Cell Stress & Chaperones (1997) **2** (1), 31–40

The molecular response to reductive stress in LLC-PK1 renal epithelial cells: coordinate transcriptional regulation of *gadd153* and *grp78* genes by thiols

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Abstract Organic thiols are toxic to eukaryotic cells. Treatment of cells with thiols activates expression of *grp78*, but it is not known if, like other forms of stress, there is a battery of stress response genes that are induced by thiols. In LLC-PK1 renal epithelial cells, mRNAs for both *grp78* and *gadd153* were induced by thiols with similar time, concentration and structure-activity dependence. Dithiothreitol (DTT) was the most potent reductant and inducer of gene expression among the thiols tested. Nuclear run-on assays demonstrated that DTT activated both *grp78* and *gadd153* genes transcriptionally. A hamster *gadd153* promoter construct which contains enhancer elements necessary for *gadd153* activation was stably integrated into the LLC-PK1 cell genome and was activated by DTT. Although auto-oxidation of thiols can generate active oxygen species, transcriptional activation of the *gadd153* promoter was not due to formation of hydrogen peroxide or superoxide since neither catalase nor superoxide dismutase prevented activation of the *gadd153* promoter by DTT. The concentration dependence for activation of the *gadd153* promoter correlated with inhibition of dome formation and protein synthesis, two toxic effects of DTT in LLC-PK1 cells. Thus, both *grp78* and *gadd153* are members of a gene battery which is responsive to reductive stress. There appears to be considerable, but not complete, overlap between the upstream signaling pathways for activation of both genes.

INTRODUCTION

Activation of gene expression is a universally conserved response to stress in prokaryotic and eukaryotic cells. Different types of stress, including oxidative stress, activate batteries of stress-response genes (Donati et al 1990; Storz et al 1990b; Holbrook and Fornace 1991; Fornace 1992). Oxidative stress activates gene expression in prokaryotes and eukaryotes including members of the heat shock protein (*hsp*) gene family, and the immediate early genes *c-jun, c-fos* and *c-myc* (Crawford et al 1988; Shibanuma et al 1988; Donati et al 1990; Shibanuma et al 1990; Storz et al 1990b; Cerutti and Trump 1991; Devary et al 1991). In some cases, oxidants modulate gene expression directly by positive or negative redox regulation through oxidation of cysteinyl residues in DNA binding proteins such as OXYR and AP-1 (Abate et al 1990; Storz et al 1990a). At low levels, oxidants may actually serve as intracellular second messengers under either stressful or physiological conditions (Schreck et al 1992; Sundaresan et al 1995). In many cases, stress-gene activation is a physiologically important protective response; accordingly, expression of stress-response genes imparts tolerance to subsequent toxicant exposure (Laszlo 1992).

Received 6 May 1996; Revised 10 September 1996; Accepted 10 September 1996

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Thiol reductants are also cytotoxic and increase expression of glucose regulated protein (*grp*) genes (Whelan and Hightower 1985; Kim et al 1987). Whelan and Hightower (1985) proposed that *grp* genes are more responsive to reductive stress while *hsp* genes respond to oxidative stress. The products of the prototypical *grp* genes *grp94* and *grp78* play important roles as chaperones during protein folding and processing in the endoplasmic reticulum (Gething and Sambrook 1992; Welch 1992). Agents that interfere with endoplasmic reticulum protein folding, including thiols, activate *grp78* transcription (Whelan and Hightower 1985; Kim and Lee 1987; Kim et al 1987; Prostko et al 1991; Wooden et al 1991; Prostko et al 1992; Li et al 1993). The high levels of oxidized glutathione in the endoplasmic reticulum provide an oxidizing redox potential that drives protein disulfide formation (Braakman et al 1992; Gething and Sambrook 1992; Hwang et al 1992; Bardwell and Beckwith 1993). Since thiols, like dithiothreitol (DTT), push the thiol redox potential of the endoplasmic reticulum in the reducing direction, protein disulfide formation and protein folding are disrupted (Kim et al 1987; Braakman et al 1992; Prostko et al 1992; Lodish and Kong 1993; Sawyer et al 1994; Losch and Koch-Brandt 1995).

Although thiols induce reductive stress, it is not known if a battery of reductive stress-responsive genes exists. However, many inducers of *grp78* also increase expression of the growth arrest and DNA damage gene 153 (*gadd153*) suggesting that induction of both genes may be linked within their upstream signaling pathways (Price and Calderwood 1992; Carlson et al 1993). *gadd* genes were originally isolated from UV-irradiated Chinese hamster ovary cells (Fornace 1992). *gadd153* is a member of the C/EBP family of leucine zipper proteins but lacks critical basic residues in the DNA binding domain (Ron and Habener 1992). This unique feature allows GADD153 to act as a negative regulator of C/EBP-mediated gene regulation, at least in cultured cells (Ron and Habener 1992; Sylvester et al 1994). A recent report suggests that GADD153 may also bind to DNA in a complex with C/EBPs, but the consequence of this interaction is not clear (Ubeda et al 1996). *gadd* genes are induced by nephrotoxic cysteine conjugates, DNA damaging agents, and growth arrest (Fornace Jr et al 1989; Bartlett et al 1992; Chen et al 1992a; Luethy and Holbrook 1992; Price and Calderwood 1992; Carlson et al 1993) and may be important in regulating growth arrest (Fornace 1992; Zhan et al 1994; Batchvarova et al 1995).

