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Coordinated Induction of the c-*jun* Gene with Genes Encoding Quinone Oxidoreductases in Response to Xenobiotics and Antioxidants

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ABSTRACT. Xenobiotics and antioxidants induce expression of detoxifying enzymes including NAD(P)H:quinone oxidoreductase (NQO1), NRH:quinone oxidoreductase (NQO2), and glutathione *S*-transferase Ya (GST Ya), presumably to provide protection to cells against electrophilic and oxidative stress. Antioxidant response elements (AREs) have been found in the promoter regions of the various detoxifying enzyme genes. An ARE is required for basal expression and induction of the various detoxifying enzyme genes in response to xenobiotics and antioxidants. In this study, we demonstrated that exposure of cells to xenobiotics [e.g. β-naphthoflavone (β-NF)] and antioxidants [e.g. *tert*-butyl hydroquinone (*t*-BHQ)] also induced the expression of the proto-oncogene c-*jun*. The induction of c-*jun* gene expression followed kinetics similar to the induction of *NQO1* and *NQO2* genes with respect to the level and time of exposure. Sequence analysis of the c-*jun* gene promoter revealed the presence of an ARE between nucleotides -538 and -514. The c-*jun* ARE was highly homologous to the AREs from genes encoding NQO1, NQO2, and GST Ya. Constructs containing the c-*jun* ARE and 1.7 and 4.5 kb of the c-*jun* promoter ligated to the chloramphenicol acetyltransferase (CAT) gene, upon transfection in human hepatoblastoma (Hep-G2) cells, expressed the CAT gene, which was inducible with β-NF and *t*-BHQ. Band shift assays indicated binding of two specific nuclear protein complexes with the c-*jun* gene ARE. The faster running c-*jun* gene ARE–nuclear protein complex was specifically competed out by unlabeled *NQO1* and *GST Ya* gene AREs. These results suggest that c-*jun* gene expression is coordinately induced and regulated with detoxifying enzyme genes in response to xenobiotics and antioxidants. The results also suggest involvement of an ARE-mediated mechanism of induction of c-*jun* gene expression. However, a comparison of fold induction of endogenous c-*jun* gene and transfected c-*jun* promoter/ARE-CAT constructs indicated involvement of another ARE upstream of the 4.5-kb promoter and/or additional mechanisms such as stabilization of c-Jun RNA in response to exposure to xenobiotics and antioxidants. BIOCHEM PHARMACOL **58**;4: 597–603, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. NAD(P)H:quinone oxidoreductases; c-*jun*; antioxidant response element; expression; induction; xenobiotics; antioxidants

Exposure of cells to xenobiotics and antioxidants leads to coordinated induction of a battery of genes encoding detoxifying enzymes [1]. The detoxifying enzymes include NQOs,† which catalyze two-electron reduction and detoxification of quinones; GSTs, which conjugate hydrophobic electrophiles and reactive oxygen species with glutathione; UDP-glucuronosyl transferases, which catalyze the conjugation of glucuronic acid with xenobiotics and drugs; and epoxide hydrolase, which inactivates epoxides and metal binding proteins [2, 3]. ARE, also referred to as electrophile response element (EpRE), has been characterized in the promoter regions of the various detoxifying enzyme genes, which regulate their expression and coordinated induction in response to xenobiotics and antioxidants [2, 3]. The ARE-mediated coordinated induction of these enzymes is considered to be one mechanism of critical importance in chemoprotection induced in cells by antioxidants and other agents. The AREs in the various genes usually contain two AP1 or AP1-like elements arranged in various orientations, separated by either three or eight nucleotides followed by a 'GC' box [2, 3]. Mutational analysis in the ARE of the human *NQO1* gene indicates that both AP1/AP1-like elements and the 'GC' box are required for optimal expression and induction of the *NQO1* gene [2, 3]. The transcription factors Jun, Fos, Fra, Nrf, Ah receptor, and YABP are known to bind to the ARE [4–9]. A role of c-Jun in the ARE-mediated expression and induction of the mouse *GST Ya* gene has been suggested [5, 6]. However, the role of c-Jun protein in the ARE-mediated regulation of human

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† *Abbreviations*: NQO1, NAD(P)H:quinone oxidoreductase$_1$; NQO2, NRH:quinone oxidoreductase$_2$; GST (Ya), glutathione *S*-transferase (subunit Ya); ARE, antioxidant response element; hARE, human antioxidant response element; CAT, chloramphenicol acetyl transferase; β-NF, β-naphthoflavone; *t*-BHQ, *tert*-butyl hydroquinone; and tk, thymidine kinase.

