Stress response elements, which mediate induction of the mouse heme oxygenase-1 (HO-1) gene by several agents, resemble the binding site for the activator protein-1 (Jun/Fos), Maf, and Cap’n’Collar/basic leucine zipper (CNC-bZIP) families of proteins. In L929 fibroblasts, significant activation of an HO-1 enhancer-reporter fusion gene was observed only with the CNC-bZIP class of proteins with Nrf2 exhibiting the highest level of trans-activation, between 25- and 30-fold. To further examine the role of this factor in HO-1 gene regulation, a dominant-negative mutant, Nrf2M, was generated and conditionally expressed in L929 cells. The mutant protein was detected in cytoplasmic and nuclear fractions but did not affect cell growth. Under conditions of Nrf2M overexpression, HO-1 mRNA accumulation in response to heme, cadmium, zinc, arsenite, and tert-butylhydroquinone was inhibited by 85–95%. In contrast, overexpression of a dominant-negative mutant of c-Jun decreased L929 cell growth but did not inhibit HO-1 gene activation. Nrf2 does not homodimerize, but CNC-bZIP-small Maf protein heterodimers and Nrf2-Jun protein complexes are proposed to function as trans-activators. Co-expression of Jun proteins or p18, however, had no significant affect or inhibited Nrf2-mediated trans-activation. Taken together, these results implicate Nrf2 in the induction of the HO-1 gene but suggest that the Nrf2 partner in this function is a factor other than p18 or Jun proteins.

Heme oxygenase enzymes catalyze the first and rate-limiting step in heme catabolism, the oxidative cleavage of b-type heme to yield equimolar quantities of iron, carbon monoxide and biliverdin. Biliverdin is subsequently converted to bilirubin by the action of biliverdin reductase. The expression of one isofrom of heme oxygenase, HO-1, is dramatically stimulated by a variety of agents including the substrate heme, heavy metals, hyperthermia, UV irradiation, and inflammatory cytokines.

The realization that most, if not all, HO-1 inducers stimulate production of reactive oxygen species or deplete glutathione levels or both, and the fact that heme is a potent pro-oxidant whereas bilirubin is an equipotent anti-oxidant, has led to the postulate that HO-1 activity is a component of the cellular defense mechanism against oxidant stress. This hypothesis has been experimentally verified by numerous studies using both in vitro and in vivo models of oxidant injury (reviewed in Ref. 1).

While the induction of HO-1 has been extensively documented and is known to be regulated primarily at the level of gene transcription, the molecular mechanism(s) underlying this response is poorly understood. Our analyses of the mouse HO-1 gene have identified two 5’-distal enhancer regions at approximately –4 and –10 kilobase pairs, termed SX2 and AB1, respectively, that mediate transcriptional activation of linked reporter genes in response to multiple agents including heme, heavy metals, 12-O-tetradecanoylphorbol 13-acetate, arsenite, hydrogen peroxide, and lipopolysaccharide (2–6). Each enhancer region contains multiple copies of a cis-acting element, termed the stress response element (StRE) (1) that are essential for inducer-dependent gene activation. The consensus StRE, (T/C)GCTGAGTCA, resembles the consensus binding site, TGA(C/G)TCA, for the AP-1 class of transcription factors, comprised of homo- and heterodimers of the Jun and Fos families of proteins, and we initially proposed that such factors were responsible for HO-1 gene activation (2, 4). This prediction was based on, among other reasons, the observations that AP-1 proteins bound to individual StREs, that the DNA binding of c-Jun-c-Fos heterodimer is subjected to redox regulation, and that expression and activities of some members of the Jun and Fos family of protein are stimulated by many of the same agents that induce HO-1 expression. A role for AP-1 proteins in HO-1 gene regulation is further supported by recent studies demonstrating that pharmacological inhibition of AP-1 activity attenuates interleukin-1α- or tumor necrosis factor-α-mediated induction of HO-1 mRNA levels in human endothelial cells (7) and ectopic expression of a dominant-negative mutant of c-Jun inhibits arsenite-mediated activation of the chicken HO-1 promoter in hepatoma cells (8).

