domain of Nrf-2 is required for the Nrf-2–PMF-1 interaction

(**A**) Two pairs of complementary oligomers (P9–P10 and P11–P12) were synthesized and used in the PCR reaction with pAS2.1/Nrf-2-LZ as the template to obtain three mutated constructs: pAS2.1/Nrf-2-LZMu1, pAS2.1/Nrf-2-LZMu3 and pAS2.1/Nrf-2-LZMu4. Mutation pAS2.1/Nrf-2-LZMu1 destroys the leucine zipper in Nrf-2 by exchanging the second and third members of the leucine-zipper motif for valine. In the mutations pAS2.1/Nrf-2-LZMu3 and pAS2.1/Nrf-2-LZMu4, amino acids Leu^{524} (Mu3) or Lys^{517}(Mu4) was mutated into proline. These mutations result in partial destruction of the coiled-coil structure in Nrf-2. (**B**) *S. cerevisiae* Y190 cells were co-transformed with the constructs as described in the Figure. The transformants were then selected on SD-Trp-Leu-His medium with 30 mM 3-amino-1,2,4-triazole for 4 days. Except wild-type Nrf-2, none of mutated fusion proteins could interact with PMF-1, suggesting the necessity for an intact leucine zipper domain in the Nrf-2–PMF-1 interaction.

to amino acids 125–160 (Figure 3). The second mutation, pACT2/PMF-1C-Mu2, reduces the coiled-coil region to amino acids 100–138. Using the yeast two-hybrid assay, only the wild-type PMF-1C construct was capable of activating the reporter genes when co-transforming yeast with pAS2.1/Nrf-2-LZ. These results indicate that the entire C-terminal coiled-coil region of PMF-1 is necessary for the transactivation of Nrf-2 by PMF-1 (Figure 5B).

An intact leucine zipper in the C-terminal region of Nrf-2 is required for the Nrf-2–PMF-1 interaction

Analysis of the leucine-zipper region in the C-terminus of Nrf-2 indicates that it possesses a relatively large region capable of forming a coiled-coil structure that would facilitate an association with PMF-1. To determine whether the coiled-coil domain alone is sufficient for the Nrf-2 interaction with PMF-1, a series of mutations were made in the leucine-zipper region of Nrf-2 to destroy the leucine zipper while maintaining the coiled-coil structure. Separate mutations were made that maintained the leucine-zipper residues, but altered the coiled-coil structure. It is important to note that, in the yeast two-hybrid system, the full-length Nrf-2 is self-activating, as we have previously reported [7]. Therefore, for the experiments detailed here, only the C-terminal region containing amino acids 434–589 was used. PCR was used to generate point mutations resulting in pAS2.1/Nrf-LZ-Mu1 (Figure 6A). Complete sequencing of the mutant confirmed that the second and third (of a total of seven) leucine residues in the leucine-zipper region were replaced by valine residues. This results in a loss of the leucine-zipper motif while maintaining the coiled-coil structure. When compared with the wild-type construct, pAS2.1/Nrf-LZ, the mutant lacking the leucine-zipper motif, was unable to associate with PMF-1 and activate transcription in the yeast two-hybrid system (Figure 6B).

The above results suggested that the leucine-zipper motif in Nrf-2 is required for Nrf-2/PMF-1 binding. However, it does not conclusively determine whether the coiled-coil motif is also required, since the above amino acid replacement does not significantly alter the coiled-coil domain. Therefore an attempt was made to interrupt the coiled-coil structure while leaving the leucine-zipper motif intact. To accomplish this, two mutations were made in the Nrf-2-LZ construct (pAS2.1/Nfr-2LZ-Mu3 and pAS2.1/Nrf-2-LZ-Mu4) using proline to interrupt the coiled-coil structure (Figure 6A). Although neither of the mutant constructs changed any of the leucine residues required for the leucine-zipper motif, neither construct was capable of interacting with PMF-1 (Figure 6B). Although these results suggest that both the leucine-zipper motif and the coiled-coil structure are required for Nrf-2–PMF-1 interaction, an alternative interpretation is that the proline substitutions not only interrupted the coiled-coil structure, but also distorted the coiled structure of the leucine zipper to a significant extent. This potential change in leucine-zipper structure may be responsible for the lack of Nrf-

2–PMF-1 interaction, rather than solely the loss of the coiled-coil structure.

DISCUSSION

We recently reported that the transcription of the SSAT gene is regulated, in part, by the association of two transcription factors bound to the PRE [7]. These two *trans*-acting transcription factors, Nrf-2 and PMF-1, appear to be required for the induction of SSAT expression in response to an increase in intracellular polyamines or exposure to polyamine analogues. Nrf-2 has previously been shown to bind to DNA only after binding to a member of the small Maf protein family [8–11]. However, Nrf-2 appears to bind to the PRE constitutively without first heterodimerizing with a Maf family member [6]. Further, the binding of Nrf-2 to Maf family proteins is dependent on the presence of leucine-zipper motifs in both Nrf-2 and the Maf family member. Here we have demonstrated a unique binding interaction in which the Nrf-2 leucine zipper is required to bind to the non-leucine-zipper, coiled-coil domain of the C-terminal region of PMF-1.