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and rat *NQO1*, *NQO2,* and *GST Ya* genes remains to be established.

In the present study, we demonstrated that the human c-*jun* gene is induced coordinately with genes encoding the detoxifying enzymes NQO1 and NQO2 in response to a xenobiotic, β-NF, and an antioxidant, *t*-BHQ. The concentration- and time-dependent induction of c-*jun* gene expression followed a pattern similar to that observed with the *NQO1* and *NQO2* genes. Nucleotide sequence analysis of 1.7 kb of the c-*jun* promoter revealed the presence of an ARE-like *cis*-element between nucleotides -538 and -514. Upon transfection into human hepatoblastoma (Hep-G2) cells, a plasmid containing 1.7 kb of the c-*jun* promoter ligated to CAT and a plasmid containing the c-*jun* gene ARE ligated to tk and CAT expressed the CAT gene, which was inducible with β-NF and *t*-BHQ. Band shift assays with the c-*jun* gene ARE indicated specific binding of nuclear protein complexes to the c-*jun* gene ARE. This binding was inhibited specifically by unlabeled human *NQO1* gene ARE and rat *GST Ya* gene ARE. These results suggest an ARE-mediated mechanism of induction of the c-*jun* gene in response to xenobiotics and antioxidants.

MATERIALS AND METHODS

Cell Culture, Xenobiotic and Antioxidant Treatment, RNA Isolation, and Northern Analysis

Human hepatoblastoma (Hep-G2) cells were obtained from the American Type Culture Collection. The cells were plated at a density of about 10^6/125-cm^2 T flask and grown as monolayer cultures at 37° in 95% air and 5% CO_2 in minimal essential medium supplemented with 10% fetal bovine serum, 40 U/mL of penicillin, and 40 μg/mL of streptomycin. Hep-G2 cell cultures were treated for 16 hr with either DMSO (control) or β-NF (Sigma) or *t*-BHQ (Sigma) dissolved in DMSO. β-NF and *t*-BHQ are highly soluble in DMSO. Therefore, we prepared high strength stock solutions, which were diluted with medium to obtain desired concentrations for treatment of cells. The control cells received the maximum amount of DMSO (10 μL) in 10 mL of medium. Various concentrations of β-NF and *t*-BHQ were used for treatment to study concentration-dependent induction of c-*jun* and detoxifying enzyme genes. Similarly, the exposure time was varied from 4 to 16 hr to study time-dependent induction of c-*jun* and detoxifying enzyme genes. After the exposure to the chemicals, the medium was removed by aspiration, and the cell monolayer surface was washed three times with 20–30 mL of ice-cold Dulbecco's PBS without calcium and magnesium. The cells were scraped from the flasks and collected by centrifugation (800 *g* for 5 min). Total RNA from DMSO-, β-NF-, and *t*-BHQ-treated cells was isolated using an RNeasy mini kit from Qiagen, Inc. RNA was treated with DNase to remove any DNA contamination, cleaned with phenol/chloroform, and precipitated with ethanol by standard procedures [10]. Ten micrograms of total RNA was run on a 1% formaldehyde agarose gel and blotted by procedures described previously [10]. cDNAs encoding c-Jun, GST Ya, and β-actin were labeled in separate reactions using a Prime-a-Gene labeling system (Promega Corp.). NQO1 and NQO2 cDNAs were labeled by nick translation (Amersham International). We used nick translation to label NQO1 and NQO2 cDNAs because these cDNAs are labeled very poorly by the random priming method. c-Jun cDNA was a gift from Dr. Kevin Ryder (NIH). The cDNAs encoding NQO1 and NQO2 were isolated and sequenced in our laboratory [11, 12]. Prehybridization and hybridization were done according to a method described previously [13].

Nucleotide Sequence Analysis

Human c-*jun* gene and promoter sequences were obtained from GenBank. A 1.7-kb region of the c-*jun* gene promoter was analyzed for the presence of ARE-like sequences using GCG software.

