Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 2965–2969, April 1993
Medical Sciences

Chemical and molecular regulation of enzymes that detoxify carcinogens

(chemoprotection/electrophiles/quinone reductase/transient gene expression/phase 2 enzymes)

TORY PRESTERA, W. DAVID HOLTZCLAW, YUESHENG ZHANG, AND PAUL TALALAY*

Department of Pharmacology and Molecular Sciences, The Johns Hopkins University School of Medicine, Baltimore, MD 21205-2185

Contributed by Paul Talalay, December 21, 1992

ABSTRACT **Inductions of detoxication (phase 2) enzymes, such as glutathione transferases and NAD(P)H:(quinone-acceptor) oxidoreductase, are a major mechanism for protecting animals and their cells against the toxic and neoplastic effects of carcinogens. These inductions result from enhanced transcription, and they are evoked by diverse chemical agents: oxidizable diphenols and phenylenediamines; Michael reaction acceptors; organic isothiocyanates; other electrophiles—e.g., alkyl and aryl halides; metal ions—e.g., $HgCl_2$ and $CdCl_2$; trivalent arsenic derivatives; vicinal dimercaptans; organic hydroperoxides and hydrogen peroxide; and 1,2-dithiole-3-thiones. The molecular mechanisms of these inductions were analyzed with the help of a construct containing a 41-bp enhancer element derived from the 5′ upstream region of the mouse liver glutathione transferase Ya subunit gene ligated to the 5′ end of the isolated promoter region of this gene, and inserted into a plasmid containing a human growth hormone reporter gene. When this construct was transfected into Hep G2 human hepatoma cells, the concentrations of 28 compounds (from the above classes) required to double growth hormone production, and the concentrations required to double quinone reductase specific activities in Hepa 1c1c7 cells, spanned a range of four orders of magnitude but were closely linearly correlated. Six compounds tested were inactive in both systems. A 26-bp subregion of the above enhancer oligonucleotide (containing the two tandem "AP-1-like" sites but lacking the preceding ETS protein binding sequence) was considerably less responsive to the same inducers. We conclude that the 41-bp enhancer element mediates most, if not all, of the phase 2 enzyme inducer activity of all of these widely different classes of compounds.**

Elevation of the activities of phase 2 detoxication enzymes† of cells provides protection against neoplasia (6). This paper analyzes the chemical and molecular specificity of the regulation of phase 2 enzymes, as part of our efforts to develop novel approaches to chemoprotection against cancer. Phase 2 enzymes, which are widely distributed in mammalian cells and tissues, include the following: glutathione (GSH) transferases, which conjugate mostly hydrophobic electrophiles with GSH; QR, which promotes obligatory two-electron reductions of quinones, preventing their participation in oxidative cycling and the depletion of intracellular GSH; epoxide hydrolase, which inactivates epoxides and arene oxides by hydration to diols; and UDP-glucuronosyltransferases, which conjugate xenobiotics with glucuronic acid, thus facilitating their excretion. The induction of these enzymes is accompanied by elevations of intracellular GSH levels which augment cellular protection (7–11).

Induction of phase 2 enzymes is evoked by an extraordinary variety of chemical agents, including Michael reaction acceptors, diphenols, quinones, isothiocyanates, peroxides, vicinal dimercaptans, heavy metals, arsenicals, and others (12–14). With few exceptions these agents are electrophiles (or can be converted to electrophiles by metabolism), and accordingly, many of these inducers are substrates for glutathione transferases (13).

The molecular basis of the regulation of phase 2 enzyme inductions has been analyzed by deletions of the 5′ upstream regulatory regions of glutathione transferase Ya subunit genes and QR genes after transfection of cells with chloramphenicol acetyltransferase (CAT) constructs (3, 15–17). The sequences of the upstream enhancer elements of the mouse and rat liver glutathione transferase Ya subunit genes that respond to the few inducers tested are very similar and have been termed the electrophile-responsive element (EpRE) (18) and the antioxidant-responsive element (ARE) (19), respectively. These elements (Fig. 1) are contained within a 41-nt segment located between base pairs -754 and -714 in the mouse, and -722 and -682 in the rat Ya gene. The critical DNA sequences responsive to monofunctional inducers appear to be the TGACAT/AT/AGC regions, which resemble AP-1 binding sites (20). Similar enhancer sequences have also been identified in the upstream regulatory regions of the rat and human QR genes (3, 4, 17). We show that all the chemical inducers of phase 2 enzymes that we tested stimulate expression of a reporter gene through this 41-bp enhancer element.

