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Nrf2 Knockout Mice that Lack Control of Drug Metabolizing and Antioxidant Enzyme Genes - Animals Highly Sensitive to Xenobiotic Toxicity

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ABSTRACT *: Xenobiotics and their reactive intermediates bind to cellular macromolecules and/or generate oxidative stress, which provoke deleterious effects on the cell function. Induction of xenobiotic-biotransforming enzymes and antioxidant molecules is an important defense mechanism against such insults. A group of genes involved in the defense mechanism, e.g. genes encoding glutathione S-transferases, NAD(P)H: quinone oxidoreductase, UDP-glucuronosyltransferase (UDP-GT) and γ-glutamylcysteine synthetase (GGCS), have a common regulatory sequence, Antioxidant or Electrophile Responsive Element (ARE/EpRE). Recently, Nrf2, discovered as a homologue of erythroid transcription factor p45 NF-E2, was shown to bind ARE/EpRE and induce the expression of these defense genes. Mice that lack Nrf2 show low basal levels of expression and/or impaired induction of these genes, which makes the animals highly sensitive to xenobiotic toxicity. Indeed, we show here that nrf2-deficient mice had a higher mortality than did the wild-type mice when exposed to acetaminophen (APAP). Detailed analyses of APAP hepatotoxicity in the nrf2 knockout mice indicate that a large amount of reactive APAP metabolites was generated in the livers due to the impaired basal expression of two detoxifying enzyme genes, UDP-GT (Ugt1a6) and GGCS, while the cytochrome P450 content was unchanged. Thus, the studies using the nrf2 knockout mice clearly demonstrate significance of the expression of Nrf2-regulated enzymes in protection against xenobiotic toxicity.*

Key Words *: Nrf2, Antioxidant responsive element (ARE), Enzyme induction, Acetaminophen*

I. INTRODUCTION

Genes that encode a group of xenobiotic-biotransforming enzymes contain a conserved sequence motif called antioxidant or electrophile responsive element (ARE/EpRE) in the gene regulatory regions. These genes are induced coordinately via this *cis*-acting motif in response to certain types of chemicals. ARE/EpRE motif has also been found in regulatory regions of the genes coding for the antioxidant enzymes, suggesting that this sequence motif enables concomitant expression of the drug metabolizing enzymes and the antioxidant enzymes for protection against the stress generated by xenobiotics. Nrf2 is a recently discovered transcription factor that regulates the ARE/EpRE-mediated gene expression.

1. A group of enzymes that protect cells from xenobiotic toxicity

Many toxicants, either parent chemicals or their metabolites, are electrophiles. Electrophiles contain an electron-deficient atom and have a partial or full positive charge. In contrast, nucleophiles containing an electron-rich atom are abundant in biomolecules. Oxygen, nitrogen and sulfur atoms in the nucleic acids, proteins and other cellular components often have a negative charge that renders them targets for electrophilic chemicals. Electrophiles either covalently bind to these nucleophilic biomolecules or extract an electron from them (*i.e.*, oxidize them), and change their structures and functions, leading to toxicity (Gregs and Klaassen, 1996).

To prevent such harmful reactions from occurring,

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List of abbreviations : ARE, antioxidant responsive element; UDP-GT, UDP-glucuronosyltransferase; GST, glutathione S-transferase; GSH, glutathione; GGCS, γ-glutamylcysteine synthetase; SOD, superoxide dysmutase; XRE, xenobiotic responsive element; BHA, butylated hydroxyanisole; tBHQ, *tert*-butylhydroquinone; HO-1, heme oxygenase-1; NQO1, NAD(P)H: quinone oxidoreductase 1; EpRE, electrophile responsive element; APAP, acetaminophen; NAPQI, *N*-acetyl-*p*-benzoquinoneimine; NPSH, non-protein sulfhydryl.

