Direct Association with Thioredoxin Allows Redox Regulation of
Glucocorticoid Receptor Function*

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Yuichi Makino, Noritada Yoshikawa, Kensaku Okamoto, Kiichi Hirota‡, Junji Yodoi§,
Isao Makino, and Hirotoshi Tanaka†

From the Second Department of Internal Medicine, Asahikawa Medical College, 4-5-3 Nishikagura, Asahikawa 078-8510, the Department of Anesthesiology, Kyoto University Hospital, Kyoto 606-8507, and the Department of Biological Responses, Institute for Virus Research, Kyoto University, Kyoto 606-8507, Japan

The glucocorticoid receptor (GR) is considered to belong to a class of transcription factors, the functions of which are exposed to redox regulation. We have recently demonstrated that thioredoxin (TRX), a cellular reducing catalyst, plays an important role in restoration of GR function in vivo under oxidative conditions. Although both the ligand binding domain and other domains of the GR have been suggested to be modulated by TRX, the molecular mechanism of the interaction is largely unknown. In the present study, we hypothesized that the DNA binding domain (DBD) of the GR, which is highly conserved among the nuclear receptors, is also responsible for communication with TRX in vivo. Mammalian two-hybrid assay and glutathione S-transferase pull-down assay revealed the direct association between TRX and the GR DBD. Moreover, analysis of subcellular localization of TRX and the chimeric protein harboring herpes simplex viral protein 16 transactivation domain and the GR DBD indicated that the interaction might take place in the nucleus under oxidative conditions. Together these observations indicate that TRX, via a direct association with the conserved DBD motif, may represent a key mediator in interplay between cellular redox signaling and nuclear receptor-mediated signal transduction.

Gene expression is regulated via interactions between factors, including DNA-binding proteins, coactivators/corepressors, histones, and DNA, and it allows fine tuning of essential cellular processes; e.g. proliferation, growth, differentiation, energy metabolism, and stress responses (1). Among others, redox regulation has now been considered to be one of the important determinants for activity of transcription factors and subsequent gene expression; DNA binding activity of a growing number of transcription factors, including AP-1 (2, 3), NFκB (4, 5), Sp1-6 (6), p53 (7), c-Myb (8), Egr-1 (9), PEBP2 (10), E2 (11), TTF-1 (12), and Ets (13), has been shown to be reduced by thioredoxin (TRX)1 is a small protein with a molecular mass of 13 kDa, and acts as a potent disulfide reductase for a variety of target proteins (14, 15). Recently, TRX has been shown to interact, directly or indirectly, with several transcription factors. For example, TRX facilitates the DNA binding and transcriptional activities of NFκB by reducing Cys62 in the DNA binding loop of p50 subunit (4, 16). TRX has also been suggested to participate in redox regulation of AP-1 via interaction with another reducing catalyst, redox factor-1 (Ref-1) (17), which reduces conserved cysteine residues within the DNA binding domains of Fos and Jun (18–20). TRX has thus been suggested as a candidate endogenous molecule operating in the redox-regulation of gene expression via modulation of many transcription factors.

The glucocorticoid receptor (GR) is a ligand-inducible transcription factor that belongs to the superfamily of the nuclear receptors, comprising a central DNA binding domain (DBD), nuclear localization signals (NLSs), a ligand binding domain (LBD), and several transactivation functions (21–23). After binding hormone and dissociation of heat shock proteins, the GR translocates into the nucleus, thereby communicating with the basal transcriptional machinery, coactivators, other transcription factors, and DNA and modulating target gene expression to produce pleiotropic glucocorticoid hormone actions (24–26). Numerous biochemical studies have demonstrated that GR function in vitro is subject to redox modulation, via reversible modification of functionally and structurally critical cysteine residues within the GR; oxidative treatment of the GR reduces both ligand binding activity (27, 28) and binding to DNA cellular (29, 30). We have previously demonstrated that metal ions that have high affinity for thiols interfere GR functions in living cells, plausibly via similar modification of cysteine thiols (31). Moreover, a recent study has shown that cellular redox state is an important determinant of GR function in vivo and that TRX is implicated in redox regulation of GR function; GR-mediated gene expression is suppressed by oxidative treatment of cells, which overexpression of TRX counteracts (32). Suggested mechanisms are 1) that TRX may preserve ligand binding activity of the GR, in accordance with previous biochemical observations showing that ligand binding activity of cytosolic GR is maintained by the presence of TRX systems (TRX and TRX reductase) (33, 34), and 2) that the nuclear translocation, DNA binding, and transactivation of the GR may also be influenced by TRX. The precise mechanisms of mole-

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¶ To whom correspondence should be addressed. Tel.: 81-166-68-2451; Fax: 81-166-68-2459; E-mail: hirotnk@asahikawa-med.ac.jp.

