Small Maf (MafG and MafK) Proteins Negatively Regulate Antioxidant Response Element-mediated Expression and Antioxidant Induction of the NAD(P)H:Quinone Oxidoreductase1 Gene*

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The antioxidant response element (ARE) is known to regulate expression and induction of NQO1, GST Ya, and other detoxifying enzyme genes in response to antioxidants and xenobiotics. The nuclear transcription factor Nrf2 and Nrf1 bind to the ARE and positively regulate expression and induction of the NQO1 and GST Ya genes. In this study, we demonstrate that overexpression of small Maf (MafG and MafK) proteins negatively regulate ARE-mediated expression and tert-butyldihydroquinone induction of the NQO1 and GST Ya genes in transfected Hep-G2 cells. In similar experiments, overexpression of small Maf proteins also repressed Nrf2-mediated up-regulation of ARE-mediated NQO1 and GST Ya genes expression in Hep-G2 cells co-transfected with Nrf2 and small Maf proteins. Band and supershift assays with the NQO1 gene ARE and nuclear proteins demonstrate that small MafG and MafK bind to the ARE as Maf-Maf homodimers and Maf-Nrf2 heterodimers. Therefore, Maf-Maf homodimers and possibly Maf-Nrf2 heterodimers play a role in negative regulation of ARE-mediated transcription and antioxidant induction of NQO1 and other detoxifying enzyme genes. In contrast to Maf-Nrf2, the Maf-Nrf1 heterodimers failed to bind with the NQO1 gene ARE and did not demonstrate the repressive effect in transfection assays.

NAD(P)H:quinone oxidoreductase1 (NQO1)† is a flavoprotein that catalyzes two-electron reductive metabolism and detoxification of quinones. This protects cells against quinone-induced oxidative stress and neoplasia (1, 2). Higher levels of NQO1 gene expression were observed in liver, lung, colon, and breast tumors, when compared with normal tissues of the same origin (3, 4). NQO1 gene transcription is coordinately activated with other detoxifying enzyme genes in response to xenobiotics (e.g. tert-naphthoflavone) and antioxidants (e.g. tert-butyldihydroquinone (t-BHQ)) (5, 6). The other detoxifying enzyme genes that are coordinately induced with NQO1 include glutathione S-transferases (GSTs), that conjugate hydrophobic electrophiles and ROS with glutathione (7, 8); UDP-glucuronosyl transferases, which catalyze the conjugation of glucuronic acid with xenobiotics and drugs for excretion (9); epoxide hydrolase, which inactivates epoxides (10); γ-glutamylcysteine synthetase (γ-GCS), which plays a role in glutathione metabolism (11); ferritin-L gene, which plays an important role in iron storage (12); and heme oxygenase-1, which catalyzes the first and rate-limiting step in heme catabolism (13). The coordinated induction of these genes, including NQO1, protects cells against free radical damage, oxidative stress, and neoplasia. It is critical in achieving chemoprevention. Deletion mutagenesis studies of the human NQO1 gene promoter identified 24 base pairs of an antioxidant response element (ARE) between nucleotides −470 and −447. This region is required for basal expression and induction of NQO1 in response to β-naphthoflavone and t-BHQ (6). ARE elements have also been found in the promoter region of the human NQO2 gene (14), the rat and mouse GST Ya subunit genes (15–19), the rat GST P gene (20), the human γ-GCS gene (11), the ferritin-L gene (12), and the human heme oxygenase-1 gene (21). Analysis of the AREs from various genes revealed that they contain AP1/AP1-like elements arranged as inverse or direct repeats. This is followed by a GC box (22). Additional cis-elements and nucleotide sequences, flanking the core sequence, have been shown to contribute to the ARE-mediated expression and induction of detoxifying enzyme genes (12, 23, 24).