biological systems have a variety of enzymes that detoxify xenobiotics. For example, phase I xenobiotic biotransformation, catalyzed by the enzymes represented by cytochome P450s, exposes or introduces a functional group to chemicals. Phase II xenobiotic biotransformation, catalyzed by a group of enzymes such as UDP-glucuronosyltransferase (UDP-GT) or glutathione S-transferases (GSTs), conjugates hydrophilic moieties such as glucuronic acid or glutathione to the functional groups, making the metabolites water-soluble and easy to be excreted. Antioxidant molecules and antioxidant enzymes also protect critical cellular components from reactive electrophiles by providing electrons to the electron-deficient (electrophilic) molecules or catalyzing such reactions, respectively. One of the important endogenous antioxidants is glutathione (GSH) that binds to various electrophiles with its nucleophilic sulfhydryl moiety either enzymatically or non-enzymatically. Rate-limiting step in GSH biosynthesis is catalyzed by γ-glutamylcysteine synthetase (GGCS), and loss of GGCS activity affects the intracellular GSH content and, thus, the antioxidant level.

Whereas many of the xenobiotic-biotransforming and antioxidant enzymes are constitutively expressed at certain levels, some are also newly synthesized after exposure to chemicals. An example for the induction is the increased expression of cytochrome P450 1A1 and other enzymes by polycyclic or polyhalogenated aromatic hydrocarbons, such as benzo[a]pyrene or tetrachlorodibenzo-*p*-dioxin (Parkinson, 1996). When these chemicals bind to a cytosolic *Ah* (Aromatic hydrocarbon) receptor (AhR), the activated AhR enters the nucleus, forms a heterodimer with *Ah*-receptor-nuclear translocator (Arnt), binds to XRE (Xenobiotic Responsive Element) in the regulatory regions of a group of genes and enhances their transcription. Several xenobiotic-biotransforming enzyme genes are known to have XRE or XRE-like sequences and concomitantly induced by the aromatic hydrocarbons.

Similarly, some chemicals such as butylated hydroxyanisole (BHA) and *tert*-butylhydroquinone (tBHQ) are known to induce a different group of enzymes. These enzymes include both xenobiotic-biotransforming and antioxidant enzymes, such as UDP-GT, GSTs, GGCS, heme oxygenase 1 (HO-1) and NAD(P)H: quinone oxidoreductase 1 (NQO1) (Borroz *et al.*, 1994; Buetler *et al.*, 1995; Prestera *et al.*, 1995). The genes encoding these enzymes have a common *cis*-acting sequence ARE/EpRE (Friling *et al.*, 1990; Moinova and Mulcahy, 1998; Mulcahy *et al.*, 1997; Prestera *et al.*, 1995; Rushmore *et al.*, 1991). In contrast to the XRE-regulated genes, however, the mechanisms by which ARE-mediated induction occurs were largely unknown until recently.

2. Nrf2 as a regulator of ARE-mediated gene expression

A candidate regulator of ARE-mediated gene expression was discovered in a field totally different from toxicology. While searching for transcription factors that regulate the erythroid gene expression, several new proteins homologous to p45 NF-E2 (Nuclear Factor Erythroid 2), the transcription factor for the β-globin gene, were isolated (Chan *et al.*, 1993; Itoh *et al.*, 1995; Kobayashi *et al.*, 1999; Moi *et al.*, 1994; Oyake *et al.*, 1996). Among these newly isolated proteins, Nrf2 (NF-E2 related factor 2) was expressed widely in various tissues, including the kidney, lung, intestine and liver (Itoh *et al.*, 1995; Moi *et al.*, 1994). Despite that it was originally discovered as a possible erythroid transcription factor, mice lacking Nrf2 exhibited no signs of anemia (Chan *et al.*, 1996; Itoh *et al.*, 1997; Kuroha *et al.*, 1998). Important finding here is that the binding sequence for the heterodimer of Nrf2 and the small Maf proteins, which is called NF-E2 binding sequence or MARE (Maf recognition sequence), is very similar to the ARE sequence. This fact led us to hypothesize that Nrf2 might be a regulator of ARE-mediated gene expression (Itoh *et al.*, 1997).