We have been using LLC-PK1, a line of renal epithelial cells with proximal tubule character (Hull et al 1976; Stevens et al 1986), to investigate mechanisms of toxicity (Chen et al 1990; Chen and Stevens 1991) and activation of stress response genes in renal epithelium (Chen et al 1992a, 1992b; Yu et al 1994; Liu et al 1996). Recently, we found that *gadd153* mRNA, but not other stress genes, is induced by DTT treatment suggesting that *gadd153* is a candidate reductive-stress responsive gene. In this study, we characterized the induction of both *grp78* and *gadd153* genes by thiols. DTT caused dome collapse and inhibition of protein synthesis in LLC-PK1 cells and activated both the *grp78* and *gadd153* genes at the transcriptional level. The promoter element(s) responsible for activation of the *gadd153* gene by DTT are contained in the same region of the promoter responsive to DNA damaging agents and growth arrest. Thus, *gadd153* and *grp78* appear to be components of a gene battery activated by thiol-induced reductive stress.

EXPERIMENTAL PROCEDURES

Materials

Dulbecco's modified Eagle medium (DMEM) and G-418 sulfate were supplied by GIBCO BRL (Grand Island, NY). Radiochemicals were obtained from New England Nuclear (Boston, MA). Other chemicals were purchased from Sigma Chemical Company (St Louis, MO) or Aldrich (Milwaukee, WI) unless otherwise noted. DCVC was synthesized as described (Hayden and Stevens 1990).

Cell culture and experimental treatment

LLC-PK1 cells (Hull et al 1976), obtained from American Tissue Type Culture (Rockville, MD), were grown to confluence in a humidified 37°C incubator in DMEM supplemented with 10% fetal bovine serum, (GIBCO BRL or Upstate Biotechnology, Lake Placid, NY) under an atmosphere of 5% CO_2: 95% air. For experiments in which Northern analysis and chloramphenicol acetyltransferase (CAT) measurements were performed, cells (1×10^6) were plated in 100 mm dishes 5 days prior to treatment. For cytotoxicity measurements, LLC-PK1*gadd*CAT cells, that had a stably integrated *gadd153* promoter-CAT construct (Luethy and Holbrook 1992) were plated at a density of 5×10^4 cells in each well of a 24-well dish 4 days prior to treatment. Media were routinely changed 3 days after seeding. The confluent cultures were rinsed with phosphate buffered saline (PBS) and treated with DTT or DCVC in Earle's balanced salt solution containing 1.8 mM $CaCl_2$, 5.4 mM KCl, 1.7 mM $MgSO_4$, 26.2 mM $NaHCO_3$, 1.0 mM NaH_2PO_4, 5.6 mM glucose and 25 mM HEPES.

Northern hybridization analysis

Poly $(A)^+$ RNA was isolated by oligo-dt cellulose (Badley et al 1988) and Northern blotting performed as described (Chen et al 1992a). RNAs were separated by electrophoresis on a 1.4% agarose denaturing gel and capillary transferred to nitrocellulose paper. After prehybridization,

blots were hybridized overnight with cDNA probes for *gadd153* (Chen et al 1992a) or *grp78* (Lee et al 1983) that had been labeled with [^{32}P]dCTP by the random priming method (Feinberg and Vogelstein 1984) using a kit (Boehringer Mannheim, Indianapolis, IN). The *grp78* cDNA was a generous gift of Dr Amy Lee. Blots were allowed to decay prior to reprobing with a 200-base pair insert for mouse *β-actin* cut from pMACTE4, an SK-based plasmid provided by Dr Dominic Eisinger. The size of the mRNA was estimated by comparison with an RNA Ladder (GIBCO BRL). After exposing blots to Dupont Cronex film with an intensifying screen, bands were quantitated using a Bio Image Densitometer (Ann Arbor, MI). To correct for differences in gel loading, integrated optical densities were normalized to the *β-actin* signal.

Cell transfections and CAT expression

LLC-PK1 cells were seeded at a density of 5×10^5 cells per 25 cm^2 flask and transfected using the calcium phosphate precipitation method. Cells which had incorporated a CAT reporter plasmid containing the region from −801 to +21 (relative to transcription start site) of the *gadd153* promoter (*gadd*-CAT) in a JymCATO vector (Luethy et al 1990) were prepared by co-transfecting cells with 5 μg of *gadd*-CAT with 5 ng of pSV2neo and selected for resistance to G-418 sulfate (400 μg/ml). To estimate CAT activity, cells were rinsed twice with PBS after treatment and allowed to recover for 24 h in DMEM supplemented with 10% FBS. Cells were harvested by scraping and centrifugation; the pellet was resuspended in PBS containing 1 mM phenylmethylsulfonyl fluoride. After lysing the cells by repeated freezing and thawing, CAT expression was measured with an enzyme-linked immunosorbent assay kit (5 Prime 3 Prime Inc., Boulder, CO) according to the manufacturer's protocol. Protein was measured with the BioRad assay (BioRad, Richmond, CA) using bovine gamma globulin as a standard.