The consensus StRE also resembles the optimal recognition sequences, TGCTGAGTCAAGC (9) and (T/C)GCTGAGTCCT (10), of the v-Maf oncprotein and of NF-E2, respectively. The v-Maf oncprotein, encoded by the avian musculoaponeurotic fibrosarcoma virus AS42 (11), is the founding member of the Maf family of sequence-specific DNA-binding proteins, of which six cellular members have also been identified to date. Maf factors, like Jun and Fos proteins, contain the basic leucine zipper (bZIP) domain for DNA binding and dimerization. Three of the Maf members, the small Maf proteins, lack
apparent trans-activation domains. NF-E2, an erythroid-specific transcription factor that is required for β-globin synthesis in mouse erythroblasts (12), is a heterodimer of an erythroid-specific 45-kDa subunit (p45) and an ubiquitous polypeptide (p18), later identified as the small Maf protein, MaFk (10, 13, 14). p45, like AP-1 and Maf proteins, is a bZIP-type factor but also contains an upstream Cap’n’Collar (CNC) domain homologous to a region within the fruit fly homeotic selector protein encoded by the cap’n’collar gene (15). Other CNC-bZIP polypeptides homologous to p45 have been identified, including Nrf1 (16), Nrf2 (17), and Nrf3 (18), which, unlike p45, are more widely expressed. Maf polypeptides resemble Jun proteins in that they can homodimerize, whereas the CNC-bZIP proteins, like Fos family members, can only form obligatory heterodimers, most prominently with the small Maf factors.

The optimal recognition sequences for v-Maf and NF-E2, as noted above, are extended AP-1 binding sites. The 3-base pair extension, (T/C)GC, is critical for high affinity binding of these factors to their target sequences. In a recent report (19), we demonstrated that mutation of this 3-base extension in the context of the StRE, while leaving intact the AP-1 heptad, abolished activation of a linked reporter gene in response to heme and cadmium, suggesting that factors other than AP-1 proteins are responsible for this induction. In the present report, we provide data that implicate Nrf2 in inducer-dependent activation of the HO-1 gene.

**EXPERIMENTAL PROCEDURES**

**Materials**—Restriction endonucleases and other DNA modifying enzymes were purchased from either Life Technologies, Inc. or New England Biolabs. Radiolabeled nucleotides were obtained from NEN Life Science Products, Inc. (now Perkin-Elmer). All other chemicals were reagent grade.

**Plasmid Constructs**—Mammalian expression plasmids were kindly provided by Dr. Stuart Orkin (p5, Nrf2, p18, and p18M), Mark Kerppola (c-Maf), and Tom Curran (c-Fos). cDNA clones were kindly provided by Dr. Minami Matsui (FRA-1 and FRA-2) or purchased from Promega Products. Enzymes and reagents for chloramphenicol acetyltransferase and luciferase assays were purchased from Sigma. All other chemicals were reagent grade.

**RESULTS**

**Trans-Activation of the SX2 Enhancer by CNC-bZIP Proteins**—Members of the AP-1 (2, 4, 5), CNC-bZIP (see below), and Maf (see below) families of proteins bind to one or more of the StREs in the HO-1 gene enhancer. The role, if any, of these sequence-specific DNA-binding proteins in HO-1 gene transcriptional regulation was examined by transient trans-activation assays. L929 cells were co-transfected with expression plasmids encoding individual factors and an SX2-dependent CAT reporter gene construct. The effect of the factors on SX2 transcriptional activity was assessed by measurement of CAT activity in cell extracts. Of the AP-1 family members tested, only the combination of c-Jun and c-Fos increased SX2 activity (~1.5-fold) (Fig. 1). Individual members decreased enhancer activity to varying degrees (20–60% of basal level). c-Maf did not alter SX2 activity, whereas small Maf/p18 inhibited SX2-dependent transcription by approximately 90%. Only members of the CNC-bZIP family of proteins exhibited significant trans-activation (≥5-fold) with Nrf2 increasing SX2 activity between 25- and 30-fold.