The analysis of *nrf2*-gene knockout mice gave rise to the first *in vivo* evidence for the Nrf2 regulation of a group of xenobiotic-biotransforming enzymes (Itoh *et al.*, 1997). When BHA was given to homozygous and heterozygous *nrf2* knockout mice in their diet, the protein level of hepatic GST alpha (Ya1) of the heterozygous mice increased 2.9 fold of that in the control-fed animals, whereas the increase was only 1.7 fold in the *nrf2*-deficient mouse liver. Impaired induction was also detected in other GST subclasses at the protein level and in NQO1 at the mRNA level in the liver and intestine of the homozygous *nrf2* knockout

mice. In addition, an *in vitro* analysis demonstrated that Nrf2-small Maf protein heterodimer binds to the ARE in the GST gene regulatory regions with high affinity (Itoh *et al.*, 1997). These results thus demonstrate that Nrf2 regulates ARE-mediated expression of xenobiotic-biotransforming enzyme genes.

3. Sensitivity of *nrf2* knockout mice to xenobiotic toxicity

Enzyme induction by BHA has been shown to be protective against xenobiotic toxicity. For instance, animals fed a diet containing BHA are more resistant to acetaminophen (APAP) toxicity than those fed a normal diet (Hazelton *et al.*, 1986; Miranda *et al.*, 1983). APAP is a widely used antipyretic and analgesic. The hypothesis here is that if Nrf2 controls the induction of xenobiotic-biotransforming enzyme genes through ARE, Nrf2-deficiency would diminish the protective effect of BHA. To test this hypothesis, we planned to compare APAP hepatotoxicity between BHA-treated mice lacking Nrf2 and those that have this protein. To our surprise, in a preliminary study in which animals were exposed to each of these chemicals at high doses, 2 of 5 homozygous *nrf2* knockout mice died after given BHA alone at a dose level of 800 or 400 mg/kg, whereas heterozygous *nrf2* knockout mice used as a control did not show any mortality (Table 1). Similarly, all homozygous *nrf2* knockout mice died after exposure to APAP at 800 mg/kg, while 2 of 5 heterozygous knockout mice escaped death at the same dose level. These results thus suggest that Nrf2-deficient mice are more sensitive to xenobiotic toxicity than those having this protein even before enzyme inducers are given.

Table 1. Mortality of homozygous and heterozygous *nrf2* knockout mice after exposure to BHA and APAP

Chemical (MG/KG)	Dose	Genotype	Mortality
BHA	800/400	-/-	0/4[a] (0%)
		+/-	2/5 (40%)
APAP	800	-/-	3/5 (60%)
		+/-	5/5 (100%)

Nine-week-old male *nrf2* knockout mice of each genotype (Itoh *et al.*, 1997) were used. BHA was dissolved in corn oil and given to the animals orally at a dose of 800 mg/kg on the first day and at 400 mg/kg on the second day. The animals were checked for mortality for 4 days after the first treatment. APAP was dissolved in 50% propylene glycol and given orally to the animals once at a dose of 800 mg/kg. Mortality was checked for 24 hours after dosing of APAP. a: number of animals died/number of animals used.

To elucidate the reason why the Nrf2-deficiency alone makes the animals sensitive to xenobiotic toxicity, closer examination of APAP hepatotoxicity in the *nrf2*-deficient mice was carried out. A major detoxification pathway of APAP is conjugation with glucuronic acid and sulfate. Although a small portion of APAP is activated by cytochrome P450s to a reactive metabolite, *N*-acetyl-*p*-benzoquinoneimine (NAPQI), this electrophilic metabolite is non-enzymatically bound to GSH and then eliminated. Only at high doses that saturate glucuronidization, sulfation and GSH conjugation pathways, sufficient amounts of the reactive APAP intermediates are generated, bind to intracellular macromolecules, and cause hepatocellular necrosis (Cohen *et al.*, 1998). Of the main factors involved in the detoxification processes, UDP-GT that conjugates glucuronic acid to the parent chemical and GGCS that catalyzes the synthetic reaction of GSH, the scavenger of the reactive APAP metabolites, are ARE-regulated enzymes.