Nuclear run-on analysis

Nuclei were prepared for run-on transcription and stored at −70°C in 40% glycerol (Ausubel et al 1991). RNA which had been newly transcribed in the presence of [^{32}P]UTP (NEN), was purified as described (Celano et al 1989) and hybridized to plasmids which had been linearized and UV-crosslinked to nylon filters. The run-on transcripts for *gadd153* and *grp78* were detected using the cDNAs described above for Northern analysis. Controls included plasmids containing cDNAs for *β-actin* and *hsp70* (plasmid pAT125, ATCC) as internal controls as well as pBluescript SK (Stratagene, La Jolla, CA) for non-specific binding.

Cytotoxicity measurements

Cytotoxicity was determined by monitoring release of the cytosolic enzyme lactate dehydrogenase into the incubation media. Lactate dehydrogenase activity was quantitated using a microtiter plate assay previously developed in this laboratory (Chen et al 1990). Dome collapse was assessed by counting the number of domes visible in a 4X field using an inverted phase contrast microscope. Numbers represent the mean ± SD of dome counts from four areas of a 100 mm dish of confluent LLC-PK1 cells following a 4-h treatment. Inhibition of protein synthesis was determined by measuring the incorporation of [^{3}H]leucine into trichloroacetic acid insoluble material as before (Yu et al 1994).

Statistics

Analysis of variance followed by the Student-Newman-Keuls method for multiple comparisons was used to compare means of three or more groups. The level of significance was set at $P < 0.05$. Groups of means which are not different are denoted by a common letter symbol.

RESULTS

Characterization of *gadd153* mRNA induction by thiols

We tested several thiols as inducers of *gadd153* and compared the response to that seen with *grp78*, a gene that is induced by thiols (Kim et al 1987). DTT was a strong inducer of *gadd153* and *grp78* mRNA (Fig. 1). 2–Mercaptoethanol also induced *gadd153* and *grp78* mRNA, but to a lesser extent than DTT; N-acetylcysteine was ineffective. Since the pattern of expression for *grp78* and *gadd153* was similar, we further characterized the response in LLC-PK1 cells with DTT. DTT increased *gadd153* and *grp78* mRNA in a time- and concentration-dependent fashion (Figs 2 & 3). After a 1-h exposure to 10 mM DTT, both mRNAs had increased and remained elevated through 5 h of continued exposure at which time cells began to detach from the dish.

Induction of *gadd153* mRNA is regulated transcriptionally by reductive stress

To determine if transcriptional events were involved in the induction of *gadd153* mRNA by reductive stress we carried out nuclear run-on experiments. Nuclear run-on transcription of *gadd153* increased by 7-fold (7 ± 2; $n = 4$), relative to *β-actin*, in the presence of DTT (Fig. 4). Transcription of *grp78* also increased (9 ± 3 fold; $n = 4$) relative to *β-actin* as expected (Kim et al 1987). In contrast, transcription of *hsp70* RNA, which is not induced by reductive stress (Whelan and Hightower 1985; Chen et al 1992b), was not affected appreciably. Only a modest

increase in *hsp70* and *β-actin* transcription, less than 2-fold, was seen in three experiments. Thus, thiol-induced reductive stress activates transcription of both *grp78* and *gadd153*.

The hamster *gadd153* promoter has been isolated and reporter constructs have been used to investigate the structure-activity relationship for *gadd153* activation (Luethy et al 1990; Luethy and Holbrook 1992). LLC-PK1 cells which had genomically integrated a CAT reporter construct containing 801 bp of DNA 5′ to the start site of the *gadd153* gene (Luethy and Holbrook 1992) were isolated by G418 selection (PK1*gadd*CAT cells). Addition of 10 mM DTT to PK1*gadd*CAT cells increased expression of CAT dramatically (Fig. 5). Both 2-mercaptoethanol and N-acetylcysteine caused only a slight induction of CAT activity in general agreement with the Northern blot data (Fig. 1).

DTT will oxidize in an aerobic environment producing reactive oxygen species such as superoxide anion (Held and Biaglow 1993). Since toxicants which cause oxidative stress induce *gadd153* mRNA (Luethy et al 1990; Chen et al 1992a), we determined if DTT was inducing *gadd153* via an indirect oxidative stress in the PK1*gadd*CAT cells. Neither superoxide dismutase or catalase prevented DTT induction of CAT activity in PK1*gadd*CAT cells (Table 1) indicating that promoter activation is not due to oxidation of DTT.