The various AREs bind to a complex of nuclear proteins from cells of different origins (15–23, 25). Analysis of ARE-nuclear protein complexes have identified several nuclear transcription factors including c-Jun, Jun-B, Jun-D, c-Fos, Fra1, Nrf1, Nrf2, YABP, ARE-BP1, MafK, Ah (aromatic hydrocarbon) receptor, and the estrogen receptor (15–32). Among these transcription factors, Nrf1 and Nrf2 have been shown to heterodimerize with c-Jun and up-regulate the ARE-mediated expression and induction of NQO1 in response to antioxidants and xenobiotics (26, 31). Recently, Nrf3, a third member of the Nrf family of transcription factors, was cloned and sequenced (33). The transcription factor Nrf2 and Nrf1, which regulate the ARE-mediated expression and induction of NQO1, were also demonstrated to regulate the expression and induction of other detoxifying enzymes such as GST Ya, γ-GCS and heme oxygenase-1 (21, 26, 31, 34–37). Recently, Nrf2-MafK heterodimer was shown to bind to the ARE of a gene encoding a subunit of GST Ya (32). It was suggested that Nrf2-MafK heterodimers up-regulate ARE-mediated expression and induction of the GST Ya gene. More recently, a repressive role for MafK and MafG in ARE-regulated expression of the γ-GCS gene was suggested (37).

Small Maf (MafG, MafK, and MafP) proteins are leucine

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† The abbreviations used are: NQO1, NAD(P)H:quinone oxidoreductase1; GST, glutathione S-transferase; γ-GCS, γ-glutamylcysteine synthetase; ARE, antioxidant response element; hARE, human NQO1 gene ARE; t-BHQ, tert-butyldihydroquinone; ROS, reactive oxygen species; PAGE, polyacrylamide gel electrophoresis.
Small Maf Repression of ARE-mediated Gene Expression

The mouse Nrf2 and Nrf1 cDNAs were kindly provided by Dr. Jefferson Y. Chan (University of California, San Francisco, CA). The full-length Nrf2 and Nrf1 cDNAs were amplified by polymerase chain reaction and subcloned separately into the mammalian expression vector pcDNA 3.1 to make the expression plasmids pcDNA-Nrf2, pcDNA-Nrf1, pcDNA-Nrf1R, and pcDNA-Nrf1C. The mouse MafG and MafK cDNAs were provided by Dr. Makoto Nishizawa (The Scripps Research Institute, La Jolla, CA). The CDNA encoding MafG and MafK were subcloned separately into the pcDNA 3.1 vector to generate the expression plasmids pcDNA-MafG, pcDNA-MafGc, pcDNA-MafK, and pcDNA-MafKc, and pcDNA-Maf-Kc. The plasmids with an R suffix contain cDNAs in the reverse orientation, and those with the suffix II in combination with those with the suffix I are in the correct orientation. The MafG and MafK cDNAs were also subcloned in-frame with the V5 epitope of the pcDNA 3.1 expression plasmid. These plasmids encode the V5-tagged MafG-V5 and MafK-V5 proteins. The V5 epitope contains 14 amino acids in the sequence Gly-Lys-Pro-Ile-Pro-Pro-Leu-Leu-Glu-Asp-Ser-Thr

Cell Culture and Co-transfection of Reporter and Expression Plasmids—Human hepatoblastoma (Hep-G2) cells were grown in six-well monolayer cultures containing 0.1% minimum essential medium supplemented with fetal bovine serum (25). The Effectene transfection reagent kit was used to perform the transfections by procedures as described in the manufacturer’s protocol. Briefly, 0.5 μg of reporter constructs hARE-Luc, mutant hARE-Luc, and GST Yα ARE-Luc were co-transfected with varying amounts of cDNAs, individually and in combinations, with the pcDNA expression plasmids (Nrf2, Nrf1, MafG, and MafK). The transfection efficiency was determined by transfection with the corresponding plasmid pRL-TK encoding Renilla luciferase (25) and analyzed by procedures as described above.

The transfection experiments were performed using pcDNA-Maf and pcDNA-Maf-V5 constructs. Both sets of expression plasmids gave similar results. The presence or absence of V5 epitope-tagged to Maf proteins had no effect on their activity/function. Therefore, we have shown transfection data only on Maf proteins without V5 tag. The addition of V5 tag to Maf proteins enabled us to use antibodies against V5 peptide in Western and band and supershift assays.