When homozygous *nrf2* knockout mice and their wild-type littermates received a single oral dose of APAP at 0, 150, 300 or 600 mg/kg, 1 of 4 knockout mice died at 300 mg/kg and 3 of 4 died at 600 mg/kg. In contrast, no mortality was observed in wild type mice at any doses. Histopathological examination revealed marked centrilobular necrosis, which is known to occur in animals after overdoses of APAP (Ginsberg *et al.*, 1982), in livers of the *nrf2* knockout mice at these two highest doses. These results thus prove the high sensitivity of *nrf2* knockout mice to APAP hepatotoxicity.

We next examined early changes that occur in the APAP-treated animals before the development of such severe hepatic necrosis. Hepatic GSH is known to decrease temporarily after APAP exposure, as GSH binds to reactive metabolites. When the time course change of hepatic GSH was monitored as non-protein sulfhydryl (NPSH) content in wild-type and homozygous knockout mice after exposure to APAP at 300 mg/kg, it decreased 2 hours after administration in the animals of both genotypes. However, while approximately 30% of the pre-dose value remained in the wild type mouse livers, GSH in the *nrf2*-deficient mice was almost depleted at this time point.

The depletion of GSH in the *nrf2* knockout mice suggests that certain amount of reactive APAP metabolites remained in their livers without conjugation with GSH. To support this notion, vacuolation of centrilobular hepatocytes was observed only in the knockout mice at this time point. This is an early histopathological change after APAP exposure (Walker *et al.*, 1980). Immunohistochemical staining using APAP antibody also demonstrated detectable levels of APAP-adducts only in the livers from the *nrf2*-deficient animals. These data thus indicate that, even after the administration of the same dose of APAP, a large amount of reactive APAP metabolites is generated and remains in the *nrf2* knockout mouse liver, while such reactive intermediates are easily detoxified and eliminated from the wild-type mouse liver.

Since the amount of reactive APAP metabolites depends on the balance between activation and detoxification pathways, we then compared the baseline levels of the major factors involved in APAP metabolism in untreated wild-type mice and homozygous *nrf2* knockout mice. The results showed that hepatic UDP-GT activity and NPSH content for detoxification of APAP were low in the knockout mice (64 and 82% of those in the wild type mice, respectively), whereas there was no difference in the activation pathway, *i.e.* cytochrome P450 content. mRNAs for Ugt1a6 and GGCS heavy chain (catalytic subunit of GGCS) in the *nrf2*-deficient mouse livers were also approximately 60% of those in the wild-type mice, indicating that the low detoxification activity of these mice is determined, if not exclusively, at gene expression level.

Taken together, these data demonstrate that the lack of Nrf2 lowers expression of detoxifying genes, resulting in an increase in generation of reactive APAP metabolites that, in turn, form sufficient amounts of APAP-adducts in hepatocytes, leading to necrosis. The low baseline expression levels of detoxifying genes in these untreated mice were considered to be due to a lack of response to natural dietary inducers or to the endogenous oxidative stress that occurs as a result of normal biological activities (Klaunig *et al.*, 1995; Martin *et al.*, 1996).

4. Molecular mechanisms of Nrf2 activation

The molecular mechanisms how Nrf2 regulates gene expression have been addressed (Ishii *et al.*, 2000; Itoh *et al.*, 1999). A negative regulator of Nrf2 that represses its transcriptional activity was recently discovered and named Keap1 (Kelch-like ECH-associated protein 1). Keap1 shares structural similarity with the *Drosophila* actin binding protein Kelch (Adams *et al.*, 2000; Itoh *et al.*, 1999; Xue and Cooley, 1993). Keap1 retains Nrf2 in the cytoplasm. When electrophiles are present, Nrf2 is released from the Keap1 repression, enters the nucleus, forms a heterodimer with a small Maf protein, binds to ARE and activates the transcription.