Induction of *gadd153* mRNA is linked to toxic effects of DTT

Although DTT did not cause an increase in lactate dehydrogenase release from LLC-PK1 cells up to 5 h after addition of DTT, it did appear to cause morphological changes in the cells. In particular, the domes, structures which are formed due to trapping of actively transported solutes between the cell layer and the plastic culture surface (Lever 1985), collapsed after DTT treatment (Fig. 6). When we compared the concentration dependence of dome collapse with induction of CAT activity, there was a good correlation (Table 2). However, cytochalasin D, which perturbs the actin cytoskeleton, also caused dome collapse (Fig. 6), but did not induce *gadd153* (data not shown) suggesting that additional factors must be involved. Since DTT inhibits protein synthesis (Prostko et al 1991; Prostko et al 1992) we also compared the concentration dependence for inhibition of protein synthesis or CAT activation and again there was a good correlation (Table 2). However, inhibition of protein synthesis was not sufficient to activate *gadd153* since cycloheximide did not induce *gadd153* mRNA in LLC-PK1 cells (Chen et al 1992a). Thus, the transcriptional activation of *gadd153* by DTT appeared to depend both on thiol reducing potential and the ability of DTT to interfere with normal cellular homeostasis at multiple levels.

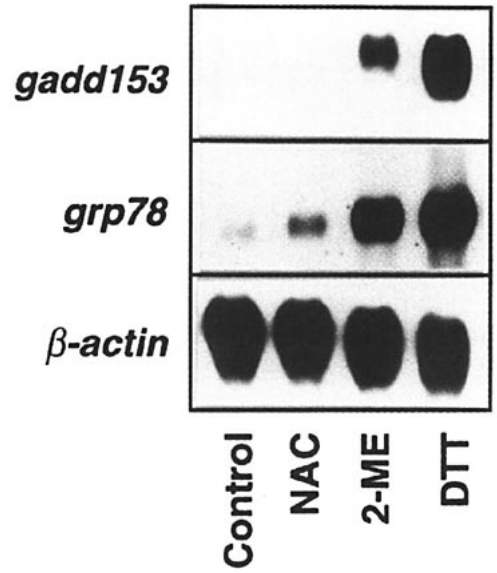

Fig. 1 Structure activity relationship for the induction of *gadd153* and grp78 by thiols. LLC-PK1 cells were treated with various thiols at a concentration of 10 mM for 5 h, and polyA+ RNA prepared for analysis of *grp78* and *gadd153* mRNA expression. The data are from a single experiment representative of four ($n = 4$). NAC, N-acetylcysteine; 2-ME, 2-mercaptoethanol.

The ability of DTT to protect against cytotoxicity is not due to induction of *grp78* or *gadd153*

When DTT is added along with or immediately after toxicant exposure, it protects LLC-PK1 cells from cytotoxicity (Chen et al 1990; Chen and Stevens 1991; Liu et al 1996). Because DTT induces *gadd153* and *grp78*, it seemed possible that the ability of DTT to protect against various toxicants could be due to induction of gene expression

Table 1 Activation of gadd153 by DTT is not due to a secondary oxidative stress

Treatment	CAT ng/mg protein	Relative induction
none	0.05 ± 0.05	1
DTT	*4.4 ± 1.2	88
DTT + catalase	*4.6 ± 1.3	92
DTT + superoxide dismutase	*4.2 ± 1.3	83

*$P < 0.05$ relative to no treatment.

LLC-PK1 cells which had stably integrated the gadd-CAT reporter construct were treated for 2 h with 10 mM DTT in the presence or absence of either superoxide dismutase or catalase (500 U/ml) and allowed to recover for 24 h in DMEM containing 10% fetal bovine serum. Chloramphenicol acetyltransferase activity was determined as described under Procedures. The increases in CAT were significant after DTT treatment; however, catalase and superoxide dismutase had no significant effect on DTT-induced CAT expression ($P < 0.05$; ANOVA). The data are a summary of two experiments ($n = 2$).