† The abbreviations used are: GR, glucocorticoid receptor; TRX, thioredoxin; DBD, DNA binding domain; GST, glutathione S-transferase; VP16, viral protein 16; Ref-1, redox factor-1; NLS, nuclear localization signal; LBD, ligand binding domain; GFP, green fluorescent protein; GRE, glucocorticoid response element; PBS, phosphate-buffered saline; NFκB, nuclear factor κB.
ular interplay between the GR and TRX, however, are not yet well understood.

To explore the molecular mechanism of redox regulation of GR function with particular reference to its interaction with TRX, we here report that the conserved DBD of the GR, independent of the LBD, is a target for redox regulation by TRX. Mechanistically, direct association between the DBD and TRX in the nucleus was shown by mammalian two-hybrid and in vitro protein-protein interaction assays. Thus, we suggest that TRX may play a critical role allowing cellular redox potential to modulate steroid hormone receptor-mediated gene expression.

**EXPERIMENTAL PROCEDURES**

**Cell Culture—**COS-7, CV-1, and HeLa cells were obtained from Riken Cell Bank (Tsukuba Science City, Japan) and maintained in Dulbecco's modified Eagle's medium (Life Technologies, Inc.), pH 7.0, supplemented with 10% heat-inactivated fetal calf serum (Cell Technologies, Inc.) and antibiotics. The human GR overexpressing (300,000–500,000 molecules/cell) Chinese hamster ovary-pMTGR cells (35), were kindly provided by Dr. S. Nilsson (Karlo Bio, Huddinge, Sweden) and maintained in Ham's F-12 medium (Life Technologies, Inc.) supplemented with antibiotics and 10% heat-inactivated fetal calf serum in the presence of cadmium and zinc ions, each at a concentration of 4 μM. In all experiments, serum was stripped with dextran-coated charcoal, and cells were cultured in a humidified atmosphere at 37 °C with 5% CO2.

**Reagents and Antibodies—**Diamide and dexamethasone were purchased from Sigma. Other chemicals were from Wako Pure Chemicals (Osaka, Japan). Recombinant TRX was produced according to the method described previously and kindly provided by Ajinomoto Co. Inc., Basic Research Laboratory (Kawasaki, Japan) (36). Monoclonal antibody against the carboxyl-terminal sequence of TRX was prepared as described previously (37). Anti-GST polyclonal antibody was obtained from Amersham Pharmacia Biotech. All enzymes were purchased from TaKaRa Syu zo (Kyoto, Japan).

**Plasmids—**The expression vectors for the wild-type and mutant GR, RSHGR and I550, respectively, have been described elsewhere (38) and were kindly supplied by Dr. R. M. Evans (Salk Institute, La Jolla, CA). The expression plasmids for TRX and antisense TRX, pcDSRαADF and pASADF, respectively, have also previously been described (32).

To construct expression plasmids for fusion protein VP16 transactivation domain and the DBD of the human GR with or without constitutive TRX, the DNA fragments encoding 129 amino acids (serine 403 to leucine 532) or 87 amino acids (serine 403 to alanine 490) of the human GR were amplified by polymerase chain reaction and ligated in frame into the expression plasmid pCMX-VP16 (39), resulting in pCMX-VP16-GR DBD NL1, the DNA fragments encoding 102 amino acids (serine 403 to alanine 504) or 87 amino acids (serine 403 to alanine 490) of the human GR were amplified by polymerase chain reaction with appropriate flanking sequences for enzymatic cleavage and inserted into the BamHI site of the parent pCMX-VP16 (39), resulting in pCMX-VP16-GR DBD and pCMX-VP16-GR DBD NL1, respectively. Construction of the expression plasmids for the fusion protein of the DNA binding domain of GAL4 (40) and TRX has been previously described (17). The expression plasmids for the green fluorescent protein (GFP)-fused chimeric protein, pCMX-GFP-VP16-GR DBD and pCMX-GFP-VP16-GR DBD NL1, were made by inserting a polymerase chain reaction-cloned DNA fragments encoding VP16-GR DBD and VP16-GR DBD NL1, respectively, into the pCMX-GFP vector (41). The glucocorticoid-responsive reporter construct pGRE-Luc (31) and the GAL4-responsive luciferase p5XGAL4X-Luc (17) were previously described. The β-galactosidase expression plasmid pCH110 (Amersham Pharmacia Biotech) was used as an internal control for transfection efficiency when appropriate.

