

## **Thioredoxin: a redox-regulating cellular cofactor for glucocorticoid hormone action. Cross talk between endocrine control of stress response and cellular antioxidant defense system.**

Y Makino, ... , I Makino, H Tanaka

*J Clin Invest.* 1996;**98**(11):2469-2477. <https://doi.org/10.1172/JCI119065>.

### Research Article

Adaptation to stress evokes a variety of biological responses, including activation of the hypothalamic-pituitary-adrenal (HPA) axis and synthesis of a panel of stress-response proteins at cellular levels: for example, expression of thioredoxin (TRX) is significantly induced under oxidative conditions. Glucocorticoids, as a peripheral effector of the HPA axis, exert their actions via interaction with a ligand-inducible transcription factor glucocorticoid receptor (GR). However, how these stress responses coordinately regulate cellular metabolism is still unknown. In this study, we demonstrated that either antisense TRX expression or cellular treatment with H<sub>2</sub>O<sub>2</sub> negatively modulates GR function and decreases glucocorticoid-inducible gene expression. Impaired cellular response to glucocorticoids is rescued by overexpression of TRX, most possibly through the functional replenishment of the GR. Moreover, not only the ligand binding domain but the DNA binding domain of the GR is also suggested to be a direct target of TRX. Together, we here present evidence showing that cellular glucocorticoid responsiveness is coordinately modulated by redox state and TRX level and propose that cross talk between neuroendocrine control of stress responses and cellular antioxidant systems may be essential for mammalian adaptation processes.

**Find the latest version:**

<https://jci.me/119065/pdf>



# Thioredoxin: a Redox-regulating Cellular Cofactor for Glucocorticoid Hormone Action

## Cross Talk between Endocrine Control of Stress Response and Cellular Antioxidant Defense System

Yuichi Makino,\* Kensaku Okamoto,\* Noritada Yoshikawa,\* Masaru Aoshima,\* Kiichi Hirota,† Junji Yodoi,‡  
Kazuhiko Umesono,§ Isao Makino,\* and Hirotohi Tanaka\*

\*Second Department of Internal Medicine, Asahikawa Medical College, Asahikawa 078, Japan; †Department of Biological Responses, Institute for Virus Research, Kyoto University, Shogoin-Kawaracho, Kyoto 660, Japan; and ‡Department of Bioscience, Graduate School of Science, Nara Institute of Science and Technology, Ikoma, Nara 630-01, Japan

### Abstract

Adaptation to stress evokes a variety of biological responses, including activation of the hypothalamic-pituitary-adrenal (HPA) axis and synthesis of a panel of stress-response proteins at cellular levels: for example, expression of thioredoxin (TRX) is significantly induced under oxidative conditions. Glucocorticoids, as a peripheral effector of the HPA axis, exert their actions via interaction with a ligand-inducible transcription factor glucocorticoid receptor (GR). However, how these stress responses coordinately regulate cellular metabolism is still unknown. In this study, we demonstrated that either antisense TRX expression or cellular treatment with H<sub>2</sub>O<sub>2</sub> negatively modulates GR function and decreases glucocorticoid-inducible gene expression. Impaired cellular response to glucocorticoids is rescued by overexpression of TRX, most possibly through the functional replenishment of the GR. Moreover, not only the ligand binding domain but the DNA binding domain of the GR is also suggested to be a direct target of TRX.

Together, we here present evidence showing that cellular glucocorticoid responsiveness is coordinately modulated by redox state and TRX level and propose that cross talk between neuroendocrine control of stress responses and cellular antioxidant systems may be essential for mammalian adaptation processes. (*J. Clin. Invest.* 1996. 98:2469–2477.)  
Key words: redox • oxidative stress • glucocorticoids • gene expression • thioredoxin

### Introduction

The mammalian stress response evokes a series of neuroendocrine responses that activate the hypothalamic-pituitary-adrenal (HPA)<sup>1</sup> axis and the sympathetic nervous system. Coordinated interactions between stress response systems, occurring at multiple levels including the brain, pituitary gland, adrenal

gland, and peripheral tissues, are required for the maintenance of homeostatic plateau (1).

Glucocorticoids, as a major peripheral effector of the HPA axis, play an essential role in reestablishing homeostatic status in every peripheral tissue in human. However, molecular mechanism for glucocorticoid-mediated attenuation of cellular stress is largely unknown (2). On the other hand, the adaptive responses also operate on various intrinsic or extrinsic forces which disturb cellular homeostasis as a part of local host-defense mechanisms at a cellular level (3). Currently, reduction/oxidation (redox) reactions are intimately involved in the control of biological processes including modulation of the function of transcription factors, e.g., AP-1 and NF- $\kappa$ B (4–9). Reactive oxygen intermediates (ROIs) are known to be generated as a consequence of respiratory chain reaction and as a result of stimulation by cytokines (e.g., TNF- $\alpha$ ) and they act as a significant stressor to cells (10–12). Cells contain endogenous buffering systems against excessive production of ROIs to preserve cellular metabolism through the expression and regulation of many enzymes (3, 4). However, it is still unknown whether the peripheral adaptive responses can be specific to each stressor or if they can be generalized and nonspecific. Moreover, how these diverse stress response systems, i.e., systemic/central and peripheral/cellular, converge and orchestrate remains to be elucidated.

Glucocorticoids, on binding to the glucocorticoid receptor (GR), promote the dissociation of heat shock proteins (HSPs), and the ligand-receptor complex translocates to the nucleus and then binds to palindromic DNA sequences, called glucocorticoid response elements (GREs) (13–15). After binding to DNA, the GR differentially regulates target gene expression to produce hormone action, interacting with or without other transcription factors and coactivators/corepressors (13–19). The GR has a modular structure mainly consisting of a central DNA binding domain (DBD), nuclear localization signals, a ligand binding domain (LBD), and several transcription activation functions (13, 14). The human GR contains 20 cysteine residues, concentrated in the central region spanning the DBD and LBD (14). The cysteine residues in each domain have

---

Address correspondence to Hirotohi Tanaka, M.D., Second Department of Internal Medicine, Asahikawa Medical College, Nishikagura-4-5-3, Asahikawa 078, Japan. Phone: 81-166-65-2111 x2454; FAX: 81-166-65-1182; E-mail: hirotnk@asahikawa-med.ac.jp

Received for publication 18 July 1996 and accepted in revised form 24 September 1996.

---

*J. Clin. Invest.*

© The American Society for Clinical Investigation, Inc.

0021-9738/96/12/2469/09 \$2.00

Volume 98, Number 11, December 1996, 2469–2477

---

1. *Abbreviations used in this paper:* ADF, adult T cell leukemia-derived factor; CHO, Chinese hamster ovary; DBD, DNA binding domain; ECL, enhanced chemiluminescence; EMSA, electrophoretic mobility shift assay; GR, glucocorticoid receptor; GRE, glucocorticoid response element; HPA, hypothalamic-pituitary-adrenal; HSP, heat shock protein; LBD, ligand binding domain; 2ME, 2-mercaptoethanol; NAC, *N*-acetyl-L-cysteine; PVDF, polyvinylidene fluoride; rADF, recombinant ADF; rGR DBD, recombinant DBD of the GR; ROI, reactive oxygen intermediate; TRX, thioredoxin.

been shown to be crucial for maintaining both structure and function of those domains (15, 20–22). For example, it has been shown already that conversion of sulfhydryls in the DBD to disulfides blocks GR binding to DNA cellulose (23), and that metal ions which have high affinity for thiols interfere with the DBD–DNA interaction (24).

An important constituent of cellular antioxidant buffering systems that controls the redox state of proteins is thioredoxin (TRX), a 13-kD protein that catalyzes thiol-disulfide exchange reactions (25, 26). In humans, TRX is discovered as adult T cell leukemia–derived factor (ADF) from human T lymphotropic virus type I–infected T cells (27). Aside from the activity as an autocrine growth factor for human T cell leukemia virus type I–infected T cells (28) and Epstein-Barr virus–transformed lymphocytes (29), numerous studies have shown the importance of ADF/TRX as a cellular reducing catalyst in human physiology (25, 26). The redox-active sulfhydryls of TRX are located in the highly conserved active-site sequence -Trp-Cys-Gly-Pro-Cys- (25). The pathway for the reduction of a protein disulfide by TRX entails nucleophilic attack by one of the active-site sulfhydryls to form a protein–protein disulfide followed by intramolecular displacement of the reduced target proteins with concomitant formation of oxidized TRX (25). Recently, TRX is considered to be involved in transcriptional processes: for example, NF- $\kappa$ B activation is inhibited, whereas AP-1 activity is induced by TRX (30, 31). Moreover, the GR in the isolated rat cytosol is shown to be stabilized and maintained in a reduced, ligand-binding form by TRX (32). However, the interplay between the GR and TRX in situ has not yet been documented.