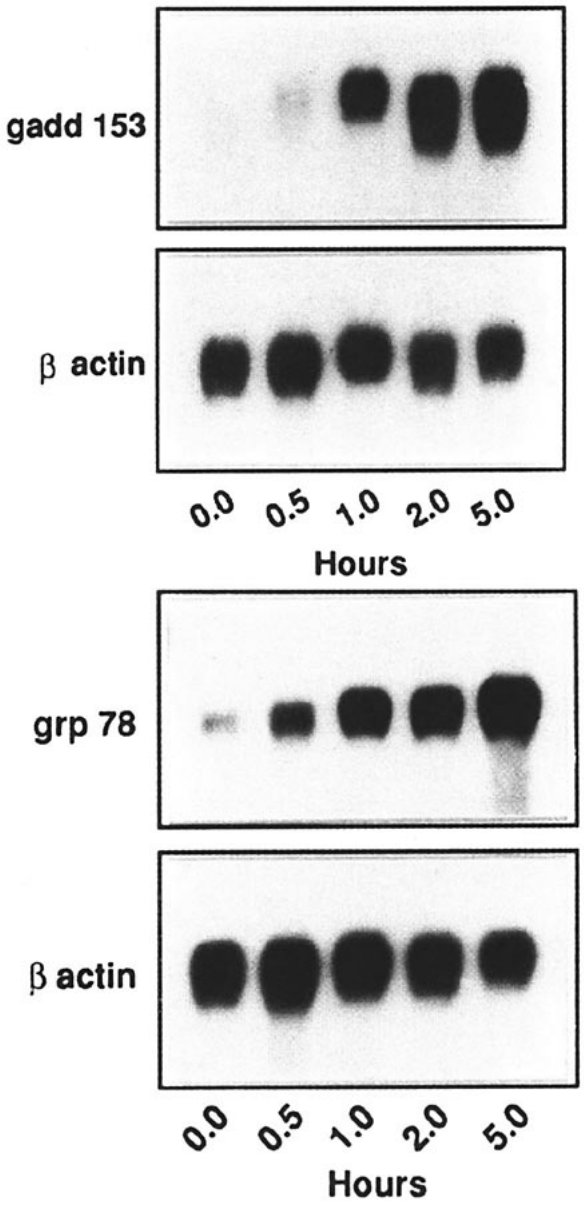

Fig. 2 Time dependence for induction of *gadd153* and *grp78* by dithiothreitol. LLC-PK1 cells were treated with dithiothreitol at a concentration of 10 mM, and polyA+ RNA prepared at various times for analysis of *grp78* and *gadd153* mRNA expression. The data are from a single experiment representative of three ($n = 3$).

rather than protection against oxidation of cellular protein and nonprotein thiols, as suggested (Chen et al 1990; Liu et al 1996). In order to determine if transcriptional activation of *gadd153* or *grp78* could contribute to cellular protection we compared the concentration dependence for CAT induction by DTT and protection against DCVC, a nephrotoxicant (Chen et al 1990) (Fig. 7). In agreement with the Northern blot data, the hamster *gadd153* promoter was activated at 0.5–1 mM DTT. However, protection against lactic dehydrogenase release caused by DCVC treatment was observed only at 10 mM

Table 2 Correlation between activation of the gadd153 promoter and toxicity

DTT (mM)	CAT ng mg/protein	DOMES	[3H]Leucine (% control)
0	<0.1	34 ± 5	100 ± 0
0.1	0.2 ± 0.2	31 ± 8	86 ± 15
1.0	*5.5 ± 0.5	*<1 ± 1	*34 ± 2
10	*3.6 ± 0.2	*<1 ± 1	*7 ± 7

*$P < 0.05$ relative to untreated.

Cells were treated with various concentrations of DTT for 4 h. At the end of the treatment period, domes were counted using a 4X objective. The counts represent the number of domes per field and are the mean ± the standard deviation of the data from 3 plates/group. Protein synthesis was determine by measuring [^{3}H]leucine incorporation as described in Procedures; the data are a summary of three experiments ($n = 3$). Significant differences ($P < 0.05$) from untreated cells were determined by ANOVA.

DTT. Thus, the immediate protective effect of DTT against DCVC toxicity is not due to activation of the *grp78* or *gadd153* genes during DCVC treatment.

DISCUSSION

Proteotoxicity, i.e. damage and/or denaturation of mature or nascent polypeptides (Hightower 1991), is linked to transcriptional activation of *grp78* as well as the *hsp70* gene (Wooden et al 1991; Gething and Sambrook 1992; Morimoto 1993). However, *grp78* transcription is more responsive to reductive stress while *hsp70* is more responsive to oxidative stress suggesting that the mechanisms must differ (Whelan and Hightower 1985). Oxidative and reductive stress both damage proteins by modifying the protein thiol/disulfide status, but by distinct mechanisms. The cytoplasm is a reducing environment (Sies 1993). Rapid loss of cytosolic GSH sensitizes cells to oxidative stress, causes disulfide cross-linking and allows aggregation of proteins, events which activate *hsp70* transcription (Lee and Hahn 1988; Beckman et al 1992; Freeman et al 1995; Liu et al 1996). On the other hand, organic thiols prevent disulfide formation in the endoplasmic reticulum by perturbing the oxidizing thiol/disulfide redox potential causing protein aggregation (Gething and Sambrook 1992; Hwang et al 1992; Lodish and Kong 1993; Sawyer et al 1994; Losch and Koch-Brandt 1995). The presence of unfolded and aggregated proteins in the endoplasmic reticulum activates *grp78* transcription (Gething and Sambrook 1992).