**Development of a Dominant-negative Mutant of Nrf2**—To further characterize the role of Nrf2 in HO-1 gene regulation, we devised a methodology to inhibit Nrf2 function. Nrf2, like Jun proteins, contains an N-terminal transcription activation domain and C-terminal dimerization and DNA-binding domains (17). Deletion of the transcription activation domain of c-Jun results in a protein that functions as a dominant-negative mutant by virtue of homodimerization with c-Jun and 2 J. Alam and D. Stewart, unpublished observations.
Nrf2 Regulation of HO-1 Gene

Fig. 1. trans-ACTIVATION of the SX2 enhancer in L929 cells. Cells were transfected with a DNA mixture consisting of 4 μg of pSX2Δ44cat, 4 μg of an empty expression vector (−) or the indicated transcription factor expression plasmid, and 2 μg of pT109luc. Aliquots representing 4% and 2% of the cell extracts were used for CAT and luciferase assays, respectively. Background CAT activity (from mock-transfected cells) was subtracted from each experimental measurement, and the resulting value was corrected for variation in transfection efficiency by normalization with background-subtracted luciferase activity in the same cell extract. Normalized activity for pSX2Δ44cat in the absence of exogenous transcription factor was arbitrarily assigned a value of 1. Each data bar represents the average ± S.D. from three to five independent experiments.

Characterization of L929 Cells Expressing the Nrf2 Dominant-negative Mutant—To examine the effect of the Nrf2 dominant-negative mutant on HO-1 gene induction, we generated L929 clones stably expressing Nrf2M. Because of the possibility that inhibition of the Nrf2 transcription factor would affect cell proliferation or viability, Nrf2M was expressed in a regulated manner. DNA encoding Nrf2M was cloned downstream of the tet operator (tetO) sequences in plasmid pUHDBG (20). The resultant clone, pUHDBG/Nrf2M, was stably transfected into E8.T4 cells, a subclone of L929 cells that expresses the tetracycline analogue, in a dose-dependent manner over a range of approximately 100-fold. The Nrf2M protein was detected in both cytoplasmic and nuclear fractions and migrated at a size corresponding to 27 kDa (predicted size of 22.5 kDa) (Fig. 3C). The predicted size of the native Nrf2 protein is 72 kDa, but the protein may migrate anomalously above 90 kDa on an SDS-polyacrylamide gel (17). Multiple, faint bands were observed in this region after longer exposure of the Western blots (data not shown). The rates of growth of N2 and N5 cells were not significantly different in the absence (Nrf2M overexpression) or presence of 1 μg/ml Tc in the culture media (Fig. 3D) and were similar to that of the parental E8.T4 cells (data not shown).