5. Importance of Nrf2 in biological defense systems

The number of genes under the Nrf2 control is increasing (Table 2). In addition to xenobiotic biotransforming enzymes, several antioxidant genes are on the list. Among them, HO-1 degrades heme, a potent pro-oxidant, to biliverdin that has antioxidant properties (Choi and Alam, 1996). Peroxiredoxin reduces hydrogen peroxide and A170 has structural domain that interacts with PKC ξ and ubiquitin; both are induced in the cell exposed to oxidative stress and considered to play a protective role under such conditions (Ishii *et al.*, 2000). Cystine membrane transporter (system X_c^-) increases the level of intracellular cysteine necessary for GSH synthesis (Bannai *et al.*, 1991). Simultaneous induction of these antioxidant genes with xenobiotic-biotransforming enzymes is considered to provide effective protection against a variety of electrophilic agents and oxidative stress generated during metabolism of chemicals.

Xenobiotics and xenobiotic-induced oxidative stress not only cause acute toxicity, but also induce chronic toxicity, carcinogenesis and other effects. Oxidative stress generated through normal metabolic processes is also related to aging and some spontaneous diseases. Nrf2 is thus considered to play a significant role in protection against these harmful events. We believe that further studies using *nrf2* knockout mice and other models would help elucidate the details of the integrated defense mechanism of biological systems, which would then provide valuable clues for protection against various toxic insults, aging and disease processes.

Table 2. Members of Nrf2-regulated defense system

Enzymes that catalyze xenobiotic biotransformation	[references]
• NAD(P)H: quinone oxidoreductase 1	Venugopal and Jaiswal, 1996 Itoh *et al.*, 1997
• Glutathione S-transferases	Itoh *et al.*, 1997 Venugopal and Jaiswal, 1998 Hayes *et al.*, 2000
•UDP-glucuronosyltransferase	Chan and Kan, 1999
Enzymes and other factors with antioxidative properties	
• γ-Glutamylcysteine synthetase	Moinova and Mulcahy, 1999 Chan and Kan, 1999
• Catalase	Chan and Kan, 1999
• Superoxide dismutase 1	Chan and Kan, 1999
• Heme oxygenase 1	Alam *et al.*, 1999 Chan and Kan, 1999 Ishii *et al.*, 2000
• Peroxiredoxin MSP23	Ishii *et al.*, 2000
• A170	Ishii *et al.*, 2000
• Cystine membrane transporter (system X_c^-)	Ishii *et al.*, 2000

REFERENCES

Adams, J., Kelso, R. and Cooley, L. (2000): The kelch repeat superfamily of proteins: propellers of cell function, *Trends Cell Biol.*, **10**, 17-24.

Alam, J., Stewart, D., Touchard, C., Boinapally, S., Choi, A.M.K. and Cook, J.L. (1999): Nrf2, a Cap'n'Collar transcription factor, regulates induction of the heme oxygenase-1 gene, *J. Biol. Chem.*, **274**, 26071-26078.

Bannai, S., Sato, H., Ishii, T. and Taketani, S. (1991): Enhancement of glutathione levels in mouse peritoneal macrophages by sodium arsenite, cadmium chloride and glucose/glucose oxidase, *Biochim. Biophys. Acta*, **1092**, 175-179.

Borroz, K.I., Buetler, T.M. and Eaton, D.L. (1994): Modulation of γ-glutamylcysteine synthetase large subunit mRNA expression by butylated hydroxyanisole, *Toxicol. Appl. Pharmacol.*, **126**, 150-155.

Buetler, T.M., Gallagher, E.P., Wang, C., Stahl, D.L., Hayes, J.D. and Eaton, D.L. (1995): Induction of phase I and phase II drug-metabolizing enzyme mRNA, protein, and activity by BHA, ethoxyquin, and oltipraz, *Toxicol. Appl. Pharmacol.*, **135**, 45-57.

Chan, J.Y., Han, X.-L. and Kan, Y.W. (1993): Cloning of Nrf1, an NF-E2-related transcription factor, by genetic selection in yeast, *Proc. Natl. Acad. Sci. USA*, **90**, 11371-11375.