Gel Shift/Supershift Assays—The nuclear extracts from Hep-G2 (Me2SO control and t-BHQ-treated (200 μM for 16 h)) cells were prepared by previously described procedures (25). The Hep-G2 cells were co-transfected with pcDNA-Nrf2C and pcDNA-Maf-G-V5 in 1:1 ratio to overexpress Nrf2 and MafK-V5. These cells were treated with Me2SO (200 μM for 16 h) and nuclear extract prepared by previously described procedures (25). The in vitro transcription/translation of the plasmids encoding Nrf1, Nrf2, MafG-V5, and MafK-V5 were performed using the TNT-coupled rabbit reticulocyte lysate system (Promega) by procedures as suggested in the manufacturer’s protocol. Redivue 1-[35S]methionine was substituted for methionine in the reactions. After the coupled transcription/translation, the proteins were checked for their correct size on a 10% PAGE and Western analysis. Briefly, 5 μl of the translated proteins were resolved on a 10% PAGE, treated with Amplify solution (NAP 100; Amersham Pharmacia Biotech) to enhance the 35S signal, dried, and exposed to x-ray film. In a similar manner, the translated proteins were band shifted with GST Yα ARE-Luc DNA annealed, phosphorylated using T4 polynucleotide kinase, and cloned at the respective sites in the pGEluc promoter vector. The GST Yα ARE was cloned at the NheI/IgH1 site. The various constructs were checked by DNA sequencing.
were then performed by previously described procedures (25, 31). 10 μg of the nuclear extract or equimolar concentrations of in vitro translated proteins were used in the gel shift and supershift experiments. The supershift assay with nuclear extracts used 20 μg of proteins. 1.5 μl of anti-V5 antibody and 3 μl of Nrf2 antibody or preimmune serum were used in the supershift assays.

RESULTS

The transfection of Hep-G2 cells with expression plasmids pcDNA-MafG-V5, pcDNA-MafK-V5, and pcDNA-Nrf2(C) led to the overexpression of these respective proteins as determined by SDS-PAGE, Western analysis, and antibody probing (data not shown). Overexpression of the various proteins was in near linear range between 0.1 and 1.0 μg of plasmids used for transfecting Hep-G2 cells. In similar experiments, transfection of Hep-G2 cells with cDNA in reverse orientation did not result in overexpression of the respective proteins.

Transfection of Hep-G2 cells with the hARE-Luc plasmid expressed luciferase activity (Fig. 2). Overexpression of MafG in Hep-G2 cells repressed hARE-mediated luciferase activity in transfected cells (Fig. 2A, left panel). This repression was MafG concentration-dependent. The transfection of 1.0 μg of pcDNA-MafG(C) plasmid repressed 83% of hARE-mediated luciferase activity in transfected Hep-G2 cells. Interestingly, transfection of Hep-G2 cells with plasmid pcDNA-MafG(R) expressing antisense MafG RNA significantly increased the hARE-mediated luciferase activity (Fig. 2A, left panel). The transfection of 0.5 μg of plasmid pcDNA-MafG(R) caused 3.4-fold increase in the hARE-mediated luciferase activity (Fig. 2A, left panel). Contrary to MafG, overexpression of Nrf2 in Hep-G2 cells led to increased expression of the NQO1 gene ARE-mediated luciferase gene (Fig. 2A). In a similar experiment, the Nrf2-mediated up-regulation of luciferase gene expression was repressed because of the overexpression of MafG (Fig. 2A, right panel). This repression was also MafG concentration-dependent. Similar results were also observed with Hep-G2 cells overexpressing MafK alone or MafK with Nrf2 (Fig. 2B). The overexpression of MafK repressed hARE-mediated luciferase gene expression and its activation by Nrf2. Replacement of the reporter plasmid hARE-Luc with mutant hARE-Luc resulted in the loss of basal expression and repression of the luciferase gene by MafG and MafK (Fig. 2C). In related experiments, the overexpression of MafG and MafK also repressed GST Ya ARE-mediated luciferase gene expression and up-regulation by Nrf2 (Fig. 3). t-BHQ treatment of Hep-G2 cells, transfected with hARE-Luc and GST Ya ARE-Luc, increased luciferase gene by approximately 2-fold (Fig. 4). Interestingly, t-BHQ induction of hARE- and GST Ya ARE-mediated luciferase gene expression was also repressed in Hep-G2 cells that overexpressed MafG and MafK (Fig. 4).
repression of t-BHQ induced expression was more or less proportional to the repression of basal expression. In other words, overexpression of small Maf proteins inhibited the basal and t-BHQ induced luciferase activity equally.

The pcDNA-Nrf2C, pcDNA-Nrf1, pcDNA-MafG-V5, and pcDNA-MafK-V5 plasmids were in vitro transcribed and translated with rabbit reticulocyte lysate system. SDS-PAGE analysis of in vitro translated Nrf2, Nrf1, and V5-tagged MafG and MafK is shown in Fig. 5. The in vitro translated Nrf2 and Nrf1 proteins migrated between the 105- and 75-kDa standards. Nrf1 migrated slower than Nrf2. The in vitro translated MafG-V5 and MafK-V5 proteins moved faster than the 25-kDa molecular mass standard. MafK-V5 moved faster than MafG-V5 in SDS-PAGE. The in vitro translated proteins were confirmed by Western blotting and probing with specific antibodies (data not shown).