Overexpression of Nrf2M Attenuates HO-1 Gene Induction—To determine if Nrf2M overexpression and, by inference, inhibition of Nrf2 function, affects HO-1 gene regulation, N2 cells were grown in the absence or presence of 1 μg/ml Tc and treated with various HO-1 inducers, including heme, cadmium, zinc, arsenite, and tert-butylhydroquinone (TBHQ). HO-1 gene induction was assessed by measuring HO-1 mRNA levels in Northern and dot blot analyses (Figs. 4 and 5). In the presence of Tc (i.e. undetectable expression of Nrf2M), these agents increased the steady-state amount of HO-1 mRNA by 15–90-fold above basal levels. The magnitude of inductions is similar using a rabbit β-globin probe. (β-Globin sequences, present in pUHDBG, are fused downstream of the Nrf2M translation termination site and provide stability to the chimeric transcript.) All six clones exhibited Tc-dependent regulation of the Nrf2M/β-globin chimera (Fig. 3A). Northern blot analysis of total RNA from N2 cells using an Nrf2 probe identified an Nrf2M transcript of the predicted size (approximately 750 bases) and the endogenous Nrf2 mRNA of 2.4 kilobases (Fig. 3B). The level of Nrf2M mRNA was regulated by doxycycline, a tetracycline analogue, in a dose-dependent manner over a range of approximately 100-fold. The Nrf2M protein was detected in both cytoplasmic and nuclear fractions and migrated at a size corresponding to 27 kDa (predicted size of 22.5 kDa) (Fig. 3C). The predicted size of the native Nrf2 protein is 72 kDa, but the protein may migrate anomalously above 90 kDa on an SDS-polyacrylamide gel (17). Multiple, faint bands were observed in this region after longer exposure of the Western blots (data not shown). The rates of growth of N2 and N5 cells were not significantly different in the absence (Nrf2M overexpression) or presence of 1 μg/ml Tc in the culture media (Fig. 3D) and were similar to that of the parental E8.T4 cells (data not shown).
to that observed in the parental E8.T4 and wild-type L929 cells (data not shown). Overexpression of Nrf2M (−Tc) inhibited HO-1 mRNA accumulation by all inducers tested by 85–90%. The basal level of HO-1 mRNA was not altered. Some of the HO-1 inducers, metals in particular, activate the c-jun and metallothionein genes. These inductions, however, were not affected by Nrf2M, indicating differences in gene activation mechanisms. Similar results were observed with N5 cells (data not shown).

A Dominant-negative Mutant of c-Jun Does Not Inhibit HO-1 Gene Activation—For comparative purposes we also generated a dominant-negative mutant of c-Jun, c-JunM, lacking the N-terminal activation domain (residues 1–148), which is similar to one previously described (31). As expected of a dominant-negative protein, c-JunM inhibited c-Jun-mediated trans-activation of a reporter gene (Fig. 6A). Initial attempts to develop an E8.T4 cell line conditionally expressing c-JunM were unsuccessful, so we subsequently transfected L929 cells with a plasmid permitting constitutive expression of the mutant protein. Of 12 G418-resistant colonies selected, 9 clones survived during subsequent culturing, two of which, cJM-9 and cJM-11, expressed the mutant protein at high levels (Fig. 6B). Compared with cells transfected with the empty vector (“Neo” cells), cJM-9 and cJM-11 cells exhibited a bias toward increased levels of HO-1 mRNA accumulation. An opposite tendency was observed with respect to c-Jun mRNA levels under basal conditions and in response to the agents tested (Fig. 6D).

Nrf2 Heterodimerizes with Small Maf/p18 but the Nrf2p18 Heterodimer Functions as a Transcription Repressor—Based on the sequence of its leucine zipper domain, Nrf2 is not expected to form homodimers (17) and does not bind to the NF-E2 class of recognition sequences (27, 28). Heterodimers of Nrf2

![Diagram](image)

**Fig. 3.** A, screening of pUHDBG/Nrf2M transfectants. Total RNA was isolated from individual clones (N1–N6) cultured in the absence or presence of 1 μg/ml Tc. RNA dot blot analysis was carried out as described in “Experimental Procedures” using the probe for β-globin. The filter was autoradiographed for 8 h. B and C, tetracycline-regulated expression of Nrf2M mRNA (B) and protein (C). N2 cells were cultured for 72 h in the absence or presence of the indicated concentration of doxycycline, a tetracycline analogue. Northern blot analysis (B) was carried out using a hybridization probe for Nrf2. Migration of the 18 and 28 S ribosomal RNAs is indicated. The filter was autoradiographed for 16 h. Western blot analysis (C) was carried out as described in “Experimental Procedures,” and the filter was exposed to film for 2 min. The size (kDa) and migration of the molecular size standards are indicated. D, expression of Nrf2M does not affect the rate of cell proliferation. 1 × 10⁵ cells were seeded (t = 0) in duplicate 60-mm plates and cultured in complete media in the absence or presence of 1 μg/ml Tc. Cells were recovered by trypsinization at the indicated times, and viable cells were quantified by the trypan blue exclusion method. Each data bar represents the average ± S.D. from three independent experiments.

![Diagram](image)

**Fig. 4.** Expression of Nrf2M inhibits induction of the HO-1 gene by multiple agents. E8.T4/Nrf2M (clone N2) cells were plated (2 × 10⁴/100 mm plate) and cultured in the absence (−) or presence (+) of 1 μg/ml Tc for 48 h. Cells were treated with vehicle, heme (10 μM), CdCl₂ (10 μM), ZnSO₄ (100 μM), sodium arsenite (100 μM) or TBHQ (50 μM) for 3 h in serum-free medium in the absence or presence of 1 μg/ml Tc. Total RNA was isolated, and 10-μg aliquots were electrophoresed and transferred to nylon membrane. The filter was hybridized to a rat HO-1 cDNA probe and autoradiographed for 18 h.
and small Maf proteins (27, 28) and Nrf2-Jun complexes (32), however, can bind DNA and are reported to function as transcription activators. Similarly, using in vitro synthesized proteins in electrophoretic mobility shift assay reactions, the Nrf2-p18 heterodimer exhibited avid binding to the HO-1 StRE, but no such binding was observed with Nrf2 and c-Jun cotranslation products or any of the individual proteins (data not shown). The lack of DNA binding by Nrf2 and c-Jun cotranslation products is consistent with a previous report demonstrating that an uncharacterized cytosolic factor is required for Nrf2-c-Jun complex formation and/or DNA binding activity (32).

To determine if the trans-activation of the SX2 enhancer by Nrf2 (see Fig. 1) is due to Nrf2-p18 heterodimers, we examined the effect of p18 on the Nrf2-independent and Nrf2-dependent expression of pSX2Δ44Luc in transient transfection assays. In the absence of exogenous Nrf2 (Fig. 7A, −Nrf2), p18 decreased luciferase activity (by 90%) in a dose-dependent manner. Although complexes of p18 with other cellular factors cannot be ruled out, this down-regulation is likely an effect of the p18-p18 homodimer as a mutant of p18, p18M, which does not homodimerize (12), did not affect pSX2Δ44Luc expression. Exogenous p18 also potently inhibited Nrf2 trans-activation of SX2 at all ratios of Nrf2 and p18 tested (Fig. 7A, +Nrf2). Co-expression of p18M also inhibited Nrf2-mediated trans-activation but to a lesser extent (75% maximum inhibition versus ≥90% inhibition for p18) suggesting that, although it cannot homodimerize, p18M does heterodimerize with Nrf2, albeit less efficiently than p18. Co-expression of Jun-B and Jun-D also decreased Nrf2-mediated trans-activation, but to a lesser extent than that observed with p18 (Fig. 7B, +Nrf2). Co-expression of c-Jun slightly enhanced (−20%) Nrf2-mediated trans-activation, but this effect was not statistically significant.

**DISCUSSION**

Induction of the mouse HO-1 gene by several diverse agents is mediated by multiple StREs located within two distal 5′ enhancer regions. The consensus sequence for the StRE resembles the binding site for AP-1, Maf, and CNC-bZIP families of transcription factors, which bind to DNA as obligate dimers. Because of intrafamily homodimerization and heterodimerization and even interfamily heterodimerization (9, 10, 13, 33–35), the number of dimeric species that can potentially bind to the StREs is quite large (at least 20 such species can be formed within and between Jun and Fos families alone), and identification of the dimeric species that regulate HO-1 gene expression is not a trivial matter, especially considering the possibility of inducer-specific utilization of distinct dimeric factors. The present study represents an initial attempt to identify, or at least narrow the list of, dimeric species that mediate HO-1 gene induction.

Given that the AP-1 transcription factor system is a primary regulator (along with NF-κB proteins) of the cellular response to alterations in redox states, we initially reasoned that one or more members from this family mediated HO-1 gene activation in response to oxidative stress. Results presented herein, however, suggest that the CNC-bZIP transcription factors play a more prominent role than AP-1 factors in HO-1 gene induction. This conclusion is based on two principal observations as discussed below.

First, in transient trans-activation experiments, Jun and Fos proteins generally do not affect or inhibit, whereas CNC-bZIP proteins potentially stimulate, the transcription activity of the SX2 enhancer in L929 cells. This pattern is also observed in other cells including Hepa (mouse hepatoma) and RAW 264.7 and MCF-7 (human breast cancer epithelial) cells (data not shown), attesting to the generality of this phenomenon. The trans-activation profile of SX2 is strikingly similar to that of the antioxidant response element (ARE) of the human NAD(P)H:quinone oxidoreductase (hNQO1) gene (36). The NQO1 ARE contains a core sequence, TGCTGAGTCA, that conforms perfectly to the consensus StRE and NF-E2 binding site and, like the StRE, binds to both AP-1 and CNC-bZIP factors, but only the latter stimulate transcription activity (36). As observed with SX2, Jun and Fos family members either had no effect or inhibited ARE-dependent reporter gene expression and Nrf2 was a more potent trans-activator than Nrf1. AREs, in response to electrophiles and antioxidants, regulate the coordinate induction of several genes encoding phase II enzymes - proteins which function in xenobiotic detoxification and as such provide protection against electrophile and oxidative toxicity. Interestingly, many of the monofunctional phase II enzyme inducers also activate the HO-1 gene by an StRE-dependent mechanism (37). Furthermore, the hNQO1 ARE (38, 39) and the HO-1 StRE (2, 5, 37) mediate induction of the hNQO1 and HO-1 genes, respectively, in response to 12-O-tetradecanoylphorbol 13-acetate, hydrogen peroxide, and TBHQ. Given the similarities outlined above, it is not unreasonable to speculate that activation of the NQO1 and HO-1 genes, at least by com-

![Fig. 5. Nrf2M expression does not inhibit induction of the c-jun and metallothionein genes.](image-url)
mon inducers, is mediated by the same transcription factor(s).

In this regard it is noteworthy that stimulation of NQO1 gene expression by the phase II enzyme inducer butylated hydroxyanisole is impaired in \textit{nrf2} null mice (40). Additional indirect evidence of a role for Nrf2 in HO-1 gene regulation is provided by the recent identification of Keap1, a protein that represses Nrf2 activity by cytoplasmic retention of the transcription factor (41). Interestingly, electrophilic agents antagonize Keap1 repression, permitting nuclear translocation of Nrf2 and subsequent activation of ARE-dependent genes.

Transient trans-activation experiments, as carried out in this study, do not directly address the mechanism of inducer-dependent HO-1 gene activation. More direct evidence for a role of Nrf2 in HO-1 gene regulation is provided by the recent identification of Keap1, a protein that represses Nrf2 activity by cytoplasmic retention of the transcription factor (41). Interestingly, electrophilic agents antagonize Keap1 repression, permitting nuclear translocation of Nrf2 and subsequent activation of ARE-dependent genes.

Transient trans-activation experiments, as carried out in this study, do not directly address the mechanism of inducer-dependent HO-1 gene activation. More direct evidence for a role of Nrf2 in this process is provided by the observed inhibition of HO-1 gene induction by a dominant-negative mutant of Nrf2. Interestingly, overexpression of Nrf2M inhibits HO-1 mRNA accumulation in response to multiple agents, implying a commonality in the induction mechanism. The simplest explanation for this observation is that a single, Nrf2-containing dimeric factor is responsible for induction by all agents tested. Or, if distinct dimeric species are utilized in an inducer-specific manner, then Nrf2 is a common subunit of such factors. An alternative explanation for the inhibition of HO-1 gene induction by all agents tested is that Nrf2M, after dimerization with cellular factors such as the small Maf proteins, binds to the StREs with such avidity as to interfere with the binding of the actual positive activators. By extension, it is formally possible that Nrf2 does not in fact mediate induction by any of the agents examined. While such a possibility cannot be ruled out, the potent trans-activation of SX2 by Nrf2 (and the lack of such activation by AP-1 members) strongly suggests that Nrf2 is a positive regulator of HO-1 gene induction.

Unlike Nrf2M, expression of c-JunM does not inhibit activation of the HO-1 gene and in fact appears to slightly enhance such induction. This latter tendency and the generally negative effect of AP-1 factors on SX2 trans-activation are more consistent with an inhibitory or neutral role of AP-1 members in HO-1 gene regulation, at least in response to the agents used in this study. In contrast to the results presented here, Elbirt \textit{et al.} (8) have recently reported that ectopic expression of a c-Jun dominant-negative mutant abrogates arsenite-mediated induction of a chicken HO-1 promoter-luciferase fusion gene in chicken embryo hepatoma cells. One explanation for this discrepancy is that a truncated chicken HO-1 promoter fragment, lacking all of the potential arsenite-responsive elements and one which is minimally responsive to arsenite, was used in the latter study. An alternative explanation for the lack of an effect of c-JunM, that stable overexpression of the dominant-negative mutant

![Fig. 6](image-url)

**A**. Inhibition of c-Jun-mediated trans-activation by c-JunM. RAW 264.7 cells were transfected with the indicated amount (in µg) of plasmid mixtures. Fold trans-activation was calculated in a manner analogous to that described in the legend to Fig. 1. Each data bar represents the average ± S.D. from three independent experiments. **B**. Identification of L929 stable transfectants expressing c-JunM. Total cellular extracts were prepared from clones transfected with the empty expression plasmid (N) or with pEF/c-JunM (clone no. indicated). Western blot analysis was carried out as described under “Experimental Procedures,” and the filter was exposed to film for 5 min. The size (kDa) and migration of the molecular mass standards are indicated. The bands between 40 and 46 kDa presumably represent endogenous Jun proteins. **C**. Expression of c-JunM decreases the rate of cell proliferation. Control (Neo) or c-JunM-expressing cells were cultured in 10% or 2% fetal bovine serum, and cell growth was quantified as described in the legend to Fig. 3. **D**, expression of c-JunM does not inhibit induction of the HO-1 gene. Cell treatment and RNA dot blot analysis were carried out as described in the legends to Figs. 4 and 5. The probe fragment used to detect endogenous c-Jun mRNA was derived from the 5’ end of the c-Jun cDNA and does not hybridize to c-JunM transcripts. The data bars represent the average of two independent experiments.
provides a less complete inhibition of AP-1 activity than transient overexpression (as in Ref. 8), is unlikely because: (1) stable expression of Nrf2M does not affect the level of nuclear Nrf1-expressed AP-1 activity at any Nrf2 to p18 ratio tested. Our experiments show that Nrf2-mediated induction of the HO-1 gene is not inhibited by any of the Jun proteins at any ratio tested, consistent with the role of Nrf2-Jun complexes as mediators of the HO-1 gene induction. This conclusion is also supported by the ineffectiveness of c-JunM in inhibiting HO-1 gene activation.

In summary, Nrf2, a CNC-bZIP factor is a potent positive regulator of the mouse HO-1 gene and mediates inducer-dependent gene activation. Nrf2 functions as an obligate heterodimer, but the partner(s) necessary for HO-1 gene regulation is not known. This protein, however, is not p18 or a member of the known Jun family of proteins. Studies to identify the Nrf2 partner are currently in progress.

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