**Partial Purification of GR—**Partially purified GR was prepared from Chinese hamster ovary-pMTGR whole cell extract essentially as described by Cairns et al. (42). Briefly, whole cell extracts were prepared in the presence of molybdate and chromatographed through a phosphocellulose column. The flow-through material was then applied to a DEAE-Sephrose column, and the absorbed material was eluted with 200 mM NaCl. Salt and molybdate were removed from the pooled, eluted material by chromatography on Sephadex G-25. After transformation (25 °C for 60 min), the receptor fraction was further purified by fast protein liquid anion-exchange Mono Q chromatography (Amersham Pharmacia Biotech). Fractions containing receptor were identified by ligand binding and specific DNA binding assays. These fractions contained 10–20% pure receptor and were used for protein-DNA interaction experiments.

**Electrophoretic Mobility Shift Assay—**Electrophoretic mobility shift assay was carried out as described previously (43). Briefly, partially purified GR (usually 10 ng of protein per reaction) was incubated with 0.2 ng of 32P-labeled GRE oligonucleotide (5′-CGAAGGTCTCATAGA- GACTGTTCTGAGG-3′) in a 20-μl reaction mixture containing 5 mM HEPES, pH 7.9, 70 mM KCl, 2.5 mM EDTA, 2.5 mM MgCl2, 10 mM spermidine, 0.25 mM dithiothreitol, 10% glycerol, and 100 ng of polyclonal antibody (Amersham Pharmacia Biotech) for 15 min on ice. The reaction was then loaded onto a 4% polyacrylamide gel containing 0.25% TBE (1× TBE is 89 mM Tris borate, 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.

**Immunocytochemical Analysis—**Cells grown on 8-chambered glass slides (Nikon, Japan) were fixed for immunostaining using a freshly prepared solution of 4% paraformaldehyde in phosphate-buffered saline (PBS) at 4 °C. Immunocytochemistry was carried out as described previously with minor modification (44). Briefly, after fixation, cells were washed five times with PBS at room temperature and incubated with anti-human TRX monoclonal mouse antibody at 1 μg/ml in PBS containing 0.1% Triton X-100 for 9 h at 4 °C. The cells were then washed again washed five times with PBS and incubated with fluorescein isothiocyanate-conjugated streptavidin at a dilution of 1:100 in PBS containing 0.1% Triton X-100 for 1 h at room temperature. The cells were then washed a final five times with PBS and mounted with GEL/MOUNT™ (Biomeda Co. Ltd., Foster City, CA) for examination by laser scanning microscope (Zeiss LSM 510, Carl Zeiss Jena GmbH, Jena, Germany).

**Transfection and Luciferase Assay—**Transient transfection was performed as described previously (31). Briefly, cells were plated on plastic culture dishes (IWK Glass, Funabashi, Japan) to 30–50% confluence and washed with PBS three times, and medium was replaced with Opti-MEM (Life Technologies, Inc.). Plasmid mixture was mixed with TransIT-LT1 transfection reagent (Pan Vera Corp., Madison, WI) and added to the culture. After 6 h of incubation, the medium was replaced with fresh Dulbecco’s modified Eagle’s medium supplemented with 2% dextran-coated charcoal-stripped fetal calf serum, and the cells further cultured in the presence or absence of various ligands for 24 h. After normalization of transfection efficiency by β-galactosidase expression, luciferase enzyme activity was determined in a luminometer (Berthold GmbH & Co. KG, Bad Wildbad, Germany) essentially as described before (32).