The binding of in vitro translated MafG-V5, MafK-V5, Nrf2, and Nrf1 to the NQO1 gene ARE was determined by band and supershift assays (Fig. 6). MafG and MafK both bound to the hARE as Maf-Maf homodimers and Nrf2-Maf heterodimers (Fig. 6). The binding of homo- and heterodimers of Maf and Nrf2 were competed with cold hARE (data not shown). The presence of V-5-tagged MafG and MafK in Maf-Maf homodimers and Nrf2-Maf heterodimers were confirmed by supershift assays with anti-V5 antibodies (Fig. 6). The antibodies against Nrf2 also supershifted Nrf2-Maf heterodimers in experiments with Nrf2-MafG and Nrf2-MafK (Fig. 6).

The results of band shift assays with hARE and nuclear extract from Hep-G2 cells treated with Me2SO (control) and t-BHQ are shown (Fig. 7A). The results demonstrated two-shifted bands as observed with in vitro translated Nrf2 and Maf proteins (Fig. 6). The upper and lower shifted bands corresponded to the Nrf2-Maf heterodimers and Maf-Maf homodimers, respectively. It may be noteworthy that both the lower and upper bands are not exclusively due to Nrf2-Maf and Maf-Maf dimers. These bands are also expected to contain positive factors including Nrf2-Jun (31). The presence of Nrf2 and Maf in the upper and lower complexes was determined by supershift assays with respective antibodies (Fig. 7B).

Small Maf proteins (MafG, MafK, and MafF) possess leucine zipper DNA binding domains but lack transactivation domains (39–41, 43). Recent studies on the role of small Maf proteins were done with NF-E2 binding site-regulated expression of the \( \beta \)-globin gene (43). The small Maf proteins bind to the NF-E2 site of the \( \beta \)-globin gene and directly control the DNA binding properties of erythroid specific factor NF-E2 p45 (43). Homodimers of the small Maf proteins act as negative regulators, whereas heterodimers of Maf and p45 support active transcription in vitro (43). Recently, small Maf proteins were also shown to homodimerize and heterodimerize with the ubiquitous factors Nrf1/Nrf2 and regulate the MARE-mediated \( \beta \)-globin gene
expression (42). Maf-Maf homodimers repressed yet Maf-Nrf heterodimers activated transcription of the β-globin gene (42). The Nrf2, Nrf1, and Maf proteins are also known to bind with AREs from several detoxifying enzyme genes (32, 37). Nrf2 and Nrf1 are known to up-regulate the ARE-mediated expression and induction of NQO1, GST Ya, γ-GCS, and heme oxygenase-1 genes (21, 26, 31, 34, 37). However, the role of small Maf proteins in the ARE-mediated detoxifying enzyme gene expression especially its mechanism of action remains unknown.

It may be noteworthy that ARE is a distinct element than MARE and TRE (22–23, 38). Therefore, the role of Maf-Maf homodimers and Nrf-Maf heterodimers in ARE-mediated regulation of detoxifying enzyme genes expression remains relatively unknown.

In the present report, we demonstrate that small Maf proteins contribute significantly to the regulation of ARE-mediated NQO1 and GST Ya gene expression. The small Maf (MafG and MafK) proteins negatively regulate ARE-mediated expression and antioxidant induction of NQO1 and GST Ya gene expression. This conclusion is based on the following observations. Overexpression of MafG and MafK repressed ARE-mediated expression, as well as induction, by t-BHQ. The repression of t-BHQ induced expression was more or less proportional to the repression of basal expression. Band and supershift assays indicated that both MafG-MafG and MafK-MafK homodimers bind with NQO1 gene ARE and participate in the repression of NQO1 gene expression. Therefore, the present studies clearly demonstrate that small Maf homodimers negatively regulate hARE-mediated NQO1 and other detoxifying enzyme gene expression.

Further support to the negative role of small Maf proteins in ARE-mediated gene expression came from the observation that the expression of antisense RNA of MafG and MafK significantly up-regulated the ARE-mediated NQO1 and GST Ya gene expression. It may be noteworthy that there are three small Maf proteins. These include MafG, MafK, and MafF. These Maf proteins are 81–83% homologous to each other. Therefore, the antisense of MafG will not only bind to MafG but also to MafK and MafF RNA leading to trans-activation of ARE-mediated gene expression. Studies on the antisense effect of individual protein are not feasible because of very high homology among the three small Maf proteins.