In this study, we present evidence for the functional interaction between cellular oxidative stress, TRX, and GR *in situ* and indicate that cellular redox state and TRX levels are important determinants of cellular sensitivity to glucocorticoids. Thus, we propose that TRX systems may control homeostasis not only by sequestering ROIs (33), for example, but also by fine tuning of hormonal signals.

## Methods

**Cell culture.** CHO-pMTGR cells, which are derived from Chinese hamster ovary (CHO)-K1 cells, were engineered to express relatively high levels of human GR (300,000–500,000 molecules/cell) as described previously (34), and were kindly provided by Dr. S. Nilsson (Karo Bio, Huddinge, Sweden). The cells were maintained in Ham's F-12 medium (Gibco Laboratories, Grand Island, NY), pH 7.4, supplemented with antibiotics and 10% heat-inactivated FCS (Gibco Laboratories) in the presence of cadmium and zinc ions, 40  $\mu$ M each, and 1 d before experiments the medium was replaced with fresh Ham's F-12 medium, both antibiotics, and FCS. COS-7 and HeLa cells were obtained from RIKEN Cell Bank (Tsukuba Science City, Japan) and maintained in DME (Gibco Laboratories), pH 7.4, supplemented with 10% heat-inactivated FCS and antibiotics. In all experiments, serum steroids were stripped with dextran-coated charcoal, and cells were cultured in a humidified atmosphere at 37°C with 5% CO<sub>2</sub>.

**Reagents and antibodies.** Diamide, 2-mercaptoethanol (2ME), N-acetyl-L-cysteine (NAC), and dexamethasone were purchased from Sigma Co. (St. Louis, MO). Other chemicals were from Wako Pure Chemical (Osaka, Japan). Recombinant ADF (rADF) was produced according to the method described previously and kindly provided by Ajinomoto Co. Inc. (Basic Research Laboratory, Kawasaki, Japan) (35). Polyclonal antibody against carboxy-terminal sequence of ADF was prepared as described previously (28). Anti-human GR antibody

PA1-512 was obtained from Affinity Bioreagents (Neshanic Station, NJ). All enzymes were purchased from TaKaRa Syuzo (Kyoto, Japan).

**Plasmids.** The expression vectors for the wild-type and mutant GR, RShGR $\alpha$ , I550, and  $\Delta$ 9-385 were described elsewhere (36) and kindly supplied by Dr. R.M. Evans (Salk Institute, La Jolla, CA). The expression plasmid for TRX, pcDSR $\alpha$ ADF, was described previously (37). For the construction of the antisense TRX expression plasmid pASADF, pcDSR $\alpha$ ADF was digested with BamHI and HindIII, and the fragment containing a part of TRX coding region was isolated and then subcloned into the HindIII-BamHI site of the parent pcDNA3 plasmid (38). The inverse orientation of the insert DNA was verified by sequencing. The construction of the glucocorticoid-responsive reporter construct pGRE-Luc was described previously (39). In brief, the oligonucleotides containing the GRE tandem repeat (noncoding strand, 5'-CGGATCCAGAACAGGATGTTCTA-GCTACGGATCCAGAACAGGATGTTCTAGCTACG-3'), and a 122-bp fragment of the human immunodeficiency virus long terminal repeat (positions -9193 to -9071 from the transcription start site) was inserted into a pGVB luciferase expression vector (Toyo Ink, Inc., Tokyo, Japan). The  $\beta$ -galactosidase expression plasmid pCH110 (Pharmacia LKB Biotechnology Inc., Uppsala, Sweden) was used as an internal control for transfection efficiency.

**RNA isolation and Northern blot analysis.** Total RNA was isolated according to the method of acid guanidinium thiocyanate phenol chloroform extraction, and Northern blot analysis was done as described previously (39). In brief, 20  $\mu$ g of total RNA was separated on a 1% formaldehyde-agarose gel, and the fractionated RNA was immobilized on a charge-modified polyvinylidene fluoride (PVDF) membrane (Immobilon-N; Millipore, Tokyo, Japan) using a pressure blot apparatus (Stratagene, La Jolla, CA), and RNA was cross-linked by means of ultraviolet irradiation. ADF cDNA probe was described elsewhere (27) and labeled with [ $\alpha$ -<sup>32</sup>P]dCTP (3,000 Ci/mmol; Amersham International, Buckinghamshire, United Kingdom) using BeBEST™ labeling kit (TaKaRa Syuzo) according to the manufacturer's protocol. The membrane was prehybridized with a QuikHyb™ hybridization solution (Stratagene) for 15 min at 68°C, and was hybridized with 2  $\times$  10<sup>6</sup> cpm/ml of <sup>32</sup>P-labeled probes for 60 min at 68°C. After the membrane was washed for 15 min in 0.1% SDS with 2 $\times$  SSC (0.3 M NaCl, 0.03 M sodium citrate) at 25°C, and for an additional 30 min in 0.1% SDS with 0.1 $\times$  SSC at 60°C, radioactivity on the membrane was quantified with a phosphorimage analyzer (BAS2000; Fuji Photo Film, Tokyo, Japan). Autoradiography was performed usually at -80°C overnight using Amersham Hyperfilm (Amersham) and intensifying screens.

**Western immunoblot analysis.** Whole-cell extracts for immunoblot analysis of TRX and GR were prepared as described previously (39). In brief, cells were washed with ice-cold PBS three times and then were homogenized with a solubilizing solution containing 10 mM Tris-HCl, 10% glycerol, and 0.4 M NaCl. The homogenates were centrifuged at 100,000 g for 1 h at 4°C, and the resultant supernatants were used as whole-cell extracts. Protein samples were dissolved in SDS-sample buffer containing 50 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 2% 2ME, and 0.001% bromophenol blue, and fractionated on SDS-polyacrylamide gels. After electrophoresis, proteins were electrically transferred onto a PVDF membrane (Bio-Rad Laboratories, Richmond, CA) and probed with appropriate antibodies. Antigen–antibody complexes were detected using an enhanced chemiluminescence (ECL) Western blot detection kit (Amersham) according to the manufacturer's instructions. Films were processed to densitometric analysis using an imaging densitometer (model GS-700; Bio-Rad Laboratories).

**Transfection and luciferase assay.** Transient transfection was performed as described previously (24). Briefly, cells were plated on plastic culture dishes (IWAKI Glass, Funabashi, Japan) to 30–50% confluency, washed with PBS three times, and medium was replaced with Opti-MEM medium (Gibco Laboratories). Plasmid cocktail was mixed with Lipofectin reagent (Gibco Laboratories) and added to the

culture. Total amount of the plasmids was kept constant (20  $\mu\text{g}$ ) by adding carrier plasmids (pGEM3Z unless otherwise specified; Promega Corp., Madison, WI). After 12 h of incubation, the medium was replaced with fresh Ham's F-12 medium or DME supplemented with 2% dextran-coated charcoal-stripped FCS, and the cells were further cultured in the presence or absence of various ligands for 24 h. After normalization of transfection efficiency by  $\beta$ -galactosidase expression, luciferase enzyme activity was determined using a luminometer (Berthold GmbH & Co. KG, Bad Wildbad, Germany) essentially as described previously (40).

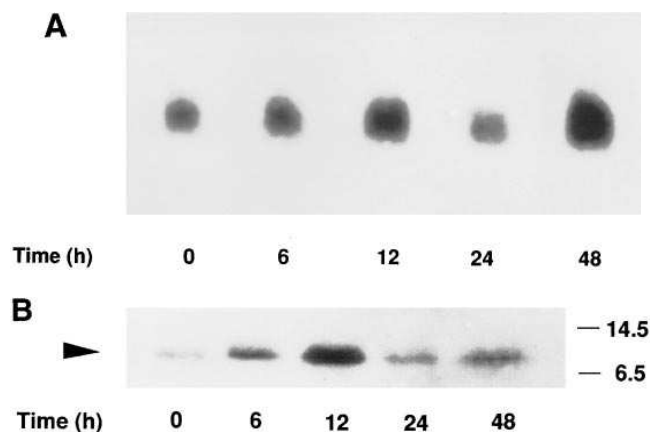
**Whole-cell hormone binding assay.** CHO $\mu$ MTGR cells were grown in 6-well flat-bottom plastic plates (IWAKI Glass) to confluence. After washing twice with PBS and once with Opti-MEM medium, the cells were incubated with 20 nM of [ $^3\text{H}$ ]dexamethasone (35–50 Ci/mmol; DuPont/New England Nuclear Research Products, Wilmington, DE) in the presence or absence of 500 M excess of radioinert dexamethasone in triplicate, for 1 h at 37°C. The monolayer was washed three times with ice-cold PBS, and the cells were dissolved in a solution consisting of 25 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate and 1% SDS. Aliquots were added to ACS II scintillation fluid (Amersham) to determine bound radioactivity. The difference between total and nonspecific binding gives the number of specific binding sites.