Construction of Recombinant Plasmids

A human liver λEMBL3 gene library was screened with full-length c-Jun cDNA to clone the human c-*jun* gene by procedures as described [14]. The λEMBL3 clone containing the human c-*jun* gene was designated as λEMBL3-hc-Jun-8. This clone was partially sequenced to determine the amount of 5′ flanking (upstream) sequence of the human c-*jun* gene. It contained 7 kb of DNA upstream to the human c-*jun* start site of transcription (data not shown). A 4.5-kb region of the human c-*jun* promoter along with 765 bp of the coding region was cloned in pBLCAT3 at the *Bam*HI site by blunt-end ligation and transfection into *Escherichia coli* DH5α. This clone was designated as pCJ4.5CAT. Recombinant plasmid pCJ1.7CAT, containing 1.7 kb of the human c-*jun* gene promoter and 760 bp of the coding sequence attached to the CAT gene, was obtained from Dr. Andrew S. Kraft. The construction of the pCJ1.7CAT plasmid has been described [15]. Both strands of the c-*jun* gene ARE (the region between -538 to -514) were synthesized with *Bam*HI ends, annealed, phosphorylated, and ligated at the *Bam*HI site of the vector pBLCAT2 to generate the recombinant plasmid c-JunARE-tk-CAT (pCJARE-tk-CAT). Several clones of pCJARE-tk-CAT were sequenced to select the clone containing one copy of the c-Jun ARE in 5′ → 3′ orientation with the tk promoter driving transcription of the CAT gene. A similar strategy was used to construct the pGST YaARE-tk-CAT plasmid. The construction of the *NQO1* gene ARE (hARE)-tk-CAT plasmid has been reported previously [4]. The nucleotide sequences of human c-*jun*, *NQO1,* and *GST Ya* gene AREs with *Bam*HI linkers at the end are shown below. Note that 'GATCC' is a *Bam*HI linker attached to the oligonucleotide.

Human c-Jun gene ARE:

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5′GATCCGGTGGCGCCGCGGTGGATGACTTCG3′
     3′CCACCGCGGCGCCACCTACTGAAGCCCTAG5′
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Human NQO1 gene ARE (hARE)

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5′GATCCAGTCACAGTGACTCAGCAGAATCT3′
     3′TCAGTGTCACTGAGTCGTCTTAGACCTAG5′
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Rat GST Ya gene ARE

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5′GATCCGAGCTTGGAAATGGCATTGCTAATGGTGACAAAGCA
     3′CTCGAACCTTTACCGTAACGATTACCACTGTTTCGT

ACTTT3′
TGAAACCTAG5′
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Transient Transfection and Measurement of CAT Activity

Ten micrograms of the pCJ4.5-CAT, pCJ1.7-CAT, and pCJ-ARE-tk-CAT plasmids were co-transfected with 5 μg of the pRSV-β-galactosidase plasmid into Hep-G2 cells by the calcium phosphate procedure [4]. Thirty-two hours after transfection, the cells were treated with DMSO, β-NF, and *t*-BHQ for 16 hr. At the end of transfection and chemical treatments, the transfected cells were harvested, homogenized, and analyzed for CAT activity by procedures described previously [4]. The CAT activities are presented as nanomoles of [^{14}C]chloramphenicol acetylated per minute per unit of β-galactosidase.

Nuclear Extract Preparation and Band Shift Assay

The nuclear extract from Hepa-1 cells was prepared according to the procedure of Dignam *et al.* [16], modified by Kadonaga [17]. The c-*jun* ARE was end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. The end-labeled c-*jun* ARE (20,000–30,000 cpm; <2 ng) was incubated with 10–20 μg of nuclear extract, and band shift assays were performed by procedures described previously [18]. Unlabeled c-*jun* ARE (100 and 250 ng) was used as specific competitor of nuclear proteins binding to the c-*jun* ARE. In addition, we also used 250 ng of unlabeled *NQO1* gene ARE, *GST Ya* gene ARE, and *AP2* oligonucleotides as competitors.

RESULTS

Northern Analysis

Northern analyses of the RNA isolated from Hep-G2 cells treated with DMSO (control), β-NF, and *t*-BHQ are shown in Figs. 1–3. Northern analysis of the RNA from Hep-G2 cells revealed the presence of one major transcript of 2.7 kb in the case of c-*jun*, two transcripts of 2.7 and 1.2 kb in the case of *NQO1*, and a single transcript of 1.2 kb in the case of the *NQO2* gene (Fig. 1). The two transcripts of the *NQO1* gene originated due to the use of two different polyadenylation signals in the 3′ untranslated region of the gene [19]. The treatment of Hep-G2 cells with β-NF and