Several lines of observation indicated that small Maf-Nrf2 heterodimers also negatively regulate ARE-mediated NQO1 gene expression. These include: 1) overexpression of MafG and MafK repressed Nrf2 up-regulation of ARE-mediated NQO1 and GST Ya gene expression; 2) the MafG-Nrf2 and MafK-Nrf2 heterodimers bound with the NQO1 gene ARE; and 3) overexpression of increasing amounts of Nrf2 with constant amount of MafG led to Nrf2 concentration-dependent increase in ARE-mediated NQO1 gene expression. In a similar experiment, an increase in Nrf2 with a constant concentration of MafG failed to increase the ARE-mediated gene expression. In essence, the increase in Nrf2 concentration with constant concentration of MafG showed small decrease in ARE-mediated gene expression. This was possible only if Nrf2-MafG acted as negative
regulator of ARE-mediated gene expression. Therefore, there is some evidence demonstrating that Nrf2-small Maf heterodimers also contribute to the negative regulation of ARE-mediated gene expression. However, the role of Nrf2-small Maf heterodimers in negative regulation of ARE-mediated gene expression remains to be confirmed by further experiments.

Our results on the repressive role of Nrf2-small Maf heterodimers is also supported by a recent report on the repressive role of Nrf2-MafK in ARE-mediated gene expression (44). This report, however, did not detect the Maf-Maf homodimers in band shift assays as observed by us in the present study. The possible explanation is that we ran our gel longer than that run in the published report. The Maf-Maf homodimers run very close to an unspecific band from rabbit reticulocyte lysate.

FIG. 6. Band shift assays. NQO1 gene ARE was end-labeled with [α-32P]ATP. 50,000 cpm of the labeled hARE was incubated with in vitro translated MafG-V5 or MafK-V5 either alone or in combination with in vitro translated Nrf2 as shown. The MafG-V5, MafK-V5, and Nrf2 alone and in combinations were preincubated at 37 °C for 15 min before incubation with the labeled hARE. The band shift experiment was performed at room temperature. The band shift reaction mixture was incubated with preimmune serum and anti-V5 antibody or Nrf2 antibody for 2 h at 4 °C in supershift assays. The band shift and supershift mixtures were analyzed on a 5% nondenaturing polyacrylamide gel. The gel was dried and autoradiographed. The asterisk denotes the nonspecific band from rabbit reticulocyte lysate.

FIG. 7. Band shift assays. A, NQO1 gene ARE was end-labeled with [α-32P]ATP. 50,000 cpm of the labeled hARE was incubated with 10 μg of nuclear extract from Hep-G2 cells treated with MeSO (control) or t-BHQ (first and second lanes). Similar experiments were also performed with nuclear extracts from Hep-G2 cells overexpressing cDNA derived Nrf2 and MafG either treated with MeSO (control) or t-BHQ (third and fourth lanes). The band shift experiment was performed at room temperature. The gel was dried and autoradiographed. B, in a similar experiment as above, 20 μg of nuclear proteins were used for the supershift assay. The Nrf2 and V5 antibodies were used to supershift the ARE-nuclear extract complex. SB, shifted bands; SSB, supershifted bands.