Our results suggest that disruption of the endoplasmic reticulum by reductive stress is a signal for activation the *gadd153* and *grp78* genes. *gadd153* is induced by insults which increase *grp78* mRNA including hypoxia, calcium ionophores, glucose deprivation, agents that block glycosylation, and thapsigargin, an inhibitor of calcium-ATPases (Bartlett et al 1992; Price and Calderwood 1992;

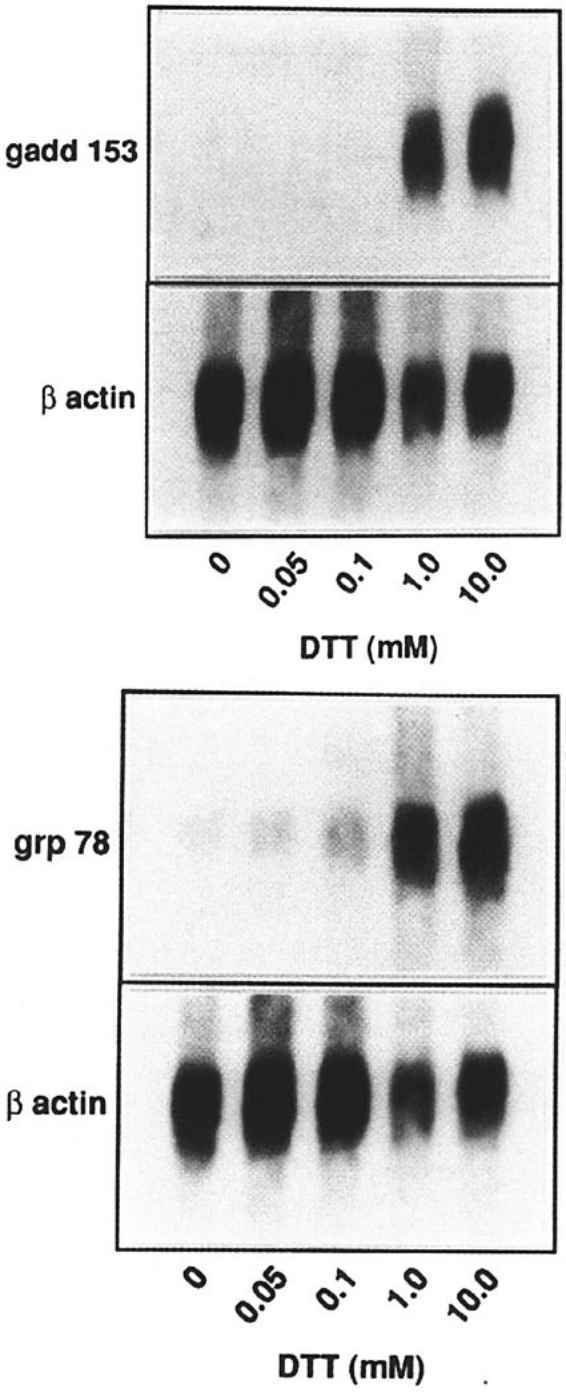

Fig. 3 Concentration dependent induction of *gadd153* and *grp78* by dithiothreitol. LLC-PK1 cells were treated with dithiothreitol at the indicated concentrations for 5 h, and polyA+ RNA prepared for analysis of *grp78* and *gadd153* mRNA expression. The data are from a single experiment representative of two ($n = 2$).

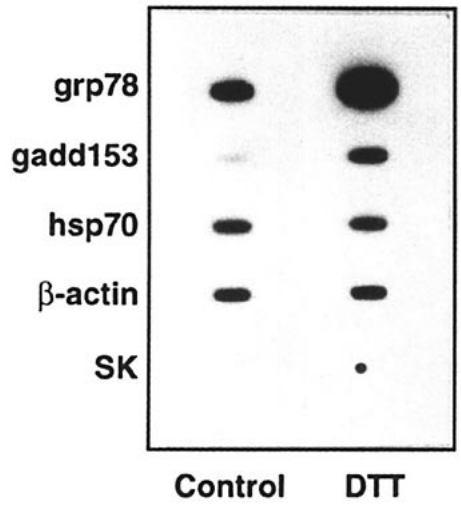

Fig. 4 Transcriptional activation of *gadd153* and *grp78* by dithiothreitol. RNA newly transcribed by isolated nuclei in the presence of [^{32}P]UTP were extracted and hybridized with inserts immobilized on nylon membranes. Nuclei were prepared from cells treated for 2.5 h with Earles balanced salt solution alone or with 10 mM DTT. SK indicates the use of pBluescript SK which served as a control for nonspecific binding. The figure is from an experiment representative of four ($n = 4$). The values for the fold induction are reported in the text.

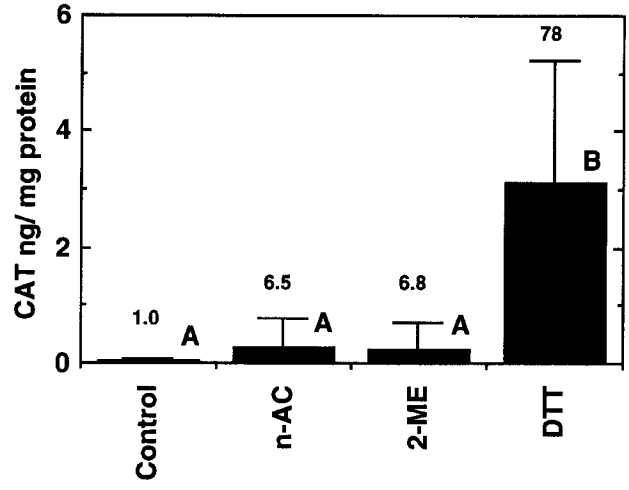

Fig. 5 Activation of a *gadd153* promoter construct by various thiols. LLC-PK1 cells which had stably integrated the *gadd*-CAT reporter construct were treated for 2 h with various thiols at a concentration of 10 mM and allowed to recover for 24 h in DMEM containing 10% fetal bovine serum. Chloramphenicol acetyl transferase activity was determined as described under Procedures. The numbers above the bars are the fold induction relative to untreated control cells. The data are a summary of two experiments ($n = 2$).