Chan, K. and Kan, Y.W. (1999): Nrf2 is essential for protection against acute pulmonary injury in mice, *Proc. Natl. Acad. Sci.*, **96**, 12731-12736.

Chan, K., Lu, R., Chang, J.C. and Kan, Y.W. (1996): NRF2, a member of the NFE2 family of transcription factors, is not essential for murine erythropoiesis, growth, and development, *Proc. Natl. Acad. Sci. USA*, **93**, 13943-13948.

Choi, A.M.K. and Alam, J. (1996): Heme oxygenase-1: function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury, *Am. J. Respir. Cell Mol. Biol.*, **15**, 9-19.

Cohen, S.D., Hoivik, D.J. and Khairallah, E.A. (1998): Acetaminophen-induced hepatotoxicity in Toxicology of the Liver (Plaa, G.L. and Hewitt, W.R. eds). Taylor & Francis, Washington DC, pp. 159-186.

Friling, R.S., Bensimon, A., Tichauer, Y. and Daniel, V. (1990): Xenobiotic-inducible expression of murine glutathione S-transferase Ya subunit gene is controlled by an electrophile-responsive element, *Proc. Natl. Acad. Sci. USA*, **87**, 6258-6262.

Ginsberg, G.L., Placke, M.E., Wyand, D.S. and Cohen, S.D. (1982): Protection against acetaminophen-induced hepatotoxicity by prior treatment with fenitrothion, *Toxicol. Appl. Pharmacol*, **66**, 383-399.

Gregus, Z. and Klaassen, C.D. (1996): Mechanisms of toxicity in Casarett and Doull's Toxicology (Klaassen, C.D. ed.). McGraw-Hill, New York, pp. 35-74.

Hayes, J.D., Chanas, S.A., Henderson, C.J., McMahon, M., Sun, C., Moffat, G.J., Wolf, C.R. and Yamamoto, M. (2000): The Nrf2 transcription factor contributes both to the basal expression of glutathione S-transferases in mouse liver and to their induction by the chemopreventive synthetic antioxidants, butylated hydroxyanisole and ethoxyquin, *Biochem. Soc. Transact.*, **28**, part2, 33-41.

Hazelton, G.A., Hjelle, J.J. and Klaassen, C.D. (1986): Effects of butylated hydroxyanisole on acetaminophen hepatotoxicity and glucuronidation *in vivo*, *Toxicol. Appl. Pharmacol.*, **83**, 474-485.

Ishii, T., Itoh, K., Takahashi, S., Sato, H., Yanagawa, T.,

Katoh, Y., Bannai, S. and Yamamoto, M. (2000): Transcription factor Nrf2 coordinately regulates a group of oxidative stress-inducible genes in macrophages, *J. Biol. Chem.*, **275**, 16023-16029.

Itoh, K., Chiba, T., Takahashi, S., Ishii, T., Igarashi, K., Katoh, Y., Oyake, T., Hayashi, N., Satoh, K., Hatayama, I., Yamamoto, M. and Nabeshima, Y. (1997): An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements, *Biochem. Biophys. Res. Commun.*, **236**, 313-322.

Itoh, K., Igarashi, K., Hayashi, N., Nishizawa, M. and Yamamoto, M. (1995): Cloning and characterization of a novel erythroid cell-derived CNC family transcription factor heterodimerizing with the small Maf family proteins, *Mol. Cell Biol.*, **15**, 4184-4193.

Itoh, K., Wakabayashi, N., Katoh, Y., Ishii, T., Igarashi, K., Engel, J.D. and Yamamoto, M. (1999): Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain, *Genes Dev.*, **13**, 76-86.

Klaunig, J.E., Xu, Y., Bachowski, S., Ketcham, C.A., Isenberg, J.S., Kolaja, K.L., Baker, T.K., Walborg, E.F.J. and Stevenson, D.E. (1995): Oxidative stress in nongenotoxic carcinogenesis, *Toxicol. Lett.*, **82/83**, 683-691.