Initial results suggested that PMF-1 might bind to Nrf-2 in a standard leucine-zipper interaction, since PMF-1 has an N-terminal leucine zipper. However, when site-directed mutagenesis was used to remove the leucine zipper from PMF-1, no effect on the interaction between PMF-1 and Nrf-2 was observed. In fact when the entire N-terminal region of PMF-1 was removed, Nrf-2 and the C-terminal region of PMF-1 were still able to interact. These results demonstrate that an intact leucine zipper of PMF-1 is not necessary for the heterodimerization of PMF-1 and Nrf-2. However, when the leucine zipper of Nrf-2 is removed by site-directed mutagenesis, it is no longer capable of interacting with PMF-1. This inability to interact occurs despite the fact that the leucine-to-valine mutation maintains the coiled-coil structure of Nrf-2. Consequently, not only does PMF-1 constitute a new transcriptional co-activator, but also the Nrf-2–PMF-1 interaction represents a unique heterodimerization of a leucine zipper directly with a non-leucine-zipper domain of a second protein.

Changes in intracellular polyamines have been implicated in altered gene expression [1,3–5]. This was first demonstrated by examining the effects of polyamine depletion on the transcription rate of several growth-related genes [2,14]. These studies demonstrated that the depletion of polyamines could lead to gene-specific changes at the transcriptional level. Additionally, we and others have demonstrated that an increase in polyamines or exposure to certain polyamine analogues can mediate an increase in the transcription and ultimately, protein expression of SSAT [15,16]. Another highly significant recent finding is that polyamine analogues can effect the expression of the p53-modulated genes, including $p21^{waf1/cip1}$. In some instances this leads to a G_1 block in the cell cycle [17]. What is not currently known is whether the polyamines or their analogues directly affect the interaction between Nrf-2 and PMF-1. It is also not known what effect, if any, expression of the Maf-family proteins has on the Nrf-2 and PMF-1.

Another important question that requires additional experimentation is what other genes are regulated by the Nrf-2–PMF-1 heterodimer. It seems unlikely that the association of Nrf-2 and PMF-1 would have evolved only to control the expression of a single gene. Since there is ample evidence that polyamine status effects the expression of several growth-related genes, we are currently attempting to identify those genes that may be under similar control. Further, the possibility that PMF-1 can also dimerize with other transcription factors cannot be excluded.

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REFERENCES

1 Lu, B., Liang, X., Scott, G. K., Chang, C. H., Baldwin, M. A., Thomas, T. and Benz, C. C. (1998) Polyamine inhibition of estrogen receptor (ER) DNA-binding and ligand-binding functions. Breast Cancer Res. Treat. **48**, 243–257

2 Celano, P., Baylin, S. B. and Casero, R. A. (1989) Polyamines differentially modulate the transcription of growth-associated genes in human colon carcinoma cells. J. Biol. Chem. **264**, 8922–8927

3 Berchtold, C. M., Tamez, P., Kensler, T. and Casero, Jr., R. A. (1998) Inhibition of cell growth in CaCO2 cells by the polyamine analogue N^1,N^{12}-bis(ethyl)spermine is preceded by a reduction in MYC oncoprotein levels. J. Cell. Physiol. **174**, 380–386

4 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

5 Wang, R. Y. H., Gehrke, C. W. and Ehrlich, M. (1980) Comparison of bisulfite modification of 5-methyldeoxycytidine and deoxycytidine residues. Nucleic Acids Res. **8**, 4777–4790

6 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 N^1-acetyltransferase gene transcription. J. Biol. Chem. **273**, 34623–34630

7 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 N^1-acetyltransferase gene. J. Biol. Chem. **274**, 22095–22101

8 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 Nrf-2. J. Biol. Chem. **272**, 16490–16497

9 Igarashi, K., Itoh, K., Hozumi, M., Norio, H., Matuzaki, Y., Nakauchi, H., Nishizawa, M. and Yamamoto, M. (1995) Activity and expression of murine small Maf family protein MafK. J. Biol. Chem. **270**, 7615–7624

10 Igarashi, K., Kataoka, K., Itoh, K., Hayashi, N., Nishizawa, M. and Yamamoto, M. (1994) Regulation of transcription by dimerization of erythroid factor NF-E2 p45 with small Maf proteins. Nature (London) **367**, 568–572

11 Toki, T., Itoh, J., Kitazawa, J., Arai, K., Hatakeyama, K., Akasaka, J., Igarashi, K., Nomura, N., Yokoyama, M., Yamamoto, M. and Ito, E. (1997) Human small Maf proteins form heterodimers with CNC family transcription factors and recognize the NF-E2 motif. Oncogene **14**, 1901–1910

12 Lupas, A. (1996) Prediction and analysis of coiled-coil structures. Methods Enzymol. **266**, 513–524

13 Lupas, A., Van Dyke, M. and Stock, J. (1991) Predicting coiled coils from protein sequences. Science **252**, 1162–1164

14 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

15 Fogel-Petrovic, M., Shappell, N. W., Bergeron, R. J. and Porter, C. W. (1993) Polyamine and polyamine analog regulation of spermidine/spermine N^1-acetyltransferase in MALME-3M human melanoma cells. J. Biol. Chem. **268**, 19118–19125

16 Xiao, L. and Casero, R. A. (1996) Differential transcription of the human spermidine/spermine N^1-acetyltransferase (SSAT) gene in human lung carcinoma cells. Biochem. J. **313**, 691–696

17 Kramer, D. L., Vujcic, S., Diegelman, P., Alderfer, J., Miller, J. T., Black, J. D., Bergeron, R. J. and Porter, C. W. (1999) Polyamine analogue induction of the p53-p21WAF1/CIP1-Rb pathway and G_1 arrest in human melanoma cells. Cancer Res. **59**, 1278–1286

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