**Preparation of recombinant DNA binding domain of GR (rGR DBD).** For the construction of the bacterial expression vector for the DBD, the DNA fragment encoding 111 amino acids (398 Methionine - 508 Serine) of the human GR was amplified by PCR with appropriate flanking sequences for enzymatic cleavage and subcloned into pMal-2 expression plasmid (New England BioLabs, Inc., Boston, MA). Subsequent to bacterial expression of the protein using *Escherichia coli*, the rGR DBD was isolated according to the manufacturer's recommendations.

**Electrophoretic mobility shift assay (EMSA).** EMSA for rGR DBD was carried out as described previously (24). Briefly, the GRE probe was end-labeled with [ $\alpha$ - $^{32}\text{P}$ ]dCTP using the Klenow fragment of DNA polymerase I. The sequence of the oligonucleotide encompassing GRE is: 5'-CGAGTAGCTAGAACAGGATGTTCTGAGG-3' (upper strand sequence). rGR DBD (30 ng protein/reaction) was incubated with 0.2 ng of  $^{32}\text{P}$ -end-labeled GRE probe ( $\sim 20,000$  cpm) in a 10- $\mu\text{l}$  reaction mixture containing 10 mM Hepes, pH 7.5, 50 mM KCl, 0.1 mM EDTA, 2.5 mM  $\text{MgCl}_2$ , 0.25 mM dithiothreitol, 10% glycerol, and 50 ng poly (di-dC) (Pharmacia LKB) for 15 min on ice. Radioinert competitor DNA was included when indicated. The reaction mixture was then loaded onto a 5% nondenaturing polyacrylamide gel containing 0.25  $\times$  TBE (1  $\times$  TBE is composed of 89 mM Tris-base, 89 mM boric acid and 2 mM EDTA). The gels were run at 350 V for 2 h and dried. Results were visualized by autoradiography using Amersham Hyperfilm and intensifying screen. For quantitation, radioactivity of appropriate bands was counted using a BAS2000 phosphorimage analyzer.

## Results

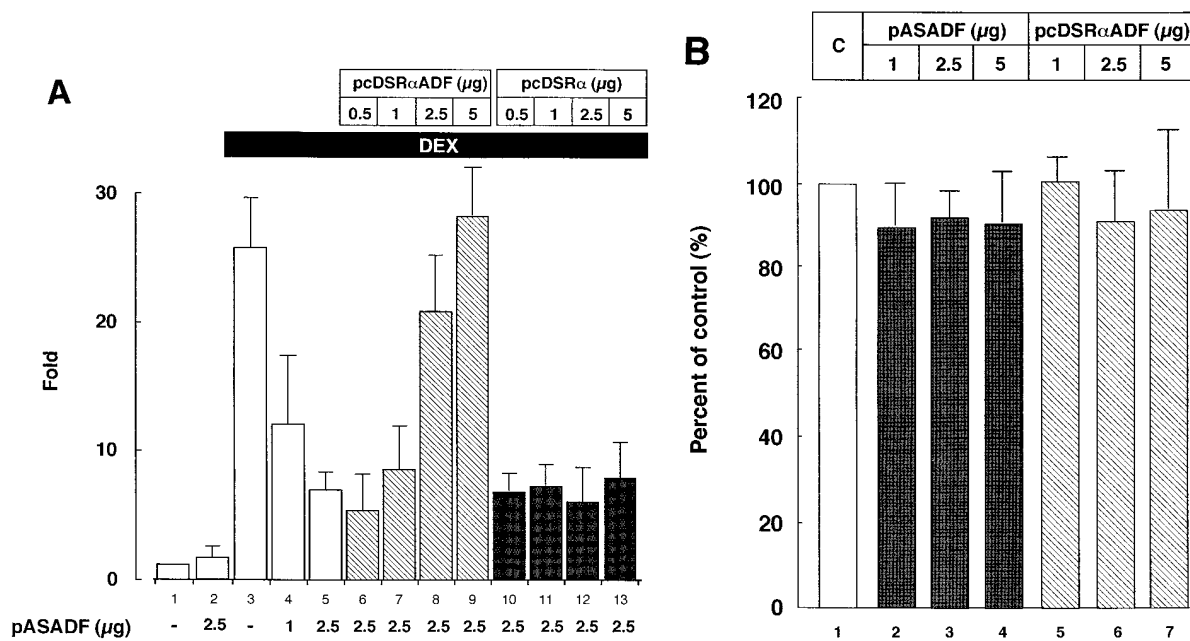
**Regulation of TRX expression under oxidative stress in HeLa cells.** Treatment of cells with  $\text{H}_2\text{O}_2$  triggers generation of ROIs and expression of a number of stress-responsive genes, so that cells are able to maintain metabolic equilibrium (3, 8, 31). Since, among others, TRX is considered to play a key role when cells are exposed to oxidative stress (25, 26), we first studied the influence of treatment with  $\text{H}_2\text{O}_2$  on both the steady state levels of mRNA and protein contents of TRX in HeLa cells. Addition of 1 mM  $\text{H}_2\text{O}_2$  to the culture medium did not cause significant cytotoxicity as assessed by trypan blue dye exclusion assay in this study (data not shown). After treatment of HeLa cells with 1 mM  $\text{H}_2\text{O}_2$ , total cellular RNA and protein were isolated at various time points as indicated (Fig.



**Figure 1.** Induction of TRX expression under oxidative stress. HeLa cells were treated with 1 mM  $\text{H}_2\text{O}_2$  for the indicated time periods and the induction of TRX expression was determined by Northern blot and Western immunoblot assays. (A) Northern blot. Total RNA was extracted, and samples of 20  $\mu\text{g}$  of RNA were separated in 1% formaldehyde-agarose gel and transferred to a PVDF filter membrane. The filter was hybridized with the cDNA probe for ADF/TRX as described in Methods. Autoradiography was performed overnight at  $-80^\circ\text{C}$ . Experiments were performed twice with identical results, and the representative autoradiogram is shown. For quantitation, a BAS2000 phosphorimage analyzer was used. (B) Immunoblot. Whole-cell extracts were prepared, and protein samples of 20  $\mu\text{g}$  were fractionated in a 15% SDS-polyacrylamide gel and transferred to a PVDF membrane. Immunoblot analysis was performed with antibody against ADF/TRX as described in Methods. For visualization, ECL system was used. Numbers depict molecular masses determined using the molecular mass marker run in parallel (not shown). Experiments were performed twice with identical results, and the representative ECL film is shown. For quantitation, the films were processed to densitometric analysis as described in Methods. The arrow denotes the position of ADF/TRX (molecular mass is 13 kD).

1). Northern blot experiments revealed that the steady state levels of TRX mRNA were significantly increased in a time-dependent fashion and peaked at 12 and 48 h (3.2- and 4-fold to control, respectively, Fig. 1 A). Western immunoblot experiments showed that TRX protein was also increased in a time-dependent manner and peaked at 12 and 48 h (4.5- and 2.6-fold to control, respectively, Fig. 1 B). These results indicate that HeLa cells may respond to oxidative stress, at least partly, via an increase in production of TRX, although critical target molecules of TRX are unknown.

**In vivo complementation assay for studying the intracellular interaction between TRX and glucocorticoid hormone action.** Since previous biochemical studies revealed the intimate association between TRX and the GR in vitro (32), we then asked whether TRX also interacts with the GR in situ to elucidate the interplay between peripheral limb of central stress response (i.e., glucocorticoids) and endogenous buffering system (i.e., TRX) in cells. For that purpose, we constructed the antisense TRX expression plasmid pASADF to manipulate cellular TRX levels, and cotransfected into HeLa cells with the GR expression plasmid RShGR $\alpha$ , and either the TRX expression plasmid pcDSR $\alpha$ ADF or its host plasmid pcDSR $\alpha$ , as indicated in Fig. 2 A. In the absence of a synthetic glucocorticoid dexamethasone, cellular luciferase activity was not significantly influenced by expression of antisense TRX (Fig. 2 A,