**Purification of GST Fusion Protein—**For the construction of the expression plasmid for GST-GR DBD fusion protein, the DNA fragment encoding the DNA binding domain (serine 403 to leucine 532) of the human GR was amplified by polymerase chain reaction and ligated in frame into the pEX2T-3 plasmid (Amersham Pharmacia Biotech). GST fusion protein was expressed in Escherichia coli BL21 (DE3) (Strategene, La Jolla, CA) by induction with 0.1 mM isopropyl-b-D-thiogalactopyranoside. The cell pellets were suspended in PBS containing 1 mM ZnCl2, 1% Triton X-100, and 5 mM dithiothreitol and subsequently sonicated. Lysates were centrifuged at 12,000 × g for 10 min at 4 °C, and supernatants were incubated with 200 μg of glutathione-Sepharose 4B beads (Amersham Pharmacia Biotech) at room temperature for 30 min. Beads were washed three times with PBS, and bound proteins were eluted with 1 μl of elution buffer (10 mM glutathione, 50 mM Tris, pH 8.0, 1 mM dithiothreitol). Eluted proteins were dialyzed against PBS containing 1 mM dithiothreitol before storage at −80 °C. The protein was characterized by Western blot analysis.

**RESULTS**

**Functional Interaction between the GR and TRX—**As described above, we hypothesized that TRX may preserve GR-de-
H2O2, the majority of the cells showed nuclear-predominant localization. Fig. 1—Effect of treatment with H2O2 and overexpression of TRX or antisense TRX on glucocorticoid-mediated gene expression. HeLa cells were transfected with 10 ng of the GR expression plasmid pRSHGRα, 5 μg of pGRE-Luc reporter plasmid, and various amounts of the TRX expression plasmid pCDSRexADF or antisense TRX expression plasmid pASADF as indicated. The cells were cultured in the presence or absence of 1 mM H2O2 and/or 100 nM dexamethasone (DEX) for 24 h, and cellular luciferase activity was determined as described under “Experimental Procedures.” 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 ± S.D. of the results are shown.

In the presence of H2O2, the repression effect of H2O2 was dose-dependently abolished (lanes 4–6, hatched columns). In contrast, reduction of cellular TRX levels, accomplished by expression of antisense TRX (32, 45), resulted in increased sensitivity to H2O2 and a further decrease in hormone induction (lanes 4–6, filled columns). This indicates that cellular TRX levels are an important determinant of glucocorticoid-mediated gene expression in oxidative conditions.

Subcellular Localization of TRX—We next studied the subcellular localization of TRX in HeLa cells using indirect immunofluorescent analysis. HeLa cells were cultured in the presence of 1 mM H2O2 for 2 h and then fixed for immuno-detection of TRX. In the absence of H2O2, TRX is mainly found in the cytoplasm, with some cells showing partial nuclear fluorescence (Fig. 2, left panel). However, in the presence of H2O2, the majority of the cells showed nuclear-predominant TRX fluorescence (right panel), indicating that TRX translocates into the nucleus under oxidative conditions.

DNA Binding Domain of the GR Is a Target of Redox Regulation—If TRX translocates into the nucleus under oxidative conditions, it is likely that TRX interacts with the GR in the nucleus to restore the transactivational function of the GR. The GR mutant I550 (which lacks the ligand binding domain, is constitutively present in the nucleus even in the absence of hormone (46), and acts as a ligand-independent transcriptional activator (47)) was shown to be sensitive to oxidative stress (32). Indeed, suppression of I550-mediated gene expression either by treatment with H2O2 (Fig. 3A) or antisense TRX expression (Fig. 3B) is similar to that seen for wild-type GR (32), further evidence for the nuclear location of the GR-TRX interaction.

Electrophoretic mobility shift assay using either partially purified full-length GR (Fig. 4) or the recombinant GR DBD (32) revealed that sequence-specific DNA binding activity of the GR is abolished by addition of the oxidative reagent diamide, and progressively restored by addition of recombinant TRX. Based on these results and the fact that the GR DBD contains several cysteine residues as a part of the zinc finger structures (22), we speculated that the DBD of the GR could be one of the targets of TRX in the nucleus. To further examine this possibility, we constructed expression plasmids for fusion proteins of the GR DBD with or without the constitutive NLS (NL1) plus the activation domain of the herpes simplex virus VP16 protein, VP16-GR DBD and VP16-GR DBDNL1, respectively (Fig. 5A). Note that transactivation domain of VP16 does not contain cysteine residues (48). When these chimeric proteins were expressed as a fusion protein with GFP in COS7 cells, VP16-GR DBD was shown to be constitutively localized in the nucleus, whereas VP16-GR DBDNL1 was exclusively cytoplasmic (Fig. 5B). Fig. 5C shows that VP16-GR DBD but not VP16-GR DBDNL1 acts as a GRE-specific constitutive transcriptional activator. Neither VP16-GR DBDNL1, nor a fusion protein of the VP16 activation domain plus GAL4 DBD (VP16-GAL4) transactivates the GRE-driven reporter plasmid (Fig. 5C).