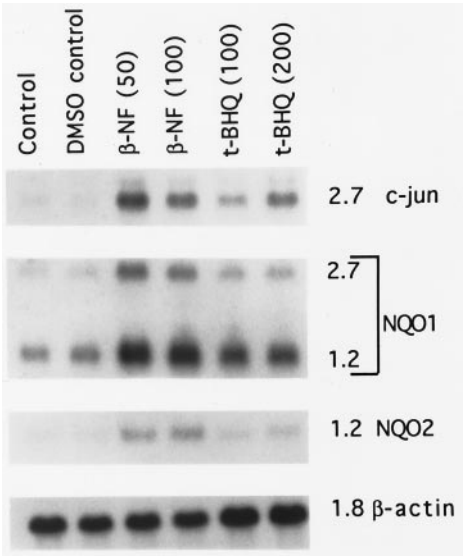

FIG. 1. Coordinated induction of c-*jun* with *NQO1* and *NQO2*. Human hepatoblastoma (Hep-G2) cells were treated with DMSO, β-NF, and *t*-BHQ for 16 hr in separate experiments. The micromolar concentrations of chemicals used for treatment are shown in parentheses. RNA was isolated from untreated and chemically treated cells. Ten micrograms of RNA was separated on an agarose gel and northern blotted. The northern blot was hybridized first with c-Jun cDNA. Then the blot was stripped of radioactivity and rehybridized with NQO1 followed by NQO2 and β-actin probes.

t-BHQ markedly increased the expression of c-*jun*, *NQO1*, and *NQO2* genes (Fig. 1). Fifty micromolar β-NF treatment resulted in a 10-fold increase in the expression of the c-*jun* gene as compared with the DMSO control. A similar increase was observed in *NQO1* gene expression. *NQO2* gene expression increased by 4-fold only. Increase in the β-NF concentration from 50 to 100 mM led to a substantial reduction in the induction of c-*jun* and *NQO1* gene expression as compared with the induction observed with 50 mM β-NF (Fig. 1). However, *NQO2* gene expression was more or less similar at 50 and 100 mM β-NF (Fig. 1). The treatment of Hep-G2 cells with 100 mM *t*-BHQ also led to a 2- to 3-fold increase in the expression of c-*jun* and *NQO1* genes (Fig. 1). An increase in *t*-BHQ concentration from 100 to 200 μM led to a further increase in c-*jun* gene expression, but *NQO1* gene expression remained unchanged. *NQO2* gene expression was slightly higher with 200 μM *t*-BHQ than with 100 μM *t*-BHQ. The treatment of Hep-G2 cells with various concentrations of β-NF (1 to 50 μM) and *t*-BHQ (10 to 200 μM) showed a concentration-dependent increase in the expression of c-*jun* and *NQO1* genes (Fig. 2). Variations in the time of exposure of Hep-G2 cells to β-NF showed a time-dependent increase in the expression of c-*jun* and *NQO1* genes (Fig. 3). The optimum induction of both the genes was observed after 8 hr of β-NF exposure. Increasing the time of exposure to β-NF from 8 to 16 hr did not result in further increases in the expression of c-*jun* and *NQO1* genes. The treatment of Hep-G2 cells with *t*-BHQ in a similar experiment showed

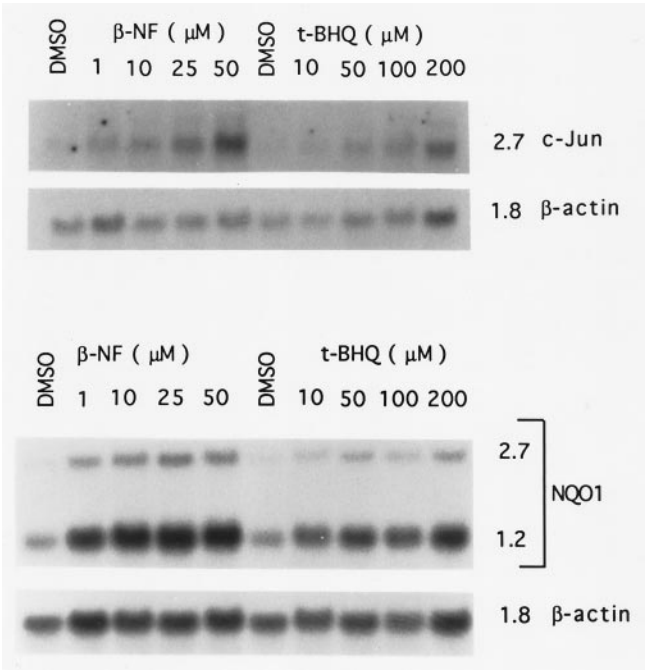

FIG. 2. Effect of different concentrations of β-NF and *t*-BHQ on the expression of c-*jun* and *NQO1* genes. Human hepatoblastoma (Hep-G2) cells were treated with DMSO and different concentrations of β-NF and *t*-BHQ for 16 hr, and RNA was isolated. Ten micrograms of RNA was analyzed by northern blotting and hybridization with probes for c-Jun and β-actin or NQO1 and β-actin.

results different from those found for β-NF (Fig. 3). Maximum expression of c-*jun* and *NQO1* genes was observed at 4 hr, and it remained unchanged at 8 hr of treatment. However, increase in the time of exposure of *t*-BHQ from 8 to 16 hr resulted in a substantial decrease in the expression of c-*jun* and *NQO1* genes.