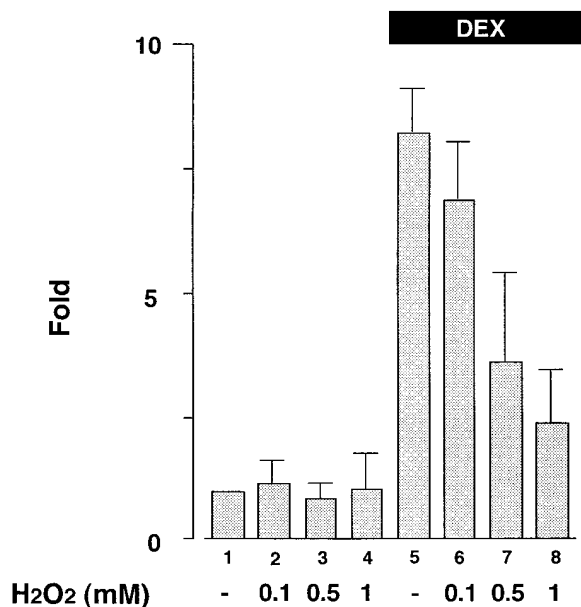
**Figure 2.** Regulation of glucocorticoid-mediated gene expression by cellular TRX levels. (A) HeLa cells were grown in 100-mm-diameter culture dishes and transfected with 10 ng of the GR expression plasmid RShGR $\alpha$ , 10  $\mu$ g of pGRE-Luc reporter plasmid, and various amounts of the antisense TRX expression plasmid pASADF, and the TRX expression plasmid pcDSR $\alpha$ ADF or its host vector pcDSR $\alpha$  as indicated. The cells were further incubated in the presence or absence of 100 nM dexamethasone (DEX) for 24 h, and cellular luciferase activity was determined as described in Methods. All results are expressed as fold induction compared with the cellular luciferase levels when the reporter and carrier plasmids PGEM3Z were transfected (column 1). Three independent experiments were performed and means $\pm$ SD of the results are shown. (B) HeLa cells were transfected with 1  $\mu$ g of a control plasmid for luciferase expression, PGV-control, and various amounts of either pASADF or pcDSR $\alpha$ ADF as indicated, and cultured for 24 h, then, cellular luciferase activity was determined as described in Methods. Expressed luciferase activity without cotransfection of either pASADF or pcDSR $\alpha$ ADF served as control (C, column 1), and results are shown as the percentage of control luciferase value. Three independent experiments were performed and means $\pm$ SD of the results are shown.

columns 1 and 2). In the presence of 100 nM dexamethasone, luciferase expression was induced by 26-fold, and antisense TRX expression repressed the hormone induction response in a dose-dependent fashion (Fig. 2 A, columns 3–5), indicating that expression of the antisense TRX selectively repressed glucocorticoid-inducible gene expression without affecting basal promoter activity. Next, to confirm the contribution of TRX to glucocorticoid-mediated gene expression *in vivo*, we cotransfected various amounts of pcDSR $\alpha$ ADF or its host plasmid pcDSR $\alpha$  with pASADF. As shown in Fig. 2 A, cotransfection of pcDSR $\alpha$ ADF reversed the negative effects of the antisense TRX and restored GR-mediated transcriptional activation in a dose-dependent manner (Fig. 2 A, columns 6–9). However, the antisense-mediated repression was not complemented by the transfection of pcDSR $\alpha$  (Fig. 2 A, columns 10–13). In separate experiments, HeLa cells were transfected with the luciferase expression plasmid driven by the strong SV40 enhancer, PGV-control, with either pASADF or pcDSR $\alpha$ ADF, and we confirmed that either antisense TRX expression or overexpression of TRX did not influence SV40 enhancer activity (Fig. 2 B). These results strongly suggest that cellular TRX level is one of the determinants of the transactivational function of the GR, and TRX acts as an endogenous auxiliary factor for the GR to augment hormone induction response through the GRE *in situ*.

*Effect of treatment with H<sub>2</sub>O<sub>2</sub> on glucocorticoid-mediated gene expression.* Next, effect of treatment with H<sub>2</sub>O<sub>2</sub> on glucocorticoid-inducible gene expression was examined. HeLa cells

were transfected with pGRE-Luc, and cultured in the absence or presence of H<sub>2</sub>O<sub>2</sub> and dexamethasone as indicated (Fig. 3). Treatment with 100 nM dexamethasone increased luciferase expression by eightfold, indicating that HeLa cells endogenously contain functional GR (Fig. 3, columns 1 and 5). Addition of H<sub>2</sub>O<sub>2</sub> into the culture medium decreased, without affecting basal promoter activity (columns 1–4), this hormone induction response in a concentration-dependent manner (columns 5–8). Since treatment of HeLa cells with H<sub>2</sub>O<sub>2</sub> increases expression of TRX (Fig. 1), depletion of TRX is not likely for the explanation of the reduction of glucocorticoid responsiveness after treatment with H<sub>2</sub>O<sub>2</sub>. In this line, we further examined the mechanism of H<sub>2</sub>O<sub>2</sub>-mediated repression of glucocorticoid hormone action.

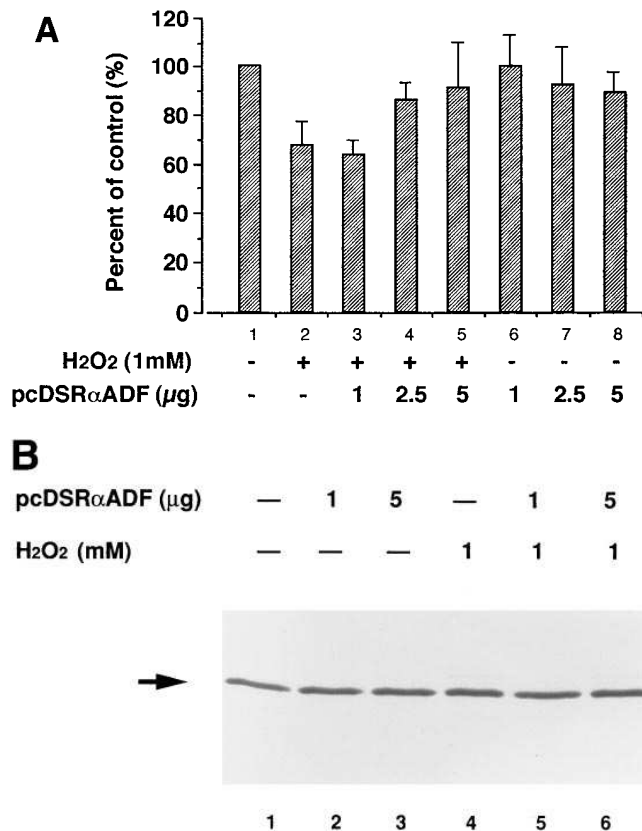
*Effect of treatment with H<sub>2</sub>O<sub>2</sub> and/or overexpression of TRX on the ligand binding activity of the GR.* To examine which function of the GR is affected by treatment with H<sub>2</sub>O<sub>2</sub> and TRX, GR-overexpressing CHO<sub>p</sub>MTGR cells, which afford sensitive measurement of GR function, were used (see Methods). CHO<sub>p</sub>MTGR cells were transfected with various amounts of TRX expression plasmid pcDSR $\alpha$ ADF and treated with 1 mM H<sub>2</sub>O<sub>2</sub> for 24 h, then cellular binding activity for tritiated dexamethasone was determined as described in Methods. As shown in Fig. 4 A, we found that the total number of binding sites for tritiated dexamethasone was decreased to ~65% of control after treatment with H<sub>2</sub>O<sub>2</sub> (Fig. 4 A, columns 1 and 2), and this suppression was rescued by overexpression of TRX in a dose-dependent manner (Fig. 4 A, columns 2–5). Overex-



**Figure 3.** Effect of treatment with H<sub>2</sub>O<sub>2</sub> on glucocorticoid-mediated gene expression. HeLa cells were transfected with 10 μg of the glucocorticoid-inducible reporter plasmid pGRE-Luc and cultured for 24 h in the presence of various concentrations of H<sub>2</sub>O<sub>2</sub> and 100 nM dexamethasone (DEX) as indicated, then, cellular luciferase activity was determined as described in Methods. Results are expressed as fold induction compared with the luciferase activity in the cells which were cultured in the absence of H<sub>2</sub>O<sub>2</sub> and DEX (column 1). Means ± SD of three independent experiments are shown.

pression of TRX did not significantly influence the ligand binding activity of the GR in the absence of H<sub>2</sub>O<sub>2</sub> (Fig. 4 A, columns 6–8). Western immunoblot analysis revealed that GR immunoreactivity apparently was not affected by either treatment with H<sub>2</sub>O<sub>2</sub> or transfection of TRX expression plasmid (Fig. 4 B), indicating that the decrease in the ligand binding activity of the GR is due not to the decrease in GR protein, but to functional alteration of the GR.

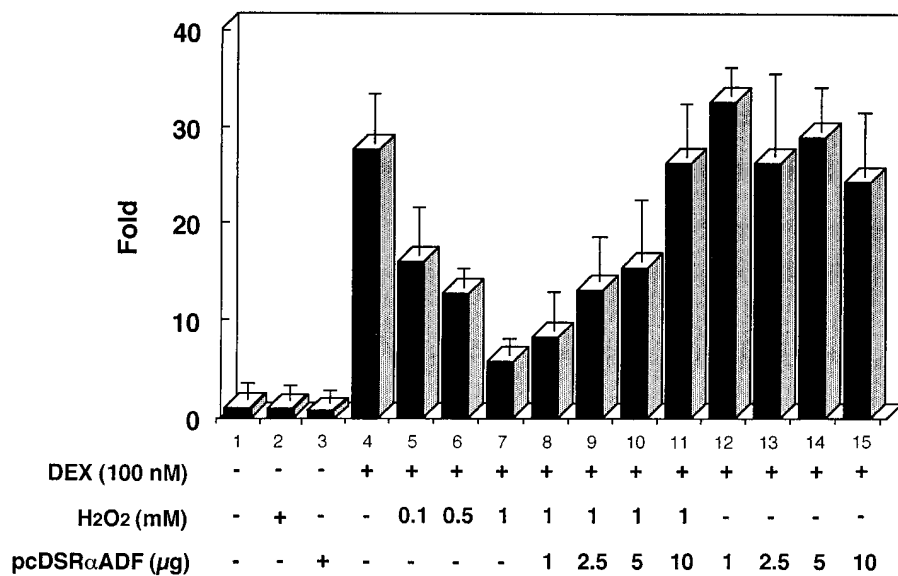
*Overexpression of TRX rescues the suppression of glucocorticoid-mediated gene expression by treatment with H<sub>2</sub>O<sub>2</sub>.* Next, we studied the effect of treatment with H<sub>2</sub>O<sub>2</sub> and/or overexpression of TRX on the glucocorticoid-mediated gene transcription using CHO<sub>p</sub>MTGR cells, in which TRX expression plasmid pcDSRαADF and the glucocorticoid-inducible reporter plasmid pGRE-Luc were transiently transfected as indicated (Fig. 5). Either treatment of the cells with 1 mM H<sub>2</sub>O<sub>2</sub> or overexpression of TRX did not affect basal level expression of luciferase (Fig. 5, columns 2 and 3). Addition of 100 nM dexamethasone resulted in an ~ 27-fold induction of the luciferase activity (Fig. 5, column 4), and treatment with H<sub>2</sub>O<sub>2</sub> suppressed the inductive effect of dexamethasone in a concentration-dependent manner (Fig. 5, columns 5–7). Treatment with 1 mM H<sub>2</sub>O<sub>2</sub> repressed the luciferase activity to 5.2-fold (Fig. 5, column 7). In the presence of H<sub>2</sub>O<sub>2</sub>, overexpression of TRX counteracted the H<sub>2</sub>O<sub>2</sub>-mediated repression of hormone-inducible gene expression in a dose-dependent manner (Fig. 5, columns 8–11). In the absence of H<sub>2</sub>O<sub>2</sub>, however, transient transfection of pcDSRαADF did not significantly affect the hormone induction response (Fig. 5, columns 12–15). Western immunoblot assay revealed that GR protein levels were not



**Figure 4.** Effect of treatment with H<sub>2</sub>O<sub>2</sub> and overexpression of TRX on the ligand binding activity of the GR in situ. CHO<sub>p</sub>MTGR cells were transfected with various amounts of the TRX expression plasmid pcDSRαADF and cultured for 24 h in the presence or absence of H<sub>2</sub>O<sub>2</sub> as indicated. (A) The ligand binding activity was determined by whole-cell assay as described in Methods. Results are expressed as the percentage of control (column 1) and means ± SD of three independently performed experiments are presented. (B) Immunoblot was performed to compare the protein content of the GR. CHO<sub>p</sub>MTGR cells were transfected with pcDSRαADF and cultured in the presence of H<sub>2</sub>O<sub>2</sub> as indicated for 24 h. Whole-cell extracts were prepared as described in Methods, and protein samples of 10 μg were fractionated in an 8% SDS-polyacrylamide gel, and transferred to a PVDF membrane. Immunoblot was performed with anti-human GR antibody as described in Methods. Relative molecular masses were determined by the marker run in parallel (not shown), and the arrow denotes the position of the GR (molecular mass is 94 kD).

significantly affected in these experiments (data not shown). Therefore, it is suggested that observed reduction in hormone-inducible gene expression after treatment with H<sub>2</sub>O<sub>2</sub> is due not to alteration in GR content, but to modification of GR protein in such a way that is complemented by TRX, for example.

The negative effect of treatment with H<sub>2</sub>O<sub>2</sub> appeared to be more prominent on the transactivation function of the GR when compared with those on the ligand binding activity (compare Figs. 4 A and 5), indicating that not only the LBD but the other domain(s) may be involved in H<sub>2</sub>O<sub>2</sub>-mediated repression of glucocorticoid-inducible gene expression. To address this issue, we cotransfected a panel of GR expression plasmids with pcDSRαADF and pGRE-Luc into COS-7 cells. RShGRα is a wild-type GR expression plasmid, I550 is a mutant GR-expression plasmid which is truncated at amino acid

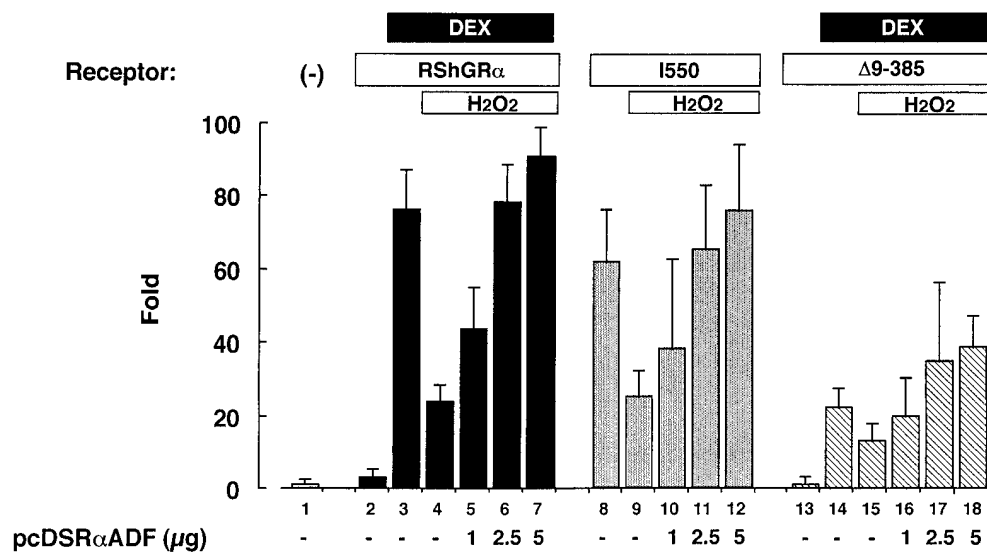


**Figure 5.** Repression of glucocorticoid-inducible gene activation by treatment with H<sub>2</sub>O<sub>2</sub> is restored by overexpression of TRX. CHOpmTGR cells were transfected with 10 μg of the glucocorticoid-responsive reporter plasmid pGRE-Luc and various amounts of the TRX expression plasmid pcDSRαADF, then cultured in the presence or absence of various concentrations of H<sub>2</sub>O<sub>2</sub> and 100 nM dexamethasone (DEX) for 24 h as indicated. Cellular luciferase activity was determined as described in Methods. Results are expressed as fold induction compared with the luciferase activity in the cells which were transfected with a carrier plasmid pGEM3Z and cultured in the absence of H<sub>2</sub>O<sub>2</sub> and DEX (column 1). Means ± SD of three independent experiments are shown.

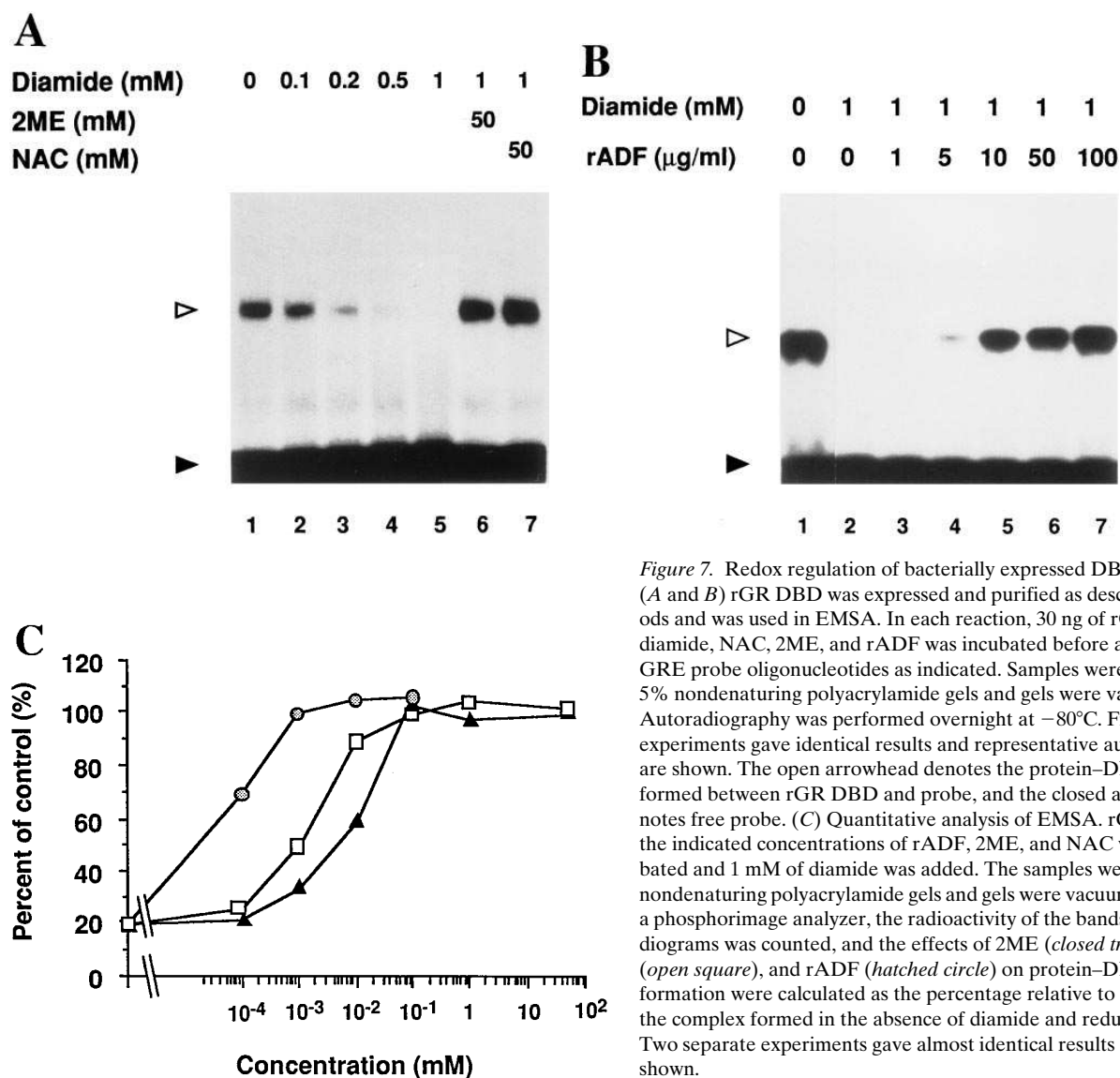
550 and lacks the LBD and is known to be a hormone-independent constitutive transcriptional activator, and Δ9-385 encodes another mutant GR containing an internal deletion from amino acids 9 to 385, which lacks τ1 transactivation domain and is known to be a weak ligand-dependent transcriptional activator (36). After transfection, the cells were cultured in the presence or absence of 100 nM dexamethasone and/or 1 mM H<sub>2</sub>O<sub>2</sub> as indicated (Fig. 6), and cellular luciferase activity was determined as described in Methods. When wild-type GR expression plasmid RShGRα was transfected, treatment with H<sub>2</sub>O<sub>2</sub> reduced hormone induction response of the luciferase expression 74- to 24-fold (Fig. 6, columns 2–4). Cotransfection of TRX expression plasmid restored dexamethasone-mediated luciferase expression in a dose-dependent fashion as in the case of CHOpmTGR cells (Fig. 6, columns 5–7, also see Fig. 5). When a ligand-independent transcriptional activator I550 was expressed, luciferase expression was strongly induced

even in the absence of dexamethasone, and treatment with H<sub>2</sub>O<sub>2</sub> decreased this induction to 40% (Fig. 6, columns 8 and 9). Overexpression of TRX counteracted the negative effect of H<sub>2</sub>O<sub>2</sub> and restored the reporter gene expression in a dose-dependent manner (Fig. 6, columns 10–12). When a ligand-inducible weak transcriptional activator Δ9-385 was cotransfected, the effects of H<sub>2</sub>O<sub>2</sub> and overexpression of TRX on the reporter gene expression were almost comparable with those when wild-type GR expression plasmid was cotransfected (Fig. 6, columns 13–18). Thus, it is indicated that H<sub>2</sub>O<sub>2</sub>-mediated repression and TRX-mediated augmentation of glucocorticoid-inducible gene expression are elicited not only by the LBD, but at least by the DBD as well.

**Effect of TRX on the DNA binding activity of the GR.** To examine direct interaction between the DBD of the GR and TRX in vitro, we prepared rGR DBD and performed EMSA. After incubation of protein samples with the thiol oxidizing re-



**Figure 6.** Domain requirement for redox regulation of the GR. COS-7 cells were transfected with 10 ng each of GR expression plasmids, RShGRα, I550, Δ9-385 (see text), 10 μg pGRE-Luc reporter plasmid, and various amounts of the TRX expression plasmid pcDSRαADF, and cultured in the presence or absence of 1 mM H<sub>2</sub>O<sub>2</sub> and 100 nM dexamethasone (DEX) for 24 h as indicated. Cellular luciferase activity was determined as described in Methods, and results are expressed as fold induction compared with the luciferase levels in the cells without either transfection of expression plasmids or treatment with reagents (column 1). Three independent experiments were performed and means ± SD of the results are shown.



**Figure 7.** Redox regulation of bacterially expressed DBD of the GR. (A and B) rGR DBD was expressed and purified as described in Methods and was used in EMSA. In each reaction, 30 ng of rGR DBD and diamide, NAC, 2ME, and rADF was incubated before addition of the GRE probe oligonucleotides as indicated. Samples were analyzed on 5% nondenaturing polyacrylamide gels and gels were vacuum-dried. Autoradiography was performed overnight at  $-80^{\circ}\text{C}$ . Five separate experiments gave identical results and representative autoradiograms are shown. The open arrowhead denotes the protein–DNA complex formed between rGR DBD and probe, and the closed arrowhead denotes free probe. (C) Quantitative analysis of EMSA. rGR DBD and the indicated concentrations of rADF, 2ME, and NAC were preincubated and 1 mM of diamide was added. The samples were run on 5% nondenaturing polyacrylamide gels and gels were vacuum-dried. Using a phosphorimage analyzer, the radioactivity of the bands of the autoradiograms was counted, and the effects of 2ME (closed triangle), NAC (open square), and rADF (hatched circle) on protein–DNA complex formation were calculated as the percentage relative to the amount of the complex formed in the absence of diamide and reducing agents. Two separate experiments gave almost identical results and means are shown.

agent diamide, DNA binding of rGR DBD was decreased in a concentration-dependent fashion (Fig. 7 A), which was restored by copresence of either 2ME or NAC (Fig. 7 A). rADF also squelched the negative effect of diamide on DNA binding of rGR DBD in a dose-dependent manner (Fig. 7 B). Quantitative analysis of the protective effects of those reducing reagents on the DNA binding activity of rGR DBD revealed that rADF is the most potent, and followed by NAC and 2ME. rADF is shown to be stronger than NAC and 2ME by  $\sim 200$  times, when the concentrations for 50% recovery are compared (Fig. 7 C). After the DNA binding activity was completely restored by 2ME or NAC, rADF did not cause an additional increase in the formation of rGR DBD–GRE complex (data not shown).

## Discussion

We here presented evidence showing that oxidative stress induces TRX expression, and glucocorticoid-inducible gene expression is coordinately modulated by cellular redox state and

TRX level in mammalian cells. Moreover, we also indicated that redox modulation of glucocorticoid hormone action is due to thiol-mediated modification of distinct domains of the GR in situ.

We showed that treatment of HeLa cells with  $\text{H}_2\text{O}_2$  induced TRX mRNA expression and increased TRX protein levels. Previously, we reported similar phenomena in keratinocytes and lymphoid cells (41), indicating that TRX is a ubiquitously inducible protein when cells are exposed to  $\text{H}_2\text{O}_2$ . Although TRX has been known to be involved in many cellular processes as a potent endogenous thiol-related reducing factor, critical target molecule of TRX in mammalian stress response remains largely unknown. Recently, it has been shown that expression of antisense TRX in human carcinoma cells results in reduction of cellular TRX levels and is associated with increased sensitivity to *cis*-diamminedichloroplatinum (II) and other superoxide-generating drugs, and  $\text{H}_2\text{O}_2$  (38). Therefore, it is suggested that TRX plays an important role in cellular defense against various oxidative stresses. In this study, we further demonstrated that, through transient expression of anti-



sense TRX and overexpression of TRX, cellular TRX levels are closely associated with glucocorticoid-mediated gene expression. Since cellular treatment with H<sub>2</sub>O<sub>2</sub> impairs glucocorticoid-inducible gene expression, we may speculate that the TRX system operates as an endogenous defense machinery for the GR, or glucocorticoid-mediated stress responses, against oxidative stress.

What is the molecular mechanism of TRX action on glucocorticoid-mediated gene regulation? Previous biochemical studies have revealed that TRX is required for generating the ligand-binding conformation of the GR. Antibody-mediated sequestration of either TRX or TRX reductase inhibited the ligand binding activity of the GR (32). We demonstrated that overexpression of TRX overcame the H<sub>2</sub>O<sub>2</sub>-mediated suppression of the ligand binding activity of the GR, thus, TRX may participate in the maintenance of the LBD function *in vivo* as well. On the other hand, the DBD of the GR involves cysteine cluster (see Introduction). Therefore, it is highly likely that redox manipulation affects DBD function independently from LBD function. In this line, using various GR expression plasmids, we showed clearly that not only the LBD but also the DBD is independently influenced by treatment with H<sub>2</sub>O<sub>2</sub> and/or overexpression of TRX (Fig. 6). Consistently, the DNA binding activity of the rGR DBD was decreased by the thiol-oxidizing reagent diamide and replenished by rADF *in vitro*. The reactivity of a thiol group is determined by its accessibility and the microenvironment within the protein, including adjacent amino acids (42, 43). Both the LBD and the DBD of the GR, when they are oxidized, may have such a structure that is sterically suitable for efficient access of TRX. On the other hand, a nuclear redox factor Ref-1 rather specifically associates with the transcription factor AP-1 (44), suggesting the presence of steric preference or specificity in the interaction between the transcription factors and cellular antioxidant proteins. Thiol groups with metal associations, like the constituent of zinc finger structures, are suggested to be the strongest nucleophiles in the cell (42, 43). The other members of the nuclear receptor superfamily, which have highly conserved zinc finger structures in the DBD (13, 45), thus, could communicate with TRX as well. Recently, the solution structure of a complex of NF- $\kappa$ B p50 subunit and TRX was documented and specificity requirements were suggested for TRX-catalyzed disulfide bond reduction of target proteins (46). Therefore, elucidation of a mixed disulfide intermediate on the reaction pathway between the GR and TRX would definitely develop our understanding of the redox regulation of the GR.

Thus, the GR (or, steroid hormone receptors) appears to be the only transcription factor that the separate domains of the molecule (i.e., LBD and DBD) are independently redox-regulated. The DBD of the GR is postulated to be sterically hindered in untransformed unliganded GR (21 and references therein). When cells are exposed to severe oxidative stress, ligand binding, which is believed to be the initial receptor function, may be rapidly repressed and unliganded GR may harbor in the cytoplasm even in the presence of the agonistic ligands (Okamoto, K., and H. Tanaka, manuscript in preparation). On the other hand, liganded GR, in which the DBD is sterically accessible to DNA, may also be functionally downregulated via oxidative modulation of the zinc finger structure. These phenomena appear to be rationale, for example, at the inflammatory tissues where cells are believed to be exposed to severe oxidative stress (5–7), since suppression of glucocorticoid ac-

tion may potentiate endogenous defense mechanisms and prevent premature termination of the cascade of inflammatory reactions for self defense. Increase in cellular TRX levels induced by oxidative stress, then, may restore the receptor activity and permit the GR to efficiently communicate with target genes. Resultant activation of antiinflammatory genes and/or repression of inflammatory genes may prevent overshoot of inflammation. This scenario would be relevant as one of the molecular mechanisms of general adaptation syndrome that was originally postulated by H. Selye (1, 2).

The harmonization of the HPA axis-mediated stress response and cellular defense system is first reported in restraint rats, in which adrenocorticotropin induced HSP70 expression in adrenal cortex (47). At the cellular level, heat shock and other metabolic stressors induce the synthesis of a set of HSPs, which interact with other cellular proteins and are considered to aid in the maintenance of cellular homeostasis. Indeed, restraint stress also induces HSP89, a rat homologue of human HSP90, which, as well as HSP70, is known to be associated with the GR in the cytoplasm (13, 14). Since HSP90 is believed to be essential for functional maintenance of the GR (48), restraint stress, through the induction of HSPs, may preserve tissue glucocorticoid sensitivity. On the other hand, we demonstrated here that increased TRX expression under oxidative stress may be important for conservation of glucocorticoid-mediated gene expression. Therefore, distinct stressors may, directly or indirectly, activate various cellular processes, some of which may converge onto eventual preservation of peripheral sensitivity to glucocorticoids.

In summary, we showed here that cellular glucocorticoid responsiveness is coordinately modulated by redox state and TRX level and we propose that cross talk between endogenous control of stress responses and cellular antioxidant defense systems may be essential for mammalian homeostatic regulation.

## Acknowledgments

We thank Dr. R.M. Evans (Salk Institute, CA) for plasmids and Dr. H. Handa (Tokyo Institute of Technology) and S. Hayashi (Saitama Cancer Center) for valuable comments.

This work was supported in part by Grant-in-Aids from the Ministry of Education, Science, Sports, and Culture of Japan, Japan Rheumatism Association, Japan Research Foundation for Clinical Pharmacology, and Akiyama Foundation.

## References

1. Selye, H. 1946. The general adaptation syndrome and the diseases of adaptation. *J. Clin. Endocrinol. Metab.* 6:117–230.
2. Munck, A., P.M. Guyre, and N.J. Holbrook. 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocr. Rev.* 5:25–44.
3. Yu, B.P. 1994. Cellular defenses against damage from reactive oxygen species. *Physiol. Rev.* 74:139–162.
4. Bauskin, A.R., I. Aikalay, and Y. Ben-Neriah. 1991. Redox regulation of a protein tyrosine kinase in the endoplasmic reticulum. *Cell.* 66:685–696.
5. Demple, B., and C.F. Amabile-Cuevas. 1991. Redox redux: the control of oxidative stress responses. *Cell.* 67:837–839.
6. Firth, J.D., B.L. Ebert, C.W. Pugh, and P.J. Ratcliffe. 1994. Oxygen-regulated control elements in the phosphoglycerate kinase and lactate dehydrogenase A genes: similarities with the erythropoietin 3' enhancer. *Proc. Natl. Acad. Sci. USA.* 91:6496–6500.
7. Devary, Y., R.A. Gottlieb, T. Smeal, and M. Karin. 1992. The mammalian ultraviolet is triggered by activation of Src tyrosine kinases. *Cell.* 71:1081–1091.
8. Schreck, R., P. Rieber, and P.A. Baeuerle. 1991. Reactive oxygen inter-

- mediates as apparently widely used messengers in the activation of the NF- $\kappa$ B transcription factor and HIV-1. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:2247–2258.
9. Abate, C., L. Patel, F.J. Rauscher III, and T. Curran. 1990. Redox regulation of Fos and Jun DNA-binding activity *in vitro*. *Science (Wash. DC)*. 249: 1157–1161.
  10. Klebanoff, S.J., M.A. Vadas, J.M. Harlan, L.H. Sparks, J.R. Gamble, J.M. Agosti, and A.M. Waltersdorf. 1986. Stimulation of neutrophils by tumor necrosis factor. *J. Immunol.* 136:4220–4225.
  11. Yoshie, O., T. Majima, and H. Saito. 1989. Membrane oxidative metabolism of human eosinophilic cell line EoL-1 in response to phorbol diester and formyl peptide: synergistic augmentation by interferon-gamma and tumor necrosis factor. *J. Leukocyte Biol.* 45:10–20.
  12. DeChatelet, L.R., P.S. Shirley, and R.B. Johnston. 1976. Effect of phorbol myristate acetate on the oxidative metabolism of human polymorphonuclear leukocytes. *Blood*. 47:545–554.
  13. Beato, M., P. Herrlich, and G. Schütz. 1995. Steroid receptors: many actors in search of a plot. *Cell*. 83:851–857.
  14. Evans, R.M. 1988. The steroid and thyroid hormone receptor superfamily. *Science (Wash. DC)*. 240:889–895.
  15. Glass, K.C. 1994. Differential recognition of target genes by nuclear receptor monomers, dimers, and heterodimers. *Mol. Endocrinol.* 15:391–407.
  16. Hörlein, A.J., A.M. Näär, T. Heinzel, J. Torchis, B. Gloss, R. Kurokawa, A. Ryan, Y. Kamei, M. Söderström, C.K. Glass, and M.G. Rosenfeld. 1995. Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature (Lond.)*. 377:397–404.
  17. Katzenellenbogen, J.A., B.W. O'Malley, and B.S. Katzenellenbogen. 1996. Tripartite steroid hormone receptor pharmacology: interaction with multiple effector sites as a basis for the cell- and promoter-specific action of these hormones. *Mol. Endocrinol.* 10:119–131.
  18. Onate, S.A., S.Y. Tsai, M.-J. Tsai, and B.W. O'Malley. 1995. Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science (Wash. DC)*. 270:1354–1357.
  19. Kamei, Y., L. Xu, T. Heinzel, J. Torchia, R. Kurokawa, B. Gloss, S.-C. Lin, R.A. Heyman, D.W. Rose, C.K. Glass, and M.G. Rosenfeld. 1996. A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell*. 85:403–414.
  20. Chakraborti, P.K., M.J. Garabedian, K.R. Yamamoto, and S.S. Simons, Jr. 1992. Role of cysteines 640, 656, and 661 in steroid binding to rat glucocorticoid. *J. Biol. Chem.* 267:11366–11373.
  21. Simons, S.S., Jr., and W.B. Pratt. 1995. Glucocorticoid receptor thiols and steroid-binding activity. *Methods Enzymol.* 251:406–422.
  22. Luisi, B.F., W.X. Xu, Z. Otwinowski, L.P. Freedman, K.R. Yamamoto, and P.B. Sigler. 1991. Crystallographic analysis of the interaction of the glucocorticoid receptor with DNA. *Nature (Lond.)*. 352:497–505.
  23. Bodwell, J.E., N.J. Holbrook, and A. Munck. 1984. Sulfhydryl-modifying reagents reversibly inhibit binding of glucocorticoid-receptor complexes to DNA-cellulose. *Biochemistry*. 23:1392–1398.
  24. Makino, Y., H. Tanaka, K. Dahlman-Wright, and I. Makino. 1996. Modulation of glucocorticoid-inducible gene expression by metal ions. *Mol. Pharmacol.* 49:612–620.
  25. Holmgren, A. 1995. Thioredoxin structure and mechanism: conformational changes on oxidation of the active-site sulfhydryls to a disulfide. *Structure (Lond.)*. 3:239–243.
  26. Holmgren, A. 1985. Thioredoxin. *Annu. Rev. Biochem.* 54:237–271.
  27. Tagaya, Y., Y. Maeda, A. Mitsui, N. Kondo, H. Matsui, J. Hamuro, N. Brown, K.-I. Arai, T. Yokota, H. Wakasugi, and J. Yodoi. 1989. ATL-derived factor (ADF), an IL-2 receptor/Tac inducer homologous to thioredoxin; possible involvement of dithiol-reduction in the IL-2 receptor induction. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:757–764.
  28. Tagaya, Y., M. Okada, K. Sugie, T. Kasahara, N. Kondo, J. Hamuro, K. Matsushima, C.A. Dinarello, and J. Yodoi. 1988. IL-2 receptor(p55)/Tac-inducing factor. Purification and characterization of adult T cell leukemia-derived factor. *J. Immunol.* 140:2614–2620.
  29. Wakasugi, N., Y. Tagaya, H. Wakasugi, A. Mitsui, M. Maeda, J. Yodoi, and T. Tursz. 1990. Adult T-cell leukemia-derived factor/thioredoxin, produced by both human T-lymphotrophic virus type I- and Epstein-Barr virus-transformed lymphocytes, acts as an autocrine growth factor and synergizes with interleukin 1 and interleukin 2. *Proc. Natl. Acad. Sci. USA*. 87:8282–8286.
  30. Schenk, H., M. Klein, W. Erdbrugger, W. Droge, and K. Schulze-Osthoff. 1994. Distinct effects of thioredoxin and antioxidants on the activation of transcription factors NF- $\kappa$ B and AP-1. *Proc. Natl. Acad. Sci. USA*. 91:1672–1676.
  31. Meyer, M., R. Schreck, and P.A. Baeuerle. 1993. H<sub>2</sub>O<sub>2</sub> and antioxidants have opposite effects on activation of NF- $\kappa$ B and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor. *EMBO (Eur. Mol. Biol. Organ.) J.* 12: 2005–2015.
  32. Grippo, J.F., A. Holmgren, and W.B. Pratt. 1985. Proof that the endogenous, heat-stable glucocorticoid receptor-activating factor is thioredoxin. *J. Biol. Chem.* 260:93–97.
  33. Sasada, T., S. Iwata, N. Sato, Y. Kitaoka, K. Hirota, K. Nakamura, A. Nishiyama, Y. Taniguchi, A. Takabayashi, and J. Yodoi. 1996. Redox control of resistance to *cis*-diamminedichloroplatinum (II) (CDDP). Protective effect of human thioredoxin against CDDP-induced cytotoxicity. *J. Clin. Invest.* 97: 2268–2276.
  34. Alksnis, M., T. Barkhem, P.-E. Strömstedt, H. Ahola, E. Kutoh, J.-Å. Gustafsson, L. Poellinger, and S. Nilson. 1991. High level expression of functional full length and truncated glucocorticoid receptor in Chinese hamster ovary cells. *J. Biol. Chem.* 266:10078–10085.
  35. Tagaya, Y., H. Wakasugi, H. Masutani, H. Nakamura, S. Iwata, A. Mitsui, S. Fujii, N. Wakasugi, T. Tursz, and J. Yodoi. 1990. Role of ATL-derived factor (ADF) in the normal and abnormal cellular activation: involvement of dithiol related reduction. *Mol. Immunol.* 27:1279–1289.
  36. Rangarajan, P.N., K. Umehono, and R.M. Evans. 1992. Modulation of glucocorticoid receptor function by protein kinase A. *Mol. Endocrinol.* 6:1451–1457.
  37. Matthews, J.R., N. Wakasugi, J.-L. Virelizier, J. Yodoi, and R.T. Hay. 1992. Thioredoxin regulates the DNA binding activity of NF- $\kappa$ B by reduction of a disulphide bond involving cysteine 62. *Nucleic Acids Res.* 20:3821–3830.
  38. Yokomizo, A., M. Ono, H. Nanri, Y. Makino, T. Ohga, M. Wada, T. Okamoto, J. Yodoi, M. Kuwano, and K. Kohno. 1995. Cellular levels of thioredoxin associated with drug sensitivity to cisplatin, mitomycin C, doxorubicin, and etoposide. *Cancer Res.* 55:4293–4296.
  39. Makino, Y., H. Tanaka, and I. Makino. 1994. Paradoxical derepression of the collagenase gene expression by the anti-rheumatic gold compound aurothiomalate. *Mol. Pharmacol.* 46:1084–1089.
  40. Tanaka, H., Y. Makino, K.-D. Wright, J.-Å. Gustafsson, K. Okamoto, and I. Makino. 1995. Zinc ions antagonize the inhibitory effect of aurothiomalate on glucocorticoid receptor function at physiological concentrations. *Mol. Pharmacol.* 48:938–945.
  41. Sachi, Y., K. Hirota, H. Masutani, K. Toda, T. Okamoto, M. Takigawa, and J. Yodoi. 1995. Induction of ADF/TRX by oxidative stress in keratinocytes and lymphoid cells. *Immunol. Lett.* 44:189–193.
  42. Cappel, R.E., and H.F. Gilbert. 1988. Thiol/disulfide exchange between 3-hydroxy-3-methylglutaryl-CoA reductase and glutathione. *J. Biol. Chem.* 263: 12201–12212.
  43. Snyder, G.H., M.J. Cennerazzo, A.J. Karalis, and D. Field. 1981. Electrostatic influence of local cysteine environments on disulfide exchange kinetics. *Biochemistry*. 20:6509–6518.
  44. Xanthoudakis, S., G. Miao, F. Wang, Y.-C.E. Pan, and T. Curran. 1992. Redox-activation of Fos-Jun DNA binding activity is mediated by a DNA repair enzyme. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:3323–3335.
  45. Mangelsdorf, D.J., C. Thummel, M. Beato, P. Herrlich, G. Schütz, K. Umehono, B. Blumberg, P. Kastner, M. Mark, P. Chambon, and R.M. Evans. 1995. The nuclear receptor superfamily: the second decade. *Cell*. 83:835–839.
  46. Qin, J., G.M. Clore, W.M.P. Kennedy, J.R. Huth, and A.M. Gronenborn. 1995. Solution structure of human thioredoxin in a mixed disulfide intermediate complex with its target peptide from the transcription factor NF $\kappa$ B. *Structure (Lond.)*. 3:289–297.
  47. Blake, M.J., R. Udelsman, G.J. Feulner, D.D. Norton, and N.J. Holbrook. 1991. Stress-induced heat shock protein 70 expression in adrenal cortex: an adrenocorticotrophic hormone-sensitive, age-dependent response. *Proc. Natl. Acad. Sci. USA*. 88:9873–9877.
  48. Picard, D., B. Khursheed, M.J. Garabedian, M.G. Fortin, S. Lindquist, and K.R. Yamamoto. 1990. Reduced levels of hsp90 compromise steroid receptor action *in vivo*. *Nature (Lond.)*. 348:166–168.