Price et al 1992; Li et al 1994; Roy and Lee 1995; Wang et al 1996). Although it is not entirely clear how events in the endoplasmic reticulum might be linked to *gadd153* activation, induction of *grp78* by unfolded proteins and/or calcium depletion requires serine-threonine kinases (Price et al 1992; Cox et al 1993; Mori et al 1993; Cao et al 1995). Likewise, both calcium depletion and DTT activate eIF2α kinase, a cytoplasmic serine-threonine kinase activated by disruption of the endoplasmic reticulum (Prostko et al 1992; Brostrom et al 1995). The

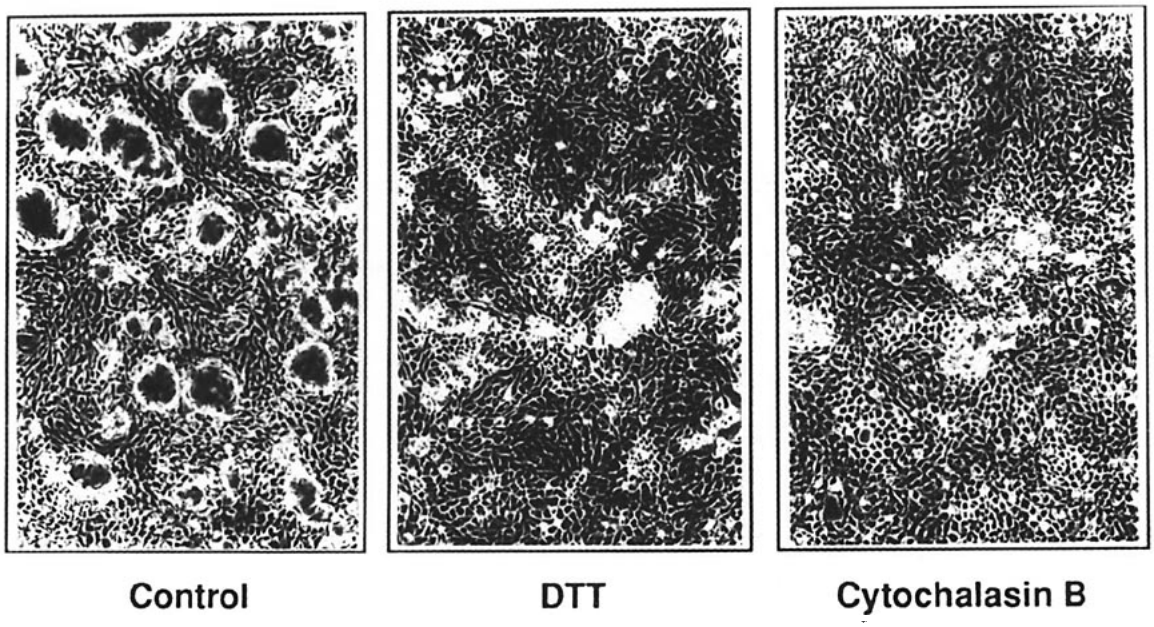

Fig. 6 Treatment of cells with dithiothreitol causes dome collapse in LLC-PK1 cells. Representative phase contrast micrographs of LLC-PK1 cells were taken using a 10X objective. Domes are apparent in cells treated with Earles balanced salt solution for 4 h, but not in cells which had been treated for the same amount of time in Earles containing 10 mM DTT or cytochalasin B. The domes were apparent again within 24 h after removal of DTT or cytochalasin B when cells were maintained in DMEM with 10% fetal bovine serum.

signals that activate *gadd153* are less clear, but serine-threonine kinase inhibitors blocks activation while okadaic acid, a phosphatase inhibitor, enhances *gadd153* mRNA accumulation (Price and Calderwood 1992; Luethy and Holbrook 1994). Thus, there is likely to be overlap in the signaling pathways that lead to increased *gadd153* and *grp78* transcription.

DNA binding proteins which interact with enhancer elements in the promoters of both the *grp78* and *gadd153* genes are the likely targets of these upstream stress-activated signaling pathways. The hamster *gadd153* promoter is complex and contains an AP-1 binding element, seven SP-1 sites, an inverted GCCAAT box, a C/EBP binding site, numerous IL-6 response elements and a consensus sequence for nuclear respiratory factor-1 (Luethy et al 1990; Virbasius et al 1993; Sylvester et al 1994). Although DNA-damaging agents increase binding to the *gadd153* AP-1 site, additional regions of the *gadd153* promoter are required for full activation (Luethy and Holbrook, 1994). Recent DNAse I foot printing data suggest that binding of C/EBPα and C/EBPβ to a C/EBP-like element increases during the acute phase response (Sylvester et al 1994). Thus, a combination of protein binding to the AP-1 and C/EBP sites may participate in induction of *gadd153* by various agents. Whether or not a similar mechanism is involved in activation of *gadd153* by DNA damage and thiol reductants remains to be determined. However, sequences outside the region of the promoter used in this study are necessary for full activation by growth arrest, glucose deprivation and Ca^{2+} ionophore treatment (Bartlett et al 1992; Carlson et al 1993), thus additional signals may well be involved.