VP16-GR DBD can thus be used in monitoring of the influence of TRX on the GR DBD and its interaction with GRE in the nucleus. To this end, VP16-GR DBD and TRX were coexpressed in COS7 cells, and the cells were cultured in the absence or presence of H2O2 as indicated in the legend for Fig. 5D. VP16-GR DBD induced reporter gene expression by 105-fold in the presence of H2O2 (Fig. 5D), indicating the presence of a productive interaction between the GR DBD and GRE. As expected, the transactivational function of VP16-GR DBD was lowered 15-fold after treatment with H2O2 (Fig. 5D) and restored by overexpression of TRX (Fig. 5D). On the other hand, when VP16-GR DBDNL1 was used, there were no effects of either H2O2 treatment or TRX overexpression (data not shown). These results strongly indicate that TRX, at least functionally, communicates with the GR DBD in the nucleus.

Demonstration of Physical Association between TRX and the GR DBD in the Nucleus—Because TRX has been shown to directly associate with target proteins in exerting its reducing function (17, 49), we postulated that TRX may physically associate with the GR DBD. This possibility was tested by using the mammalian two-hybrid assay, in which a cDNA of the GR DBD or TRX was subcloned downstream of the GAL4 DBD (harbor-
ing an NLS capable of taking fusion proteins to the nucleus (40)) or the transactivation domain of VP16 in frame. These plasmids were then cotransfected in CV-1 cells with a reporter plasmid driving luciferase gene expression under the control of GAL4 binding sites. When GAL4-TRX, which is constitutively in the nucleus (data not shown), was coexpressed with either VP16 or VP16-GR DBD NL1, luciferase activity was not induced (Fig. 6). In contrast, coexpression of increasing levels of GAL4-TRX and VP16-GR DBD significantly induced luciferase expression in a dose-dependent manner (Fig. 6). Because VP16-GR DBD constitutively localizes in the nucleus, communication between GR-mediated signal and TRX would appear to occur in the nucleus via physical association between the GR DBD and TRX.

Finally, we analyzed direct protein-protein interaction between the GR DBD and TRX using GST pull-down assay in combination with diamide cross-linking (17). For this purpose, a cDNA encoding the GR DBD was subcloned downstream of GST cDNA in frame, which resulted in GST-GR DBD. Recombinant TRX was mixed with either Sepharose, GST-bound Sepharose, or GST-GR DBD-bound Sepharose in the absence or presence of diamide, and the formation of the complex consisting of TRX and GST-GR DBD was analyzed by Western immunoblot assay as described under “Experimental Procedures.” When either Sepharose or GST-bound Sepharose was added, TRX was not detected (Fig. 7, lanes 2–5), indicating that neither Sepharose itself nor GST binds TRX. In contrast, when GST-GR DBD-bound Sepharose was added, modest levels of TRX were detected in the absence of diamide, and much higher levels in the presence of diamide (lanes 6 and 7), strongly suggesting direct protein-protein interaction between TRX and the GR DBD under oxidative conditions in vitro.

DISCUSSION

We here demonstrate that TRX directly interacts with the GR in the nucleus, allowing restoration of sequence-specific DNA binding and subsequent transcriptional activation of GR under oxidative conditions. In addition, we have shown that the DBD of the GR is the target of TRX.

Treatment of cells with H2O2 induced translocation of TRX from the cytoplasm to the nucleus. Although TRX has no authentic NLS and the mechanism of the translocation is to be elucidated, UV irradiation also results in accumulation of TRX into the nucleus in HSC-1 keratinocytes and HeLa cells (51). Similarly, in mouse Fe-NTA-induced renal tubular damage models, TRX has been largely recovered from nuclear fractions (52), suggesting that nuclear translocation of TRX may be a physiological cue in a variety of cellular stress responses and that nuclear TRