Hemin-induced Activation of the Thioredoxin Gene by Nrf2

A DIFFERENTIAL REGULATION OF THE ANTIOXIDANT RESPONSIVE ELEMENT BY A SWITCH OF ITS BINDING FACTORS

Yong-Chul Kim, Hiroshi Masutani, Yoshimi Yamaguchi, Ken Itoh, Masayuki Yamamoto, and Junji Yodoi

From the Institute for Virus Research, Kyoto University, 53 Shogoin, Kawahara-cho, Sakyo, Kyoto 606-8507, Japan and the §Center for Tsukuba Advanced Research Alliance and Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba 305-8577, Japan

Thioredoxin plays an important role in various cellular processes through redox regulation. Here, we have demonstrated that thioredoxin expression is transcriptionally induced in K562 cells by hemin (ferriprotoporphyrin IX) through activation of a regulatory region positioned from −452 to −420 bp of the thioredoxin gene. Insertion of a mutation in the antioxidant responsive element (ARE)/AP-1 consensus binding sequence in this region abolished the response to hemin. With electrophoretic mobility shift and DNA affinity assays, we have shown that the NF-E2p45/small Maf complex constitutively binds to the ARE. The binding of the Nrf2/small Maf complex to ARE was induced by hemin, whereas the binding of Jun/Fos proteins to ARE was induced by phorbol 12-myristate 13-acetate, but not hemin. Hemin induced nuclear translocation of Nrf2 but did not affect nuclear expression of Jun/Fos proteins. Overexpression of Nrf2 augmented the response to hemin in a dose-dependent manner. In contrast, overexpression of the dominant negative mutant of Nrf2 suppressed hemin-induced activation through the ARE. We show here hemin-induced activation of the thioredoxin gene by Nrf2 through the ARE and propose a novel mechanism of the regulation of the ARE through a switch of its binding factors.

Received for publication, January 5, 2001, and in revised form, February 28, 2001
Published, JBC Papers in Press, March 1, 2001, DOI 10.1074/jbc.M100103200

© 2001 by The American Society for Biochemistry and Molecular Biology, Inc.
regulated through the ARE by the binding of NF-κB and small Maf under unstimulated conditions, that of Nr2f2/small Maf with hemin stimulation, and that of the Jun/Fos proteins with PMA stimulation. The binding of these factors to the ARE correlated well with their nuclear expression pattern. We also present evidence that Nr2f2 plays a role in the hemin-induced activation of the thioredoxin gene. We here propose a novel mechanism of the regulation of the ARE by a switch of binding factors including CNC-bZIP/small Maf transcription factors and the Jun/Fos proteins, depending on different stimuli.

**EXPERIMENTAL PROCEDURES**

Materials, Cell Lines, and Cell Culture—Hemin and PMA were purchased from Sigma (St. Louis, MO). Hemin stocks were prepared as reported previously (31). PMA was dissolved in dimethyl sulfoxide (Me2SO). The final concentration of Me2SO was kept to 0.1%. K562 (erythroleukemic cell line) cells were cultured in RPMI 1640 medium, supplemented with 10% heat-inactivated fetal calf serum, 100 units/ml (erythroleukemic cell line) cells were cultured in RPMI 1640 medium, reported previously (31). PMA was dissolved in dimethyl sulfoxide and the Jun/Fos proteins, depending on different stimuli.

**Plasmids**—The pTrXCAT plasmids were constructed as described previously (14). The HindIII-BamHI inserts from the pTrXCAT vectors were subcloned into pBlueScriptII KS (+) (pTrXblue vectors). The pTrXblue vectors pTrX(239–453), pTrX(352–453), pTrX(352–574), and the pTrXR (−453, −352)–Luc vectors were constructed by ligating the KpnI-BamHI fragments of the pTrXblue vectors to the KpnI-BglII sites of the pGL3 basic vector (Promega, WI). The pTrXR(−463, −352)–Luc vector was excised by HindIII, filled in, and then excised by XhoI. The insert was ligated to the Small/XhoI site of the pGL3 promoter vector to produce the pTrXR(−463, −352)–Luc vector. The BamHI-XhoI or NheI-BamHI insert of the pTrXR (−463, −352)–Luc vector was excised, filled in, and self-ligated to produce the pTrXR (−463, −447)–Luc or the pTrXR (−452, −352)–Luc vector, respectively. The pTrXR(−468, −435)–Luc, the pTrXR (−452, −420)–WT-Luc, the pTrXR (−452, −420)–M-Luc, the pTrXRAREWT-Luc, the pTrXRAREM1-Luc, the pTrXRAREM2-Luc, the pTrXRAREM3-Luc, and the pTrXRAREM4-Luc vectors were constructed by inserting oligonucleotides (OLIGO1, OLIGO2, OLIGO3, AREW, AREM1, AREM2, AREM3, and AREM4) into the KpnI-NheI site of the pGL3 promoter vectors, respectively. All constructs were controlled by direct nucleotide sequencing using a Thermo Sequenase II dye terminator cycle sequencing kit (Amersham Pharmacia Biotech). The pRL-CMV, the pRL-SV40, and the pRL-TK constructs were controlled by direct nucleotide sequencing using a Thermo Sequenase II dye terminator cycle sequencing kit (Amersham Pharmacia Biotech). The pTrxCAT plasmids were constructed as described previously (32).

**Identification of the Hemin Responsive Region in the Thioredoxin Gene**—To investigate the mechanism of activation of hemin, we first tested the response of the thioredoxin gene regulatory region to hemin treatment by a luciferase reporter assay. An activation of the thioredoxin gene was observed in a luciferase reporter construct containing whole thioredoxin promoter (pTrXR(−1148)–Luc). To analyze the region responsible for the response to hemin, we used luciferase reporter genes containing a series of deletions of the thioredoxin promoter region. As shown in Fig. 1A, 3.6, 5.5, 2.9, or 3.5-fold induction of relative luciferase activity was observed using reporter genes (pTrXR(−1148)–Luc, pTrXR(−980)–Luc, pTrXR(−874)–Luc, or pTrXR(−483)–Luc) in response to hemin, respectively. The response was dose-dependent (data not shown). In contrast, no significant induction was observed using a reporter gene (pTrXR(−352)–Luc), suggesting that −463 to −352 of the thioredoxin upstream sequence is important for the hemin response (Fig. 1A). To further determine the region required for hemin responsiveness, we used reporter vectors containing various lengths of nucleotides derived from −463 to −352 of the thioredoxin upstream sequence. Hemin activated both pTrXR(−483, −352)–Luc and pTrXR(−452, −352)–Luc vectors. In addition, the response to hemin hex (a constitutive inducer of pTrXR(−468, −435)–Luc) was not pTrXR(−463, −447)–Luc. These findings showed that −452 to −352 region is important for hemin responsiveness. Indeed, experiments using pTrXR(−452, −420)–Luc showed a 25-fold response to hemin treatment, revealing that the −452 to −420 sequence is necessary for the hemin response (Fig. 1B).

**Marked Reduction in Responsiveness to Hemin by the Insertion of a Mutational Region of the ARE Overlapping with the AP-1 Site**—In the hemin responsive region,
the typical consensus sequence for binding of HSFs is not included. However, the region contains a sequence TGCTGAGTAAC, which resembles the optimal recognition sequence, TGCTGA(C/G)TCAGCA (21) and (T/C)GCTGA(G/C)TCA(C/T) (22) of v-Maf and NF-E2p45, respectively. The sequence is also similar to the ARE, (A/G)GTGACNNN GC (23) and the AP-1 consensus binding sequence, TGA(C/G)TCA. We therefore tested the involvement of the sequence in hemin responsiveness. Activation of the thioredoxin gene by hemin was markedly reduced in a vector (pTRX(2452,-2420)-M-Luc), which has a mutation in the region (Fig. 2A). As the effect of the mutation was partial, we further analyzed the hemin response, using constructs with various mutations in the hemin responsive region. A 10-fold activation was observed with a reporter vector containing wild-type ARE (pTrxAREWT-Luc) in response to hemin. The response was reduced in the pTrxAREM1-Luc, pTrxAREM2-Luc, or pTrxAREM3-Luc vector and was almost abolished in the pTrxAREM4-Luc vector (Fig. 2B). Collectively, these results showed the involvement of the ARE sequence overlapping with the AP-1 site in the response to hemin. In addition, the deletion of the ARE resulted in decrease of the basal activity of the thioredoxin promoter, showing that the ARE also contribute to the basal activity of the thioredoxin gene (data not shown).

Involvement of NF-E2p45 and Nrf2 in Binding Complexes to the ARE in the Thioredoxin Promoter—We next analyzed ARE-binding proteins by the EMSA. A constitutively bound complex (complex I), hemin-induced complex (complex II), and PMA-induced complex (complex III) to the ARE were detected in nuclear extracts of K562 cells. The binding of these complexes appeared to be specific because the binding of complex I, II, and III was abrogated by the addition of an excess amount of the wild-type oligonucleotides (Fig. 3) but not by the M4 mutant of the ARE (data not shown). Faster migrating complexes seemed nonspecific because they were not competed by wild-type oligonucleotides. Complex I and II binding showed no competition by an excess of oligonucleotides encompassing the heat shock element (HSE), and no supershift was induced by either anti-HSF-1 antibody or anti-HSF-2 antibody. In contrast, hemin-induced specific binding to the HSE was abolished by an excess amount of oligonucleotide encoding the HSE and was supershifted by these antibodies (Fig. 3A).

FIG. 1. Determination of the thioredoxin promoter region required for hemin response. A, hemin-induced activation of the thioredoxin gene. K562 cells were transfected with the pTRX-Luc vectors indicated on the left, together with pRL-CMV, then treated with 30 µM hemin. B, identification of the region responsible for the response to hemin in the thioredoxin promoter. K562 cells were transfected with the pTRX-Luc vectors as indicated in the upper panel, together with pRL-CMV. Values shown represent the ratio of luciferase activity of 30 µM hemin-treated cells to that of untreated cells. The result is representative of three independent experiments.

FIG. 2. Mutational analysis of the ARE of the thioredoxin gene. A, decrease of hemin responsiveness in a vector harboring a mutation in the ARE/AP-1-like sequence. The wild-type and mutated sequences are indicated in the left panel. A n x below the sequence indicates mutated bases. K562 cells were transfected with the indicated plasmids together with pRL-CMV and then treated with 30 µM hemin. Results shown are the -fold activation of luciferase activity, compared with that of untreated cells.

FIG. 3. EMSA of the ARE from the thioredoxin promoter. Lane 1, pTRX-Luc (negative control); lane 2, complex I; lane 3, complex II; lane 4, complex III; lane 5, PMA-induced complex; lane 6, heme-induced complex; lane 7, wild-type oligonucleotides; lane 8, M4 mutant of the ARE (data not shown).
AP-1 site. Complex I was supershifted by the addition of anti-NF-E2p45 or anti-MafK antibodies, but not by antibodies against Nrf2 (Fig. 3B), Jun, Fos (Fig. 3D), Nrf1, or v-Maf (data not shown), suggesting that complex II includes Nrf2 and small Maf proteins. We also observed a PMA-induced binding complex to the ARE. The PMA-induced complex (complex III) was abrogated or supershifted by the addition of anti-Jun or anti-Fos antibodies but not by anti-Nrf2 antibodies (Fig. 3D), suggesting that complex III includes the Jun/Fos proteins.

**Analysis of ARE-binding Proteins Using DNA Affinity Purification Assay**—We performed the DNA affinity purification assay to further analyze binding factors to the ARE. NF-E2p45
was detected in eluates from affinity beads conjugated with wild type ARE (AREW), but not from those with mutated ARE (AREM4), using nuclear extracts from untreated or hemin-treated cells. Nrf2 was only detected in eluates from AREW affinity beads using nuclear extracts from hemin-treated cells but not from untreated cells. We observed MafK in eluates from AREW beads using nuclear extracts from untreated or hemin-treated cells. In contrast, Jun and Fos proteins were only detected in eluates using nuclear extracts from PMA-treated cells (Fig. 4).

**Nuclear Expression of ARE and AP-1 Binding Factors**—As nuclear translocation is considered to be an important mechanism of activation of transcription factors including Nrf2, we tested nuclear expression of Nrf2, NF-E2p45, small MafK, and the Jun/Fos proteins. The Jun and Fos proteins were detected in nuclei only after PMA stimulation. Staining with anti-NF-E2p45 or anti-MafK antibodies was shown in nuclei before and after hemin treatment. In contrast, Nrf2 proteins were stained in nuclei only after hemin treatment (Fig. 5A). Because thioredoxin is translocated into nucleus by PMA (37), we also tested nuclear expression of thioredoxin in hemin treatment. Thioredoxin translocated into nuclei after PMA or hemin stimulation in K562 cells (Fig. 5A). We then examined nuclear expression of these factors by Western blotting. NF-E2p45 expression was detected in nuclear extracts without stimulation. However, after hemin or PMA treatment, the expression decreased. Nrf2 expression was augmented by hemin. In contrast, expression of the Jun/Fos proteins was augmented by PMA, not by hemin (Fig. 5B). MafK remained in the nuclei before and after stimulation. Either hemin or PMA treatment augmented thioredoxin nuclear expression (Fig. 5B).

**Activation of the Thioredoxin Promoter by Overexpression of Nrf2 and MafK**—To further test whether Nrf2 and MafK was involved in the hemin-induced activation of the thioredoxin gene, we used transient transfection experiments in K562 cells using pTrxAREWT-Luc. Overexpression of both Nrf2 and MafK augmented hemin-induced activation of the thioredoxin promoter in K562 cells, dependent on the dose of Nrf2 (Fig. 6A). In addition, overexpression of the dominant negative mutant of Nrf2 suppressed hemin-induced activation of the thioredoxin gene (Fig. 6B). These data collectively showed that Nrf2 and the small Maf proteins mediate the hemin-induced activation of the thioredoxin gene.

**DISCUSSION**

In the present study, we have shown that a region from −452 to −420 bp of the gene regulatory region of the thioredoxin gene is required for hemin responsiveness and is also important for basal expression of the thioredoxin gene. The sequence from position −452 to −420 bp of the human thioredoxin promoter is highly homologous to that from position −607 to −575 bp of the mouse thioredoxin gene (15), indicating that the sequence in the thioredoxin promoter is conserved in both the human and mouse thioredoxin genes. We previously reported an oxidative responsive element (ORE), which is the sequence from −953 to −930 bp and mediates the stress response of hydrogen peroxide (14). The possible contribution of ORE to the hemin response should be further examined. Sistonen and co-workers (18) sug-
gested that HSF-2 mediates hemin-induced activation of the thioredoxin gene. However, as observed in EMSA, specific binding complexes to the hemin responsive sequence were not affected by the addition of either unlabeled oligonucleotides encompassing HSE or antibodies against HSF-1 or HSF-2 (Fig. 3A). These observations showed that HSFs are not included among proteins that bind to the sequence. Indeed, no typical consensus sequence for HSFs was identified in the thioredoxin gene. The possible involvement of HSFs in hemin-induced activation of the thioredoxin gene should be further tested. In contrast, we found the TGCTGAGTAAC sequence, which resembles the ARE and AP-1 binding site, in the hemin responsive region of the thioredoxin promoter. Mutation in the ARE core sequence abolished the hemin response (M4 mutant in Fig. 2B), showing that the ARE is important for hemin-induced thioredoxin gene activation. We also observed a decrease of the hemin response with the M1 mutant (Fig. 2B), suggesting that the extended ARE sequence is necessary to be fully functional. This observation is consistent with a previous report (38), further indicating that hemin-induced thioredoxin gene activation is mediated by the ARE.

In the EMSA and DNA affinity binding assays, we showed that NF-E2p45/small Maf complex constitutively binds to the ARE and that Nrf2/small Maf complex is induced to bind to the ARE by hemin (Figs. 3, B and C; and 4). Other members of small Maf proteins may also contribute to these complexes, although the level of MafK is higher than MafG in K562 cells (39). The Jun and Fos proteins have been reported to be involved in the binding complex to the ARE (21, 29, 40–42). In our experiment, we could not detect members of the Jun/Fos proteins in constitutively bound or hemin-induced binding complexes to the ARE (Figs. 3 and 4). Taken together, we here propose a model that the ARE of the thioredoxin gene is regulated by a switch of its binding proteins (Fig. 7).

This change of binding proteins seemed to be regulated by the control of nuclear expression of ARE and AP-1 binding factors, because their nuclear expression pattern (Fig. 5) correlated well with the binding to the ARE (Figs. 3 and 4). Nuclear expression of these factors may be regulated by several mechanisms. Cycloheximide treatment abolished hemin-induced binding to the ARE, suggesting an involvement of protein synthesis. Induction of small Maf protein synthesis is reported in β-naphthoflavone-induced activation of the γ-glutamylcysteine synthetase subunit gene (43). In our data, the nuclear expression of MafK was unchanged or slightly augmented by hemin treatment. In contrast, Nrf2 nuclear expression was significantly augmented by hemin. The hemin-induced binding of Nrf2 to the ARE of thioredoxin gene was not preceded by augmentation of Nrf2 mRNA level (data not shown). Our results using confocal microscopy showed hemin-induced up-regulation of Nrf2 expression in nuclei. These data are consistent with a previous study that reports exposure to electrophilic agents does not change the Nrf2 steady-state mRNA level and liberates Nrf2 from Keap1, leading to Nrf2 nuclear translocation (30). Thus, the control of translocation and turnover of Nrf2 protein seems to be an important mech-

---

**FIG. 6.** Effect of overexpression of Nrf2, and MafK on the transactivation of the thioredoxin promoter. A, augmentation of hemin-induced activation by Nrf2 and MafK. K562 cells were transfected with the indicated plasmids, together with the pTrxAAREWT-Luc and the pRL-TK vector. The amount of vector was normalized using pEFBOS. Transfected cells were cultured in the presence and absence of 5 μM hemin. The results are representative of two independent experiments. Values shown represent the relative luciferase activity normalized with Renilla luciferase activity.

B, suppression of hemin-induced activation by a dominant negative mutant of Nrf2. Magnetic separated cells from transiently transfected K562 cells with either pcDNA3 or pcDNA3-Nrf2-M (pcDNA3-dnNrf2) were again transfected with the pTrxAAREWT-Luc and the pRL-TK vector. Transfected cells were cultured in the presence and absence of 5 or 30 μM hemin. The result is representative of two independent experiments.

**FIG. 7.** Schematic model of the regulation of the ARE of the thioredoxin promoter. The thioredoxin gene is regulated through the ARE in K562 cells by the binding of NF-E2p45/small Maf under unstimulated conditions, of Nrf2/small Maf in hemin stimulation, and of the Jun/Fos proteins in PMA stimulation.

---

2 H. Masutani, unpublished observations.
anism for hemin-induced augmentation of nuclear Nrf2 expression. Meanwhile, the reason for the decrease of nuclear expression of NF-κB45 after hemin or PMA treatment (Fig. 5) is currently unclear. Regulation of nuclear export of Bach2 by oxidative stress has been reported (44). Nuclear expression of NF-κB45 may also be regulated at the level of nuclear export.

Overexpression of both Nrf2 and small Maf protein augmented hemin-induced thioredoxin gene activation, which was dependent on the dose of Nrf2. In contrast, overexpression of a dominant negative mutant of Nrf2 suppressed hemin-induced activation of the thioredoxin gene (Fig. 6). Therefore, the major stimulatory effect on the thioredoxin gene by hemin seemed to be mediated by Nrf2 and small Maf proteins. Meanwhile, the role of the Jun/Fos proteins in the regulation of the ARE remains to be clarified. PMA stimulation did not change the luciferase activity using the pTrxAREWT-Luc vector (data not shown), suggesting that the PMA-induced switch from NF-κB45/small Maf to the Jun/Fos proteins does not change the activation status of the thioredoxin gene in K562 cells. In other tissues or developmental stages, however, the Jun/Fos proteins might be important for basal and inducible thioredoxin gene activation. In addition, we can also speculate that in a pathological condition, perturbation of signal to the ARE by dysregulated activation of the AP-1 system causes dysregulation of differentiation process, resulting in oncogenesis.

It is important to note that the phase II enzyme genes such as the human and rat NAD(P)H:quinone oxidoreductase genes, the rat and murine glutathione S-transferase Ya genes, and the human γ-glutamylcysteine synthetase subunit gene all contain the ARE. Nrf2 has been shown to be a regulator of the phase II enzyme genes (30, 43, 45). Thus, thioredoxin and these redox enzymes all have a common regulatory mechanism and may have co-ordinated roles against oxidative stress. Moreover, thioredoxin may be involved in cytoprotection against heme- and iron-related oxidative stress. Hemin, iron, and hemoglobin are promoters of free radical formation (46, 47). Heme release from hemoglobin has been implicated in the pathogenesis of reperfusion injury (48). Previously, we showed that thioredoxin expression is induced in ischemic reperfusion (13). Thioredoxin is also reported to facilitate the induction of the heme oxygenase-1 (49). Collectively, study of the regulation of hemin-induced thioredoxin gene activation may contribute to the regulation of ARE binding factors.

Finally, we have previously reported that thioredoxin negatively regulates p38 MAP kinase activation (50). We observed suppression of hemin-induced activation of the thioredoxin gene by p38 MAP kinase inhibitors in K562 cells, suggesting the involvement of the p38 MAP kinase system in the activation of the thioredoxin gene.2 It is possible to speculate that ARE-mediated thioredoxin gene activation is a negative feedback mechanism. Meanwhile, a recent report showed that the heme oxygenase-1 gene is activated through the ARE by MEKK1, TAK1, and ASK-1, but not by p38 MAP kinase (51). In addition, another report showed that protein kinase C-mediated Nrf2 phosphorylation is involved in the PMA-induced activation of the ARE (52), although we observed only slight augmentation of nuclear expression of Nrf2 after PMA treatment (Fig. 5). Our results and these studies indicate that several distinct signaling pathways lead to the ARE, depending on individual stimulus and cell types. Further work is in progress to elucidate the upstream pathway to the ARE of the thioredoxin gene.

Acknowledgments—We thank Y. Kanekiyo for secretarial help, Dr. F. Hossi for preparation of antibody, and Drs. H. Nakamura, Y. Ishii, and A. Nishiyama for discussion.

REFERENCES

1. Holmgren, A., and Björnstedt, M. (1995) Methods Enzymol. 252, 199–208
2. Tagaya, Y., Maeda, Y., Mitsu, A., Kono, N., Matsu, H., Hamuro, J., Brown, N., Arai, K., Yokota, T., Waksasugi, H., and Yodoi, J. (1989) EMBO J. 8, 767–774
3. Waksasugi, H., Waksasugi, N., Tursz, T., Tagaya, Y., and Yodoi, J. (1989) J. Immunol. 142, 2569–2570
4. Wollman, E. D., Auron, L., Rimsky, L., Shaw, A., Jaquot, J. P., Wigfield, P., Graber, P., Dessars, F., Robin, P., Gaubert, F., Bertolino, J., and Fradelizi, D. (1988) J. Biol. Chem. 263, 15506–15512
5. Packer, L., and Yodoi, J. (ed) (1999) Redox Regulation of Cell Signaling and Its Clinical Application, pp. 177–277, Marcel Dekker, New York
6. Ueno, M., Masutani, H., Arai, R. J., Yamauchi, A., Hirota, K., Sakai, T., Inamoto, T., Yamaoka, Y., Yodoi, J., Sakai, T., and Nikaide, T. (1999) J. Biol. Chem. 274, 35809–35815
7. Mitsui, A., Hirakawa, T., and Yodoi, J. (1992) Biochem. Biophys. Res. Commun. 186, 1220–1226
8. Masutani, M., Masutani, H., Nakamura, H., Miyajima, S., Yamauchi, A., Yonehara, S., Uchida, I., Irimajiri, K., Horiiuchi, A., and Yodoi, J. (1991) J. Immunol. 147, 3837–3841
9. Nakamura, H., Matsu, M., Furuke, K., Kitao, Y., lwata, S., Toda, K., Inamoto, T., Yamaoka, Y., Ozawa, K., and Yodoi, J. (1994) Immunol. Lett. 42, 75–80
10. Wada, H., Muro, K., Hirata, T., Yodoi, J., and Hitomi, S. (1995) Chest 108, 800–814
11. Takagi, Y., Mitusi, A., Nishiyama, A., Nazaki, K., Sono, H., Gon, Y., Hashimoto, N., and Yodoi, J. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 4311–4316
12. Ohira, A., Honda, O., Gauissant, C. T., Yamamoto, M., Hori, K., Masutani, H., Yodoi, J., and Honda, Y. (1994) Lab. Invest. 70, 279–285
13. Masutani, H., Ueno, M., Ueda, S., and Yodoi, J. (1999) in Antioxidant and Redox Regulation of Genes (Sen, C. K., Sies, H., Baeuerle, P. A., eds), pp. 297–311, Academic Press, San Diego
14. Taniguchi, Y., Taniguchi, U. Y., Mori, K., and Yodoi, J. (1996) Nucleic Acids Res. 24, 3746–3753
15. Matsui, M., Taniguchi, Y., Hirota, K., Takeo, M., and Yodoi, J. (1995) Gene (Amst.) 152, 165–171
16. Tonissen, K. F., and Wells, J. R. (1991) Gene (Amst.) 102, 221–228
17. Inamoto, T., Yamaoka, Y., Yodoi, J., Sakai, T., and Nikaido, T. (1999) Biochem. Biophys. Res. Commun. 209, 40–46
18. Chui, D. H., Tang, W., and Orkin, S. H. (1995) Biochem. Biophys. Res. Commun. 209, 40–46
19. Ishi, K., Igarashi, K., Hayashii, N., Nishizawa, M., and Yamamoto, M. (1995) Mol. Cell. Biol. 15, 4814–4820
20. Moi, P., Chan, K., Asunis, I., Cao, A., and Kan, Y. W. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 9926–9930
21. Matsui, H., Magnaghi Jaudin, L., Ali, S. A., Grosman, R., Robin, P., and Harel Bellan, A. (1997) Oncogene 15, 1661–1669
22. Savovsky, E., Mizuno, T., Sowa, Y., Watanabe, H., Sawada, J., Nomura, H., Ohmagi, Y., Hando, H., and Sakai, T. (1994) Oncogene 9, 1639–1646
ARE-mediated Regulation of the Thioredoxin Gene

37. Hirota, K., Matsui, M., Iwata, S., Nishiyama, A., Mori, K., and Yodoi, J. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 3633–3638
38. Wasserman, W. W., and Fahl, W. E. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 5361–5366
39. Toki, T., Itoh, J., Kitazawa, J., Arai, K., Hatakeyama, K., Akasaka, J., Igarashi, K., Nomura, N., Yokoizama, M., Yamamoto, M., and Ito, E. (1997) Oncogene 14, 1901–1910
40. Venugopal, R., and Jaiswal, A. K. (1998) Oncogene 17, 3145–3156
41. Venugopal, R., and Jaiswal, A. K. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14980–14985
42. Kataoka, K., Igarashi, K., Itoh, K., Fujiwara, K. T., Noda, M., Yamamoto, M., and Nishizawa, M. (1995) Mol. Cell. Biol. 15, 2180–2190
43. Wild, A. C., Moinova, H. R., and Mukahy, R. T. (1999) J. Biol. Chem. 274, 33627–33636
44. Hashino, H., Kobayashi, A., Yoshida, M., Kudo, N., Oyake, T., Motohashi, H., Yamamoto, M., and Igarashi, K. (2000) J. Biol. Chem. 275, 15370–15376
45. Ishii, T., Itoh, K., Takahashi, S., Sato, H., Yanagawa, T., Katoh, Y., Bannai, S., and Yamamoto, M. (2000) J. Biol. Chem. 275, 16023–16029
46. Tappel, A. J. (1955) J. Biol. Chem. 217, 721–733
47. Gutteridge, J. M., and Smith, A. (1988) Biochem. J. 256, 861–865
48. Balla, J., Jacob, H. S., Balla, G., Nath, K., Eaton, J. W., and Vercellotti, G. M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 9285–9289
49. Wiesel, P., Foster, L. C., Pellacani, A., Layne, M. D., Hoeh, C.M., Huggins, G. S., Straus, P., Yet, S-F., and Perrella, M. A. (2000) J. Biol. Chem. 275, 24840–24846
50. Hashimoto, S., Matsumoto, K., Gon, Y., Furuichi, S., Maruoka, S., Takeshita, I., Hirota, K., Yodoi, J., and Horie, T. (1999) Biochem. Biophys. Res. Commun. 258, 443–447
51. Yu, R., Mandlekar, S., Tan, T.-H., and Kong, A. N. T. (2000) J. Biol. Chem. 275, 9612–9619
52. Huang, H.-C., Nguyen, T., and Pickett, C. B. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 12475–12480