FIG. 8. Effect of varying concentrations of Nrf1 and Nrf2 with a constant concentration of MafG on NQO1 gene ARE-mediated luciferase gene expression. The Hep-G2 cells were co-transfected with 0.5 μg of reporter plasmid NQO1 gene hARE-Luc, a constant concentration of expression plasmid pcDNA-MafG(C) and varying concentrations of pcDNA-Nrf1(C) or pcDNA-Nrf2(C). The concentrations (μg) of plasmids used in the various transfections are also shown. 0.01 μg of plasmid pRL-TK encoding Renilla luciferase was used as the internal control in each transfection. The cells were harvested 48 h after transfection and analyzed for luciferase activity. The values represent the means ± S.E. of three independent transfection experiments.
haved differently than Nrf2. Interestingly, overexpression of Nrf1, along with MafG, did not reveal a role of Nrf1-MafG in the negative regulation of hARE-mediated NQO1 gene expression. Overexpression of increasing concentration of Nrf1 and Nrf2 with a constant concentration of MafG showed different results. The increase in Nrf1 and not Nrf2 resulted in increased expression of ARE-mediated gene expression in transfected cells. In addition, Nrf1-MafG and Nrf1-MafK heterodimers were not detected in the band and supershift assays. These results led to the conclusion that unlike Nrf2, Nrf1-small Maf heterodimers do not bind to the ARE and have more or less no effect on the gene expression. Nrf1 is known to form heterodimers with hMaf, which binds to NF-E2 binding site and γ-GCS ARE (42, 35). It is possible that Nrf1 formed heterodimers with MafG and MafK in our studies. However, Nrf1-MafG and Nrf1-MafK heterodimers failed to bind with NQO1 gene ARE. Nrf1 has also been shown to bind with γ-GCS ARE as homodimer (35). However, Nrf1 does not bind with NQO1 gene ARE either as homodimer (Ref. 31 and present studies) or as heterodimer (present studies) with small Maf proteins. The significance of this difference between NQO1 and γ-GCS ARE remains unknown. In addition, the differential effect of Nrf2 and Nrf1 with Maf proteins on NQO1 gene ARE also remains unknown.

A model to demonstrate the negative role of small Maf (MafG and MafK) proteins in ARE-mediated expression and induction of NQO1 and other detoxifying enzyme genes is depicted in Fig. 10. The metabolism of antioxidants and xenobiotics generates electrophiles and ROS that signal the activation of a battery of genes including NQO1 and GST Ya (1). Electrophiles and/or ROS lead to the transcriptional activation and/or post-transcriptional modification of positive regulatory factors including Nrf2, c-Jun, and unknown factors. The positive factors bind to the ARE and activate transcription of NQO1, GST Ya, and other detoxifying enzyme genes by unknown mechanism(s). Electrophiles and/or ROS may also lead to the transcriptional activation and/or post-transcriptional modification of negative regulatory factors including the small Maf proteins, c-Fos, Fra, and unknown factors, which also bind to ARE and down-regulate the expression of detoxifying enzyme genes. It is possible that the activation of positive factors is an early response and is followed by a late response activation of negative regulators. However, this chain of events requires further study. It is also possible that the concentrations/activities of negative regulatory factors remain unaffected because of a signal from electrophiles and ROS. This would indicate that ARE-mediated gene expression might be a balance between positive and negative regulators. Further studies are required to test these hypotheses.

It has recently been proposed that Nrf-Maf heterodimers could also possibly activate ARE-mediated γ-GCS gene expression (35, 37). Another report also suggested the positive role of Nrf2-Maf in ARE-mediated gene expression in general (45). However, these reports only speculated the role of Nrf2-Maf in positive regulation of ARE-mediated gene expression (35, 37). Another report also suggested the positive role of Nrf2-Maf in ARE-mediated gene expression in general (45). This speculation was exclusively based on in vitro binding of these factors to the ARE. No in vivo transfection data were shown in support of a positive role of Nrf2-Maf in regulation of ARE-mediated gene expression. In contrast, the Nrf2 and Maf heterodimers have been shown to negatively regulate ARE-mediated GST Ya and QG genes expression and induction (44). This conclusion on the negative role of Nrf2-Maf was supported by in vitro gel shift as well as in vivo transfection experiments (44). Our data in the present manuscript support negative regulation by Maf-Maf homodimers and possibly by Maf-Nrf2 heterodimers. Further studies should reveal more information on the negative or positive role of Nrf2-Maf heterodimers in ARE-mediated gene regulation. The difference in responses among the various genes ARE to Nrf2-Maf heterodimers also remains to be studied.

From this and previous studies, the small Maf proteins join the previously identified negative regulators c-Fos and Fra1 as repressors of ARE-mediated gene expression (26, 31, 46). These results raise an interesting question regarding the requirement of positive and negative regulators in normal cells. One hypothesis is that small amounts of superoxide and related reactive species are consistently required for keeping cellular defenses active. Because the activation of detoxifying and defensive proteins leads to a significant reduction in the levels of free radicals, cells may require negative regulatory factors to keep the levels of superoxide from falling below the level needed to keep cellular defenses active.
In conclusion, small Maf protein homodimers and possibly Nrfl2/small Maf heterodimers negatively regulate ARE-mediated expression and antioxidant induction of NQO1 and other detoxifying enzyme genes. Further studies are required to understand the role of positive and negative factors in ARE-mediated expression and induction of detoxifying enzyme genes.

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