Nucleotide Sequence Analysis of c-Jun Promoter

Nucleotide sequence analysis of 1.7 kb of the c-Jun promoter indicated the presence of a single copy of ARE between nucleotides -538 and -514 (Fig. 4). The alignment of the c-*jun* gene ARE with the AREs from six of the detoxifying enzyme genes including *NQO1* and *NQO2* showed a high degree of homology between the AREs of c-*jun* and detoxifying enzyme genes (Fig. 4). The c-*jun* gene ARE contained two AP1-like elements followed by a 'GC' box. However, the 'GC' box sequence of the c-*jun* gene ARE was 'GGC' as compared with 'GCA' in the detoxifying enzyme genes (Fig. 4). It may be noteworthy that the c-*jun* gene ARE sequence was closest to the rat *GST Ya* gene ARE. This is because the nucleotide sequence and the spacing of two AP1-like elements within the c-*jun* gene ARE were very similar to those of the rat *GST Ya* gene ARE.

CAT Assays

Treatment of Hep-G2 cells transfected with *NQO1* gene ARE (hARE)-tk-CAT plasmid and *GST Ya* gene ARE-tk-CAT plasmid with β-NF and *t*-BHQ produced the expected results. Both *NQO1* and *GST Ya* gene AREs mediated high levels of CAT gene expression, which were induced several-fold in response to β-NF and *t*-BHQ (Fig. 5). The c-*jun* gene ARE-mediated CAT gene expression in transfected cells was much lower than that mediated by the AREs from the *NQO1* and *GST* genes (Fig. 5). β-NF and *t*-BHQ treatment of Hep-G2 cells transfected with a c-*jun* ARE-tk-CAT plasmid resulted in increased expression of the ARE-mediated CAT gene. However, the fold induction of c-*jun* gene ARE-mediated CAT gene expression was significantly lower than the fold induction of the endogenous c-*jun* gene in Hep-G2 cells (compare Fig. 5 with Fig. 1). Interestingly, transfection of Hep-G2 cells with the plasmids pCJ4.5CAT (4.5 kb of the c-*jun* promoter regulating the CAT gene) and pCJ1.7-CAT (1.7 kb of the c-*jun* promoter regulating the CAT gene) expressed the CAT gene at a level 20-fold higher than c-*jun* ARE-tk-CAT (Fig. 5). However, β-NF induction of expression of the CAT gene mediated by 4.5 and 1.7 kb of the c-*jun* promoter was also markedly lower than β-NF induction of the endogenous c-*jun* gene (compare Fig. 5 with Fig. 1).

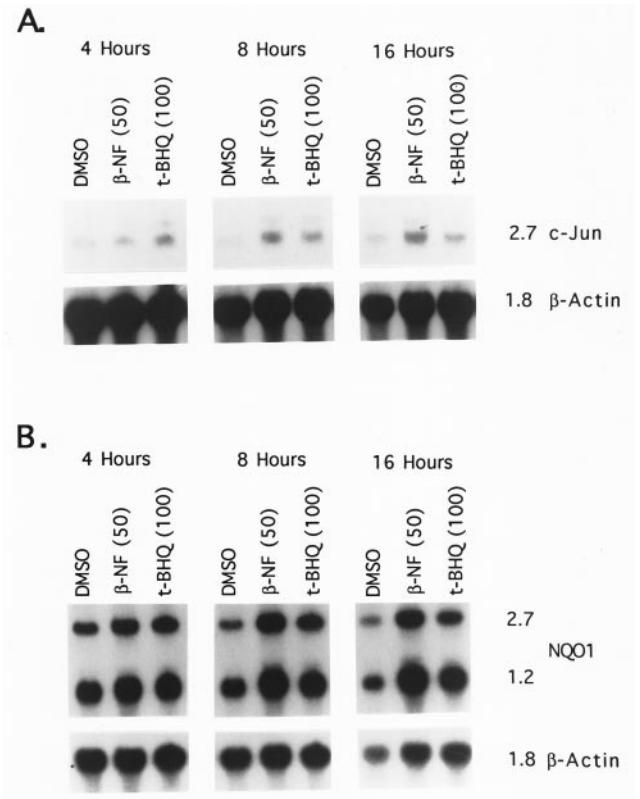

FIG. 3. Time course of β-NF and *t*-BHQ induction of c-*jun* and *NQO1* gene expression. Human hepatoblastoma (Hep-G2) cells were treated with DMSO, β-NF, and *t*-BHQ at micromolar concentrations as shown above the lanes for different time intervals. RNA was isolated, and 10 μg of RNA was analyzed by northern blotting. Northern blots were hybridized with probes for c-Jun and β-actin or NQO1 and β-actin.