The regions of the rat and yeast *grp78* promoters that confer basal and stress inducible activation have been identified (Wooden et al 1991; Liu et al 1993; Li et al 1994; Roy and Lee 1995). In mammals and yeast, an

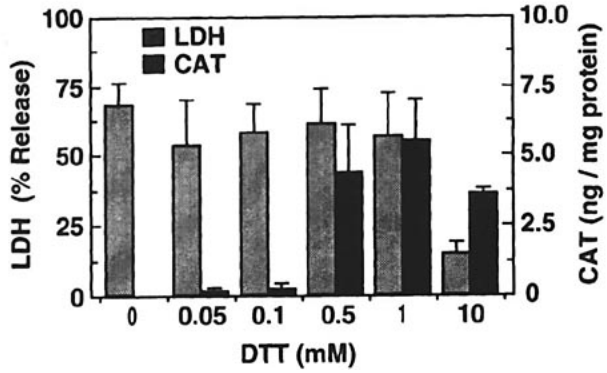

Fig. 7 The ability of dithiothreitol to protect against S-(1, 2-dichlorovinyl)-L-cysteine toxicity is not due to gene activation. PK1*gadd*CAT cells were treated with DCVC (0.5 mM) for 5 h, in the presence or absence of increasing concentrations of DTT and release of lactate dehydrogenase was determined (% LDH release). For comparison, the concentration dependence of activation of the *gadd*-CAT reporter in the PK1gaddCAT cells was determined in cells treated with DTT at various concentrations (CAT ng/mg protein). The data are a summary of two experiments ($n = 2$).

unfolded protein response element (UPR) or *grp* core sequence is necessary for transcriptional activation of the *grp78* gene (Kohno et al 1993; Li et al 1994). The binding of a 70 kDa protein, p70CORE, and CBF to the *grp* core and a proximal CCAAT box, respectively, are necessary for transcriptional activation of the rat *grp78* gene by calcium stress (Li et al 1994; Roy and Lee 1995). Although activation of these elements by thiols has not been reported, they appear to be involved generally in *grp78* activation since the same region of the promoter is required for *grp78* activation by calcium stress, brefeldin A and unfolded proteins (Wooden et al 1991; Liu et al 1993; Li et al 1994; Roy and Lee 1995). There is no evidence for participation of either C/EBP or AP-1 DNA binding proteins (Roy and Lee 1995), thus the available information suggests that the convergence in the signaling pathway for activation of *grp78* and *gadd153* occurs upstream of protein binding to the respective promoters.

Disruption of the endoplasmic reticulum by DTT leads to inhibition of protein synthesis (Brostrom et al 1995), an effect which might also be linked to the signaling pathway for activation of the *grp78* (Brostrom et al 1995) or *gadd153* genes. However, since cycloheximide does not induce *gadd153* or *grp78* in LLC-PK1 cells (Chen et al 1992a; M. M. Halleck, unpublished results), inhibition of protein synthesis per se is not responsible for transcriptional activation of either gene by thiols. On the other hand, considerable evidence suggests that inhibition of protein processing perturbs calcium homeostasis in the endoplasmic reticulum and calcium ionophores are potent activators of both *grp78* and *gadd153* transcription (Bartlett et al 1992; Brostrom et al 1995; Roy and Lee 1995). Thus disruption of protein synthesis, protein processing and calcium homeostasis in the endoplasmic reticulum could all be linked to transcriptional activation of *gadd153* and *grp78* by DTT.

Although the data suggest that there are common signals which activate *grp78* and *gadd153* in response to reductive stress, this is not to say that all conditions which activate one of the genes will activate the other. For example, in LLC-PK1 cells, t-butylhydroperoxide is a potent inducer of *gadd153* but not *grp78* (Liu and Stevens, unpublished results). Moreover, induction of *grp78* and *gadd153* shows differential sensitivity to cycloheximide and tyrosine kinase inhibition in NIH-3T3 cells (Price and Calderwood 1992). Thus, *grp78* and *gadd153* activation can be separated in some cells and with some inducers. However, the data with LLC-PK1 cells suggest that *gadd153* and *grp78* induction are tightly linked and constitute part of a genomic response to reductive insults. Further work will be necessary to elucidate the respective role of the two genes in the physiological response to reductive stress.

ACKNOWLEDGMENTS

These studies were supported by PHS grants DK46267 and DK38965 to JLS and a National Research Services Award (ES05569) to MH. The authors thank Dr Amy Lee for discussing unpublished data and for helpful comments.

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