Kobayashi, A., Ito, E., Toki, T., Kogame, K., Takahashi, S., Igarashi, K., Hayashi, N. and Yamamoto, M. (1999): Molecular cloning and functional characterization of a new Cap'n'collar family transcription factor Nrf3., *J. Biol. Chem.*, **274**, 6443-6452.

Kuroha, T., Takahashi, S., Komeno, T., Itoh, K., Nagasawa, T. and Yamamoto, M. (1998): Ablation of Nrf2 function does not increase the erythroid or megakaryocytic cell lineage dysfunction caused by p45 NF-E2 gene disruption, *J. Biochem. (Tokyo)*, **123**, 376-379.

Martin, G.M., Austad, S.N. and Johnson, T.E. (1996): Genetic analysis of ageing: role of oxidative damage and environmental stresses, *Nature Genet.*, **13**, 25-34.

Miranda, C.L., Henderson, M.C., Schmits, J.A. and Buhler, D.R. (1983): Protective role of dietary butylated hydroxyanisole against chemical-induced acute liver damage in mice, *Toxicol. Appl. Pharmacol.*, **69**, 73-80.

Moi, P., Chan, K., Asunis, I., Cao, A. and Kan, Y.W. (1994): Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the β-globin locus control region, *Proc. Natl. Acad. Sci. USA*, **91**, 9926-9930.

Moinova, H.R. and Mulcahy, R.T. (1998): An electrophile responsive element (EpRE) regulates β-naphthoflavone induction of the human γ-glutamylcysteine synthetase regulatory subunit gene. Constitutive expression is mediated by an adjacent AP-1 site, *J. Biol. Chem.*, **273**, 14683-14689.

Moinova, H.R. and Mulcahy, R.T. (1999): Up-regulation of the human γ-glutamylcysteine synthetase regulatory subunit gene involves binding of Nrf-2 to an electrophile responsive element., *Biochem. Biophys. Res. Commun.*, **261**, 661-668.

Mulcahy, R.T., Wartman, M.A., Bailey, H.H. and Gipp, J.J. (1997): Constitutive and β-naphthoflavone-induced expression of the human γ-glutamylcysteine synthetase heavy subunit gene is regulated by a distal antioxidant response element/TRE sequence, *J. Biol. Chem.*, **272**, 7445-7454.

Oyake, T., Itoh, K., Motohashi, H., Hayashi, N., Hoshino, H., Nishizawa, M., Yamamoto, M. and Igarashi, K. (1996): Bach proteins belong to a novel family of BTB-basic leucine zipper transcription factors that interact with MafK and regulate transcription through the NF-E2 site, *Mol. Cell. Biol.*, **16**, 6083-6095.

Parkinson, A. (1996): Biotransformation of xenobiotics in Casarett and Doull's Toxicology (Klaassen, C.D. ed.). McGraw-Hill, New York, pp. 113-186.

Prestera, T., Talalay, P., Alam, J., Ahn, Y.I., Lee, P.J. and Choi, A.M.K. (1995): Parallel induction of heme oxygenase-1 and chemoprotective phase 2 enzymes by electrophiles and antioxidants: regulation by upstream antioxidant-responsive elements (ARE), *Mol. Med.*, **1**, 827-837.

Rushmore, T.H., Morton, M.R. and Pickett, C.B. (1991): The antioxidant responsive element, *J. Biol. Chem.*, **18**, 11632-11639.

Venugopal, R. and Jaiswal, A.K. (1996): Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H: quinone oxidoreductase$_1$ gene, *Proc. Natl. Acad. Sci. USA*, **93**, 14960-14965.

Venugopal, R. and Jaiswal, A.K. (1998): Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes, *Oncogene*, **17**, 3145-3156.

Walker, R.M., Racz, W.J. and McElligott, T.F. (1980): Acetaminophen-induced hepatotoxicity in mice, *Lab. Invest.*, **42**, 181-189.

Xue, F. and Cooley, L. (1993): *kelch* Encodes a component of intercellular bridges in Drosophila egg chambers, *Cell*, **72**, 681-693.