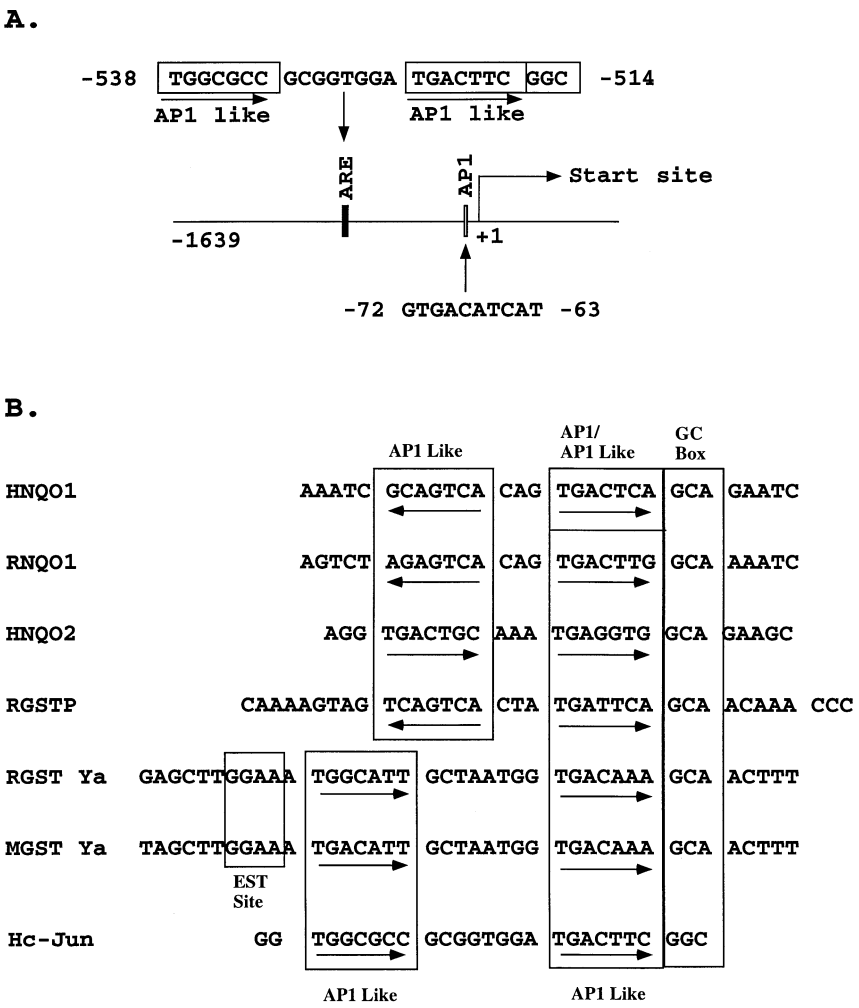

FIG. 4. (A) Nucleotide sequence and location of the c-*jun* ARE in the human c-*jun* promoter. (B) Alignment of the human c-*jun* ARE with detoxifying enzyme gene AREs. HNQO1, human NAD(P)H:quinone oxidoreductase 1 gene ARE; RNQO1, rat NAD(P)H:quinone oxidoreductase 1 gene ARE; HNQO2, human NRH:quinone oxidoreductase 2 gene ARE; RGSTP, rat glutathione *S*-transferase P subunit gene ARE; RGSTYa, rat glutathione *S*-transferase Ya subunit gene ARE; MGSTYa, mouse glutathione *S*-transferase Ya subunit gene ARE; Hc-Jun, human c-*jun* gene ARE.

Band Shift Assays

Incubation of the c-*jun* gene ARE with increasing concentrations of nuclear extract from Hepa-1 cells showed increasing amounts of nuclear proteins binding to the c-*jun* gene ARE (Fig. 6). Two complexes of c-*jun* gene ARE with Hepa-1 nuclear proteins (upper and lower complexes) were clearly visible in band shift assays (Fig. 6). The binding of nuclear proteins was specifically competed out by unlabeled c-*jun* ARE but not by nonspecific competitor AP2 oligonucleotides. The binding of nuclear proteins to the c-*jun* gene ARE in the lower band (faster moving complex) was also competed out by *NQO1* and *GST Ya* gene AREs. The competition of the upper band (slower moving complex) was much lower with *NQO1* and *GST Ya* gene AREs.

DISCUSSION

Exposure of cells to environmental and synthetic chemicals results in the generation of electrophiles and reactive oxygen species, leading to electrophilic and oxidative stress, cytotoxicity, mutagenicity, and carcinogenicity [3]. Cellular mechanisms have evolved that protect the cells against electrophilic and oxidative stress and other adverse effects of these chemicals. One such mechanism is the coordinated induction of a battery of detoxifying enzymes including quinone oxidoreductases (NQO1 and NQO2) in response to exposure to xenobiotics, drugs, and carcinogens [3, 20–22]. Antioxidants that are well known protectors of cells against oxidative stress and cellular transformation also function by similar mechanisms involving induction of detoxifying and chemopreventive proteins.

In the present report, we demonstrated that expression of the transcription factor c-Jun is coordinately induced with genes encoding detoxifying enzymes in response to xenobiotics and antioxidants. Analysis of 1.7 kb of the c-*jun* gene promoter sequence identified an ARE that upon transfection into Hep-G2 cells regulated expression and β-NF and *t*-BHQ induction of the c-*jun* gene. This indicated that xenobiotic and antioxidant induction of the c-*jun* gene may involve an ARE element(s) similar to those found in *NQO1* and *GST Ya* gene promoters. The c-*jun* gene ARE bound to two specific complexes from Hepa-1 nuclear extract. The binding of the lower complex was specifically competed out by unlabeled *NQO1* and *GST Ya* gene AREs. These results indicated that c-*jun* gene ARE-mediated CAT gene expression is regulated, at least in part,

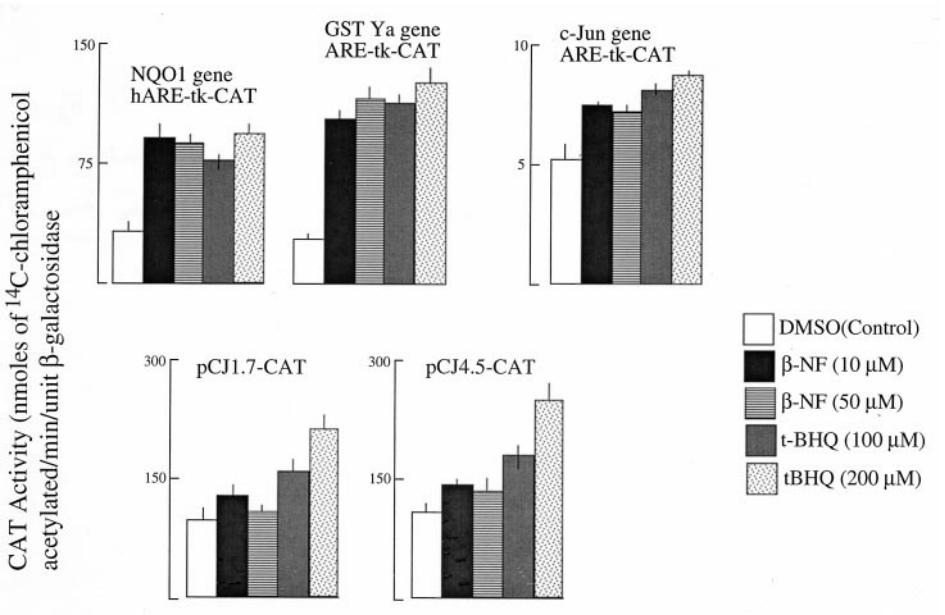

FIG. 5. Transient transfection and CAT activity. Ten micrograms of plasmid hARE-tk-CAT, GST Ya ARE-tk-CAT, pCJ4.5CAT, pCJ1.7CAT, or pCJ-ARE-tk-CAT was co-transfected with 5 μg of pRSV-β-galactosidase into Hep-G2 cells in separate experiments. Thirty-two hours after transfection, the cells were treated with β-NF and *t*-BHQ for 16 hr. At the end of the incubation period, the cells were harvested and analyzed for β-galactosidase and CAT activities. The data are presented as means ± SEM of three independent transfections.

by similar nuclear proteins that control the ARE-mediated expression of *NQO1* and *GST Ya* genes. The identity of nuclear proteins in the upper band shifted with c-*jun* ARE remains unknown. Interestingly, induction of CAT gene expression mediated by 4.5 and 1.7 kb of the c-*jun* promoter as well as by the c-*jun* ARE in response to β-NF and *t*-BHQ was of markedly lower magnitude than that observed for the endogenous c-*jun* gene. Therefore, it is possible that sequences 5′ to 4.5 kb contain additional ARE elements. It may be noteworthy that the γ-glutamylcysteine synthetase gene promoter contains an ARE (ARE1) at nucleotide position -862 that is much less active than ARE4, located at nucleotide position -3148 [23]. Because 4.5 kb of the c-*jun* promoter did not demonstrate a β-NF/*t*-BHQ response equivalent to that of the endogenous c-*jun* gene, we expect that an additional ARE may be located 5′ to 4.5 kb in the c-*jun* promoter. Alternatively, it is possible that β-NF and *t*-BHQ also induce a post-transcriptional mechanism(s) that stabilizes c-Jun RNA, leading to increased expression of c-Jun. This mechanism is expected to be in addition to the ARE-mediated transcription mechanism.

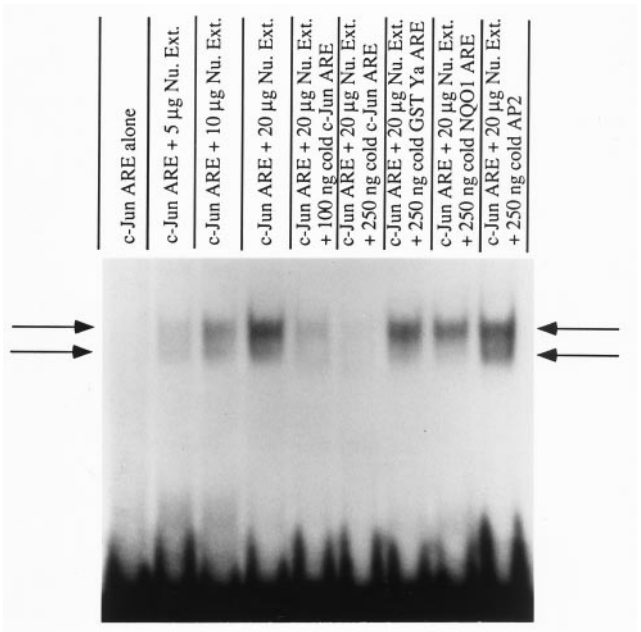

FIG. 6. Band shift assays. The human c-*jun* ARE was end-labeled with [γ-^{32}P]ATP. Human c-Jun (50,000 cpm) was incubated with various concentrations of Hepa-1 nuclear extract in the absence and presence of unlabeled (cold) competitors. The mixture was analyzed on a 6% nondenaturing polyacrylamide gel. The arrows indicate the shifted bands.

The coordinated induction of the c-*jun* gene with the various detoxifying enzyme genes raises an important question regarding the role of induced c-Jun protein. Does it play a role in ARE-mediated regulation of detoxifying enzyme gene expression, or is it induced for some unknown function? A role of c-Jun in the ARE-mediated regulation of mouse *GST Ya* gene expression has been suggested [5, 6]. A role of c-Jun in the regulation of ARE-mediated expression and induction of other detoxifying enzyme genes including *NQO1* is expected, since c-Jun is known to bind with the AREs of human *NQO1* and other detoxifying enzyme genes [4, 18]. Therefore, it is likely that induction of c-Jun is one of the many steps that leads to activation of the various detoxifying enzyme genes. However, this remains to be investigated by further experiments. The

question also arises if ARE-mediated induction of c-*jun* gene expression is similar to ARE-mediated induction of detoxifying enzyme genes. The observation that the upper complex of proteins that bind to c-*jun* ARE was not competed out by *NQO1* and *GST Ya* AREs may indicate some differences between ARE-mediated expression of c-*jun* and detoxifying enzyme genes.

In conclusion, we have demonstrated that the transcription factor c-Jun is coordinately induced with genes encoding the detoxifying enzymes NQO1 and NQO2 in response to xenobiotics and antioxidants. In addition, we presented evidence to suggest that xenobiotic- and antioxidant-mediated expression of the c-*jun* gene is, at least in part, mediated by ARE and ARE-binding proteins. We further demonstrated that additional ARE sequences upstream to the 4.5 kb c-*jun* promoter and/or post-transcriptional stabilization of c-Jun RNA are expected to play an important role in the overall increase of the expression of c-*jun* gene in response to xenobiotics and antioxidants.

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