MATERIALS AND METHODS

Cell Culture. For the growth hormone (GH) transient gene expression assays the cells were grown in Eagle's minimal

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations and definitions: AP-1, a family of transcriptional activator DNA-binding proteins that bind to the consensus sequence TGAC/GTC/AA; CAT, chloramphenicol acetyltransferase; CD_{GH}, concentration of an inducer that doubles the production of growth hormone in a transient gene expression assay; CD_{QR}, concentration of an inducer that doubles the quinone reductase specific activity in Hepa 1c1c7 cells; DMSO, dimethyl sulfoxide; ETS, a family of transcriptional activator DNA-binding proteins; GH, growth hormone; QR, quinone reductase [NAD(P)H:(quinone-acceptor) oxidoreductase, EC 1.6.99.2]; sulforaphane, 1-isothiocyanato-(4*R*)-(methylsulfinyl)butane [CH_3—SO—$(CH_2)_4$—NCS].

*To whom reprint requests should be addressed.

†Two broad classes of enzymes metabolize xenobiotics: (*i*) phase 1 enzymes, which functionalize molecules by introducing hydroxyl or epoxide groups and (*ii*) phase 2 enzymes (1), which detoxify either by conjugating these functionalized molecules with endogenous ligands (e.g., glutathione), thus facilitating their excretion, or by destroying their reactive centers by other reactions [e.g., hydrolysis of epoxides by epoxide hydrolase or reduction of quinones by quinone reductase (QR)]. Reasons for considering QR a phase 2 enzyme are presented elsewhere (2–4). Inducers of enzymes of xenobiotic metabolism belong to two families (5): (*i*) bifunctional inducers, which bind to the aryl hydrocarbon (Ah) receptor and induce certain phase 1 enzymes and phase 2 enzymes and (*ii*) monofunctional inducers, which induce phase 2 enzymes independently of the Ah receptor.

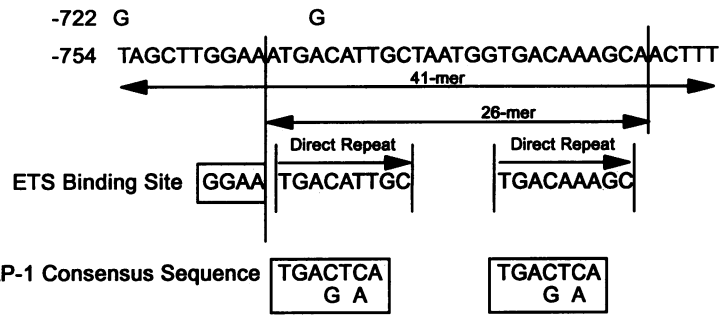


FIG. 1. Highly homologous 41-bp enhancer sequences from the upstream region of the mouse and rat glutathione transferase Ya subunit genes, representing bases -682 to -722 (rat) and -714 to -754 (mouse) from the origins of replication. Two "AP-1-like" regions are present with a core ETS protein binding site located next to the first AP-1 site in the mouse sequence.

essential medium supplemented with Earle's balanced salt solution, nonessential amino acids, sodium pyruvate, glutamine, and 10% fetal calf serum. All cells were maintained in a humidified atmosphere of 5–7% CO_2 at 37°C. Cell lines were free from mycoplasma.

Compounds. Most inducers were obtained commercially. Racemic sulforaphane was synthesized by C.-G. Cho and G. H. Posner (21).

Plasmids and Their Constructions. The plasmids 41YaCAT and -187YaCAT (15) and RSVgal were gifts of Violet Daniel (The Weizmann Institute of Science, Rehovot, Israel). The plasmid pCH110 was obtained from Pharmacia. p41YaCAT contains a portion of the upstream sequence of the mouse glutathione transferase Ya gene from -1594 to the *Bgl* II site at -1272 where the 41-bp EpRE is present in the reverse orientation (20). The EpRE is linked directly to the intact *Bgl* II site at nucleotide -187 in the upstream region. p284YaGH was prepared by ligating a 284-nt fragment (representing the sequence from -186 to +98) containing the mouse glutathione transferase Ya minimal promoter (as in -187 YaCAT) region into the *Bam*HI site of plasmid p0GH (22). The 284-nt fragment was generated by PCR from the plasmid 41YaCAT by use of the primers 5′-GGC TTC ACT CCA TCT AGA AAG GG-3′ and 5′-TTG CAG TGC TGC AGA CCT GGG AA-3′. The fragment was gel purified, its ends were blunted, and *Bam*HI linkers were added. It was then digested with *Bam*HI and *Bgl* II to generate two fragments, the smaller, 284-nt, fragment containing 186 nucleotides of the upstream region, the first exon, and 56 nucleotides of the first intron of the mouse glutathione transferase Ya gene. The plasmid p26-284GH was prepared by first ligating the oligonucleotide 5′-agc ttA TGA CAT TGC TAA TGG TGA CAA AGC Ag-3′ (lowercase letters indicating restriction overhangs) and its complement 5′-gat ccT GCT TTG TCA CCA TTA GCA ATG TCA Ta-3′ into p0GH (which had been cleaved with *Hin*dIII and *Bam*HI) to provide p26GH. The 284-nt fragment containing the glutathione transferase Ya minimal promoter was then inserted into the *Bam*HI site of the plasmid p26GH. The plasmid p41-284GH was prepared by ligating the oligonucleotide 5′-agc tTA GCT TGG AAA TGA CAT TGC TAA TGG TGA CAA AGC AAC TTT g-3′ and its complementary oligonucleotide 5′-tcg acA AAG TTG CTT TGT CAC CAT TAG CAA TGT CAT TTC CAA GCT A-3′ into the *Hin*dIII and *Sal* I sites of p284GH. The structures of all DNA constructs were confirmed by automated sequencing.

Transfections and Transient Gene Expression Assays. Transfections were performed by the calcium phosphate method (23). Briefly, the cells were plated at a density of 3.5×10^6 (Hepa 1c1c7) or 7×10^6 (Hep G2) in 10-cm plates and medium was replaced after 14–16 hr. After a further 3 hr, the transfection mixture containing 20 μg of the specified GH construct and 12 μg of the β-galactosidase construct (RSVgal for Hep G2 and pCH110 for Hepa 1c1c7) was added. Five hours later the cells were shocked with 15% (wt/vol) glycerol for 2 min and then allowed to recover for 16–18 hr. The cells from each 10-cm plate were trypsinized and pooled. One-quarter of the cells from each plate were replated onto a 10-cm dish for β-galactosidase assay, and the remaining cells were distributed among the wells of three 24-well plates containing 1.5 ml of medium per well. After 3–4 hr the cells were treated with three or more concentrations of inducers dissolved in either dimethyl sulfoxide (DMSO) or water (arsenicals and metal salts). A final concentration of 0.2% (vol/vol) DMSO was present in all assays. After a further 48 hr, 100 μl of medium was removed from each duplicate well and assayed for GH (Allégro HGH Transient Gene Expression Assay Kit; Nichols Institute, San Juan Capistrano, CA). CAT and β-galactosidase assays were performed (23), and viability was determined by staining with crystal violet (24).

Standardization of GH Gene Expression Assay. Basal GH secretions in six independent transfections with p41-284GH in Hep G2 cells were as follows (ng of GH secreted per ml of medium in 48 hr; means of n replicates ± the coefficient of variation): 2.20 ± 2.3% (n = 6); 2.67 ± 6.9% (n = 6); 2.80 ± 5.2% (n = 6); 3.27 ± 5.4% (n = 5); 4.93 ± 12.5% (n = 4); and 6.54 ± 4% (n = 4). The mean GH production in these six transfections was 3.92 ng/ml with an uncorrected interassay coefficient of variation of ±45.7%; after normalization for transfection efficiency by β-galactosidase measurements and for cell number (by staining with crystal violet), the interassay coefficient of variation decreased to ±17%. Similar results were obtained in transfections of Hepa 1c1c7 cells. Before transfection, neither Hep G2 nor Hepa 1c1c7 cells expressed detectable GH. Furthermore, GH added to the assay systems (0–10 ng/ml; n = 6) was recovered quantitatively from the medium. GH addition did not alter the expression of GH by Hep G2 cells transfected with p41-284GH.

Comparison of Human GH and CAT Measurements. DNA elements involved in transcriptional regulation of phase 2 enzymes were previously identified by use of constructs containing the CAT reporter gene (3, 4, 15–17). To perform large numbers of assays rapidly and reproducibly, we chose the human GH gene as reporter (22) because the GH radioimmunoassay is simple, extremely sensitive, and highly quantitative. Transfection of cells in a single 10-cm culture plate permits more than 100 measurements and avoids problems associated with differences in transfection efficiencies. To verify that GH and CAT assays gave directly comparable results with our specific enhancer elements, we showed that GH and the CAT assays performed in both Hepa 1c1c7 and Hep G2 cells transfected with the p41YaCAT and p41-284GH plasmids and treated with 2,3-dimercapto-1-propanol gave parallel inductions (Table 1). In both cell lines, however, the GH assay was much more sensitive and the inductive response range (expressed as treated-to-untreated ratios) was much higher in the GH assay than in the CAT assay. Similar response patterns were observed with several other chemically unrelated inducers such as sulforaphane (data not shown). The expression of GH by Hepa 1c1c7 and Hep G2 cells transfected with the enhancerless but promoter-containing plasmids (187YaCAT and p284GH) was not increased by any of the inducers.

Table 1. Responses to 2,3-dimercapto-1-propanol of CAT and human GH transient gene expression assays in Hepa 1c1c7 and Hep G2 cells

2,3-Dimercapto-1-propanol, μM	Response ratio (treated/untreated) of cells: CAT Hepa 1c1c7	CAT Hep G2	GH Hepa 1c1c7	GH Hep G2
25	1.6	1.3	2.8	2.7
50	2.5	2.6	5.0	6.9
100	3.5	4.7	6.9	18.5

The cells were transfected with the CAT reporter p41YaCAT or the GH reporter p41-284GH.

We conclude that the GH transient gene expression assay in Hep G2 cells is a highly sensitive, quantitative, and reproducible measure of transcriptional regulation and that the results obtained parallel those of CAT assays.

Measurement of Potency for Induction of QR. The inducer potency of all compounds was determined with Hepa 1c1c7 cells grown in 96-well microtiter plates (24, 25). The inducers were added in either DMSO or water. A final concentration of 0.2% DMSO was present in all wells. The CD_{QR} (concentration required to double QR specific activity) values shown in Table 2 are lower than those reported previously (12), probably due to minor modifications [use of fetal calf serum treated with charcoal (1 g/100 ml) for 90 min at 55°C, and the inclusion of 0.2% DMSO in all assays].

RESULTS AND DISCUSSION

Comparison of Efficiencies of Inducer Responses of Plasmids p26-284GH and p41-284GH in Transient Gene Expression Assays. Prior studies of the mouse enhancer sequence used the entire 41-nt segment containing both AP-1-like sites and additional flanking sequences (Fig. 1). To determine whether the two AP-1 sites are sufficient for maximal induction, we compared the expression of GH by the complete construct p41-284GH and by p26-284GH, which contains both of the AP-1-like sites but lacks 10 of the 5′ base pairs and 5 of the 3′ base pairs of the 41-mer (Fig. 1) originally identified to contain the enhancer element in the mouse and rat upstream regions (16, 18, 26, 27). GH expression was measured with a series of concentrations of the following inducers (for structures, see Table 2): 1-nitro-1-cylcohexene (**1**), *trans*-4-phenylbut-3-en-2-one (**10**), *tert*-butylhydroquinone (**14**), sulforaphane (**15**), 2,3-dimercapto-1-propanol (**21**), phenylarsine oxide (**26**), sodium arsenite (**27**), mercuric chloride (**28**), phenylmercuric chloride (**31**), 1,2-dithiole-3-thione (**33**), and β-naphthoflavone (**34**) (Fig. 2).

The basal levels of GH production by both plasmids were essentially identical when corrected for cell number and transfection efficiency. All of these compounds produced concentration-dependent inductions of GH synthesis. Surprisingly, the maximal elevations of GH produced by these compounds in cells transfected with p26-284GH were low compared with experiments with p41-284GH (Fig. 2). However, the results obtained with p26-284GH were comparable to those observed by us (data not shown) and others with similar enhancer sequences in CAT assays (19). Thus, maximal inductions obtained with p26-284GH were 3.5-fold with 60 μM *trans*-4-phenylbut-3-en-2-one and 100 μM 1,2-dithiole-3-thione. The absolute induction ratios obtained with the plasmid containing the larger insert were dramatically higher; the highest induction ratios were 24.6-fold for 60 μM *tert*-butylhydroquinone and 22.5-fold for 6 μM sulforaphane. All compounds tested showed this difference in induction ratios for the two constructs, but the effects with phenylarsine

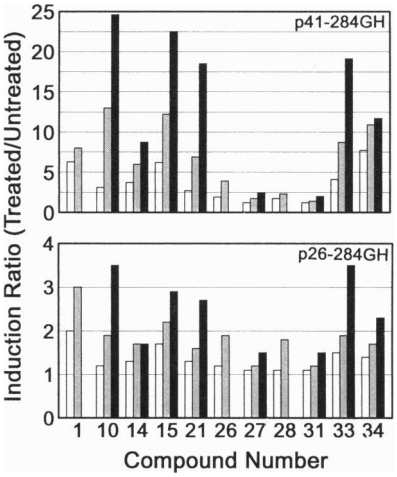


FIG. 2. Effect of different concentrations of inducers on GH production in Hep G2 cells transfected with p41-284GH (*Upper*) or p26-284GH plasmids (*Lower*). The compounds are numbered as in Table 2 and their concentrations (μM) were as follows: **1,** 1-nitro-1-cyclohexene (2.5, 5.0); **10,** *trans*-4-phenylbut-3-en-2-one (20, 40, 60); **14,** *tert*-butylhydroquinone (20, 40, 60); **15,** sulforaphane (1.5, 3.0, 6.0); **21,** 2,3-dimercapto-1-propanol (25, 50, 100); **26,** phenylarsine oxide (0.05, 0.10); **27,** sodium arsenite (2.5, 5.0, 10.0); **28,** mercuric chloride (1.25, 2.5); **31,** phenylmercuric chloride (0.5, 1.0, 2.0); **33,** 1,2-dithiole-3-thione (25, 50, 100); **34,** β-naphthoflavone (0.5, 1.0, 2.0). Open bars, low concentration; shaded bars, double the low concentration; solid bars, high concentration.

oxide, sodium arsenite, $HgCl_2$, and phenylmercuric chloride were smaller (Fig. 2). There were also large differences in the responses to inducers when p41-284GH and p26-284GH were transfected into Hepa 1c1c7 cells, although the magnitudes of induction ratios in this cell line were somewhat smaller.

In similar experiments with the rat enhancer sequence, Rushmore *et al.* (19) obtained only a 2- to 2.5-fold enhancement of CAT expression. In contrast, Friling *et al.* (18), using the 41-bp mouse enhancer sequence and the same inducers, obtained a 5- to 6-fold elevation in CAT activity, which is in accord with our results (Fig. 2). The responses of the mouse and rat enhancer sequences to inducers may differ because the 5′ region of the mouse 41-bp enhancer contains the core ETS protein DNA-binding sequence GGAA (28) near the first AP-1-like site. Adjacent ETS and AP-1 sites are known to confer dramatic synergism on gene expression (29). The rat gene lacks the first AP-1-like site, because the critical A of the AP-1 consensus is replaced by G (Fig. 1). Whether the

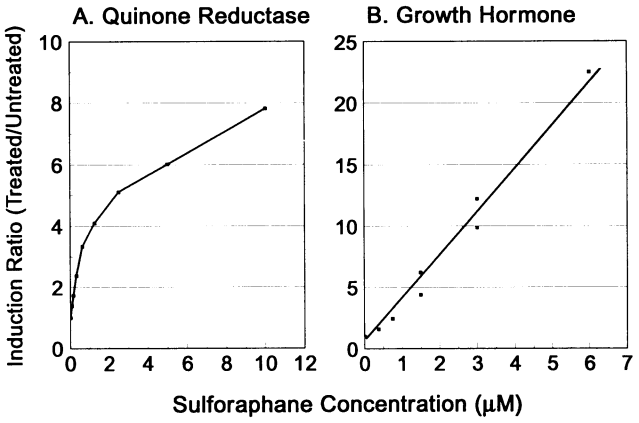
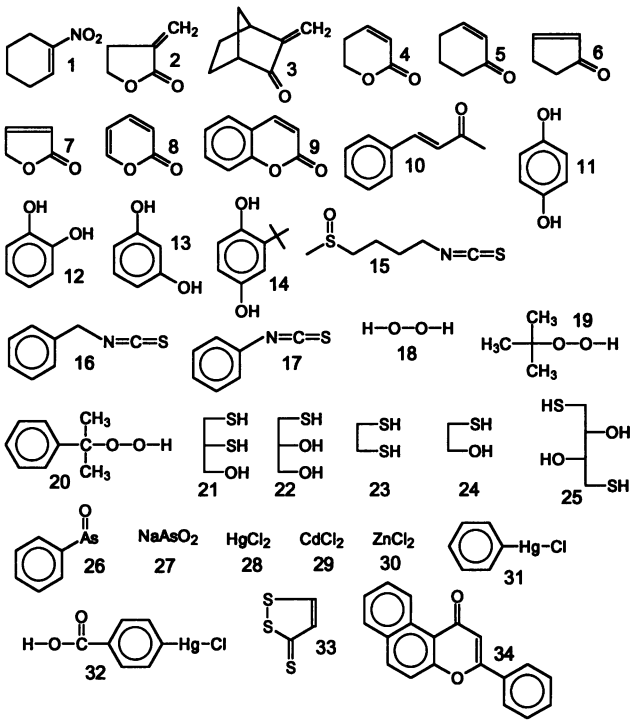


FIG. 3. Effect of increasing concentrations of sulforaphane on QR specific activity (*A*) and GH production (*B*). *B* includes data from two independent transfections, normalized for transfection efficiency.

Table 2. Potencies of inducers in enhancing GH production in Hep G2 cells transfected with p41-284GH and in elevating QR activity in Hepa 1c1c7 cells

Inducer No.	Name	CD_{GH}, μM	Rank order	CD_{QR}, μM	Rank order
Michael reaction acceptors					
1	1-Nitro-1-cyclohexene	0.98	5	0.46	5
2	2-Methylene-4-butyrolactone	2.4	8	4.5	12
3	3-Methylene-2-norbornanone	3.1	9	1.5	9
4	5,6-Dihydro-2*H*-pyran-2-one	8.8	12	6.7	16
5	1-Cyclohexen-2-one	15	17	9.1	17
6	1-Cyclopenten-2-one	80	25	32	22
7	2(5*H*)-Furanone	240	28	36	23
8	2*H*-Pyran-2-one	In	29	In	29
9	Coumarin	In	29	In	29
10	*trans*-4-Phenylbut-3-en-2-one	16	20	15	20
Diphenols and quinones					
11	Hydroquinone	12	16	5.3	14
12	Catechol	8.5	11	4.5	12
13	Resorcinol	In	29	In	29
14	*tert*-Butylhydroquinone	11	14	6.0	15
Isothiocyanates					
15	Sulforaphane	0.43	3	0.21	4
16	Benzyl isothiocyanate	0.70	4	3.7	11
17	Phenyl isothiocyanate	In	29	In	29
Peroxides					
18	Hydrogen peroxide	210	27	560	28
19	*tert*-Butyl hydroperoxide	29	23	140	24
20	Cumene hydroperoxide	21	21	210	26
Mercaptans					
21	(±)-2,3-Dimercapto-1-propanol	26	22	12	19
22	3-Mercaptopropane-1,2-diol	In	29	In	29
23	1,2-Ethanedithiol	15	17	21	21
24	2-Mercaptoethanol	180	26	170	25
25	(±)-1,4-Dithiothreitol	In	29	In	29
Trivalent arsenicals					
26	Phenylarsine oxide	0.047	1	0.057	2
27	Sodium arsenite	11	14	2.4	10
Heavy metal salts					
28	$HgCl_2$	1.9	6	0.52	6
29	$CdCl_2$	7.3	10	11	18
30	$ZnCl_2$	73	24	220	27
31	Phenylmercuric chloride	2	7	0.12	3
32	*p*-Chloromercuribenzoate	9.2	13	1.1	8
Other inducers					
33	1,2-Dithiole-3-thione	15	17	1.0	7
34	β-Naphthoflavone	0.051	2	0.029	1

Rank order refers to potencies. When two compounds were equipotent they were assigned the same rank, and the subsequent rank was omitted. Inactive (In) is defined as less than a 20% increase in the induction ratio (treated/untreated) at the highest concentration at which there was less than 50% cell toxicity. *(Structures are shown at top of next column.)*



differences in the inducer response of the mouse and rat enhancers can be attributed to this change requires further mutation and deletion experiments.

Comparison of Potencies of Inducers in Enhancing GH Production in Hep G2 Cells Transfected with p41-284GH and in Elevating QR Activities in Hepa 1c1c7 Cells. To determine whether the transcriptional activation mediated through the 41-bp enhancer element accounted for the entire phase 2 enzyme induction produced by all classes of inducers, we compared the concentrations of inducers required to double GH production (CD_{GH}) and QR activity (CD_{QR}) in the two systems. Typical response curves for sulforaphane (0–10 μM) are shown in Fig. 3*A* (QR induction) and Fig. 3*B* (GH production). Notably, the response ratios at high concentrations of sulforaphane (6 μM) were much higher in the GH assay (22-fold) than in the QR assay (6.4-fold). These graphs generated $CD_{QR} = 0.21 \pm 0.05$ μM and $CD_{GH} = 0.42 \pm 0.18$ μM for sulforaphane (Table 2).

An extraordinary diversity of chemical compounds are active in both systems. Various chemical classes of compounds were tested (Table 2): (*i*) *Michael reaction acceptors* (olefins conjugated to electron-withdrawing functions). As shown for QR induction (12, 13), the potency orders for GH production paralleled the electrophilicity of these compounds. For example, 1-nitro-1-cyclohexene ($CD_{GH} = 0.98$ μM; $CD_{QR} = 0.46$ μM), with the olefin conjugated to the powerful electron-withdrawing nitro group, is much more potent in both systems than coumarin (inactive) which is an olefinic lactone. (*ii*) *Diphenols*. Oxidizable diphenols—hydroquinone, catechol, and *tert*-butylhydroquinone—were all comparably potent in both systems, whereas the nonoxidizable resorcinol was inactive (30). (*iii*) *Isothiocyanates*. Sulforaphane was very potent, benzyl isothiocyanate less potent, and phenyl isothiocyanate inactive (12). (*iv*) *Peroxides*. These compounds were all weakly active. Cumene hydroperoxide was slightly more active than *tert*-butyl hydroperoxide, and both compounds were considerably more active than hydrogen peroxide, which induced weakly in both systems (13). (*v*) *Mercaptans*. Mercaptans (which are not electrophiles) were especially active when two thiol groups were adjacent, as in 1,2-ethanedithiol and 2,3-dimercapto-1-propanol (14). 2-Mercaptoethanol was only weakly active, and both dithiothreitol and 3-mercaptopropane-1,2-diol were inactive. Thus two adjacent thiol groups appear to lead to significant inductive potency (14). (*vi*) *Trivalent arsenicals*. Phenylarsine oxide was the most potent inducer tested and was very much more potent than sodium arsenite (14). (*vii*) *Heavy metal salts*. $HgCl_2$, $CdCl_2$, and $ZnCl_2$ were also inducers, with potencies decreasing in this order, which parallels their binding affinity for sulfhydryl groups (14). (*viii*) *Other inducers*. The metabolizable polycyclic aromatic hydrocarbon β-naphthoflavone, a bifunctional inducer, was also a very potent transcriptional activator, doubling the GH production at a concentration of only 0.051 μM. Furthermore, 1,2-dithiole-3-thione also enhanced transcriptional activation through the same enhancer element.

Table 2 shows that 6 of 34 compounds from the eight chemically dissimilar classes were inactive in both systems and none was inactive in only one system. The remaining 28 active inducers ranged in potencies over nearly four orders of magnitude from phenylarsine oxide (CD_{GH} = 0.047 μM; CD_{QR} = 0.057 μM) to hydrogen peroxide (CD_{GH} = 210 μM; CD_{QR} = 560 μM), and many compounds were nearly equipotent in the two assay systems. A plot of potencies of QR induction with respect to potencies of GH production for all active inducers (Table 2) gave a good linear correlation, with an r value of 0.89 and a slope of 0.89 (Fig. 4). We conclude that the induction of QR by all of the very different types of inducers is probably mediated entirely through the 41-bp enhancer element and that GH production and QR induction are controlled by the same or very similar rate-limiting processes. Furthermore, comparison of absolute CD values in the two assays gave a linear correlation (r = 0.64), and the slope of the correlation line was 1.17, indicating that the compounds were nearly equipotent in the two assays.

Conclusions. We have demonstrated that a 41-bp enhancer element from the 5′ upstream region of the mouse glutathione transferase Ya gene (20) is responsive to a wide variety of xenobiotic compounds that also induce phase 2 detoxication enzymes in cultured cells and in animals. Transcriptional activation through this element accounts for most, if not all, of the enzyme elevations produced by these inducers. The inducers belong to many different chemical classes; most contain electron-deficient centers and their potencies parallel the strengths of the electron-withdrawing functions. Furthermore, inducers are also substrates for glutathione transferases, thus emphasizing their electrophilicity (13). Paradoxically, dimercaptans were also found to be inducers. The only apparently universal property of all inducers is their capacity for reaction with sulfhydryl groups (by oxidoreduction or alkylation). We suggest, therefore, that a mechanism involving protein thiol modifications modulates the transcriptional activations mediated by the 41-bp enhancer element. In this connection, it is of considerable interest that the redox state of sulfhydryl groups has been implicated in AP-1 binding to DNA (31–33).

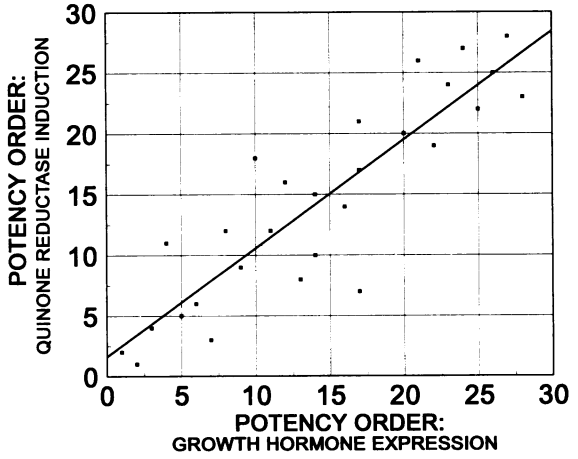


FIG. 4. Order of potencies of 28 compounds in inducing QR (CD_{QR}) and in stimulating growth hormone production (CD_{GH}). The 28 active compounds (Table 2) were ranked from 1 to 28 in order of their potencies in the QR (ordinate) and GH (abscissa) assays. Inactive compounds were excluded. There is a good linear correlation (r = 0.89 and slope = 0.89).

These studies were supported by Grants from the National Cancer Institute, Department of Health and Human Services (PO1 CA 44530) and the American Institute for Cancer Research. T.P. is a Trainee of the National Institutes of Health Medical Scientist Training Program (T32 GM 07309). Y.Z. is a Fellow of the Cancer Research Foundation of America.

1. Jakoby, W. B. & Ziegler, D. M. (1990) *J. Biol. Chem.* **265,** 20715–20718.
2. Prochaska, H. J. & Talalay, P. (1991) in *Oxidative Stress: Oxidants and Antioxidants*, ed. Sies, H. (Academic, London), pp. 195–211.
3. Favreau, L. V. & Pickett, C. B. (1991) *J. Biol. Chem.* **266,** 4556–4561.
4. Jaiswal, A. K. (1991) *Biochemistry* **30,** 10647–10653.
5. Prochaska, H. J. & Talalay, P. (1988) *Cancer Res.* **48,** 4776–4782.
6. Talalay, P., De Long, M. J. & Prochaska, H. J. (1987) in *Cancer Biology and Therapeutics*, eds. Cory, J. G. & Szentivanyi, A. (Plenum, New York), pp. 197–216.
7. Batzinger, R. P., Ou, S.-Y. L. & Bueding, E. (1978) *Cancer Res.* **38,** 4478–4485.
8. Benson, A. M., Cha, Y.-N., Bueding, E., Heine, H. S. & Talalay, P. (1979) *Cancer Res.* **39,** 2971–2977.
9. De Long, M. J., Dolan, P., Santamaria, A. B. & Bueding, E. (1986) *Carcinogenesis* **7,** 977–980.
10. Bannai, S. (1984) *J. Biol. Chem.* **259,** 2435–2440.
11. Bannai, S., Sato, H., Ishii, T. & Taketani, S. (1991) *Biochim. Biophys. Acta* **1092,** 175–179.
12. Talalay, P., De Long, M. J. & Prochaska, H. J. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8261–8265.
13. Spencer, S. R., Xue, L., Klenz, E. M. & Talalay, P. (1991) *Biochem. J.* **273,** 711–717.
14. Prestera, T., Zhang, Y., Spencer, S. R., Wilczak, C. & Talalay, P. (1993) *Adv. Enzyme Regul.*, in press.
15. Daniel, V., Sharon, R. & Bensimon, A. (1989) *DNA* **8,** 399–408.
16. Rushmore, T. H. & Pickett, C. B. (1990) *J. Biol. Chem.* **265,** 14648–14653.
17. Li, Y. & Jaiswal, A. K. (1992) *J. Biol. Chem.* **267,** 15097–15104.
18. Friling, R. S., Bensimon, A., Tichauer, Y. & Daniel, V. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6258–6262.
19. Rushmore, T. H., Morton, M. R. & Pickett, C. B. (1991) *J. Biol. Chem.* **266,** 11632–11639.
20. Friling, R. S., Bergelson, S. & Daniel, V. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 668–672.
21. Zhang, Y., Talalay, P., Cho, C.-G. & Posner, G. H. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 2399–2403.
22. Selden, R. F., Howie, K. B., Rowe, M. E., Goodman, H. M. & Moore, D. D. (1986) *Mol. Cell. Biol.* **6,** 3173–3179.
23. Gorman, C. (1985) in *DNA Cloning*, ed. Glover, D. M. (IRL, Oxford), Vol. 2, pp. 143–190.
24. Prochaska, H. J. & Santamaria, A. B. (1988) *Anal. Biochem.* **169,** 328–336.
25. Prochaska, H. J., Santamaria, A. B. & Talalay, P. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 2394–2398.
26. Daniel, V., Sharon, R., Tichauer, Y. & Sarid, S. (1987) *DNA* **6,** 317–324.
27. Rushmore, T. H., King, R. G., Paulson, K. E. & Pickett, C. B. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 3826–3830.
28. Macleod, K., Leprince, D. & Stehlin, D. (1992) *Trends Biochem. Sci.* **17,** 251–256.
29. Wasylyk, B., Wasylyk, C., Flores, P., Begue, A., Leprince, D. & Stehlin, D. (1990) *Nature (London)* **346,** 191–193.
30. Prochaska, H. J., De Long, M. J. & Talalay, P. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8232–8236.
31. Abate, C., Patel, L., Rauscher, F. J., III, & Curran, T. (1990) *Science* **249,** 1157–1161.
32. Xanthoudakis, S. & Curran, T. (1992) *EMBO J.* **11,** 653–665.
33. Xanthoudakis, S., Miao, G., Wang, F., Pan, Y.-C. E. & Curran, T. (1992) *EMBO J.* **11,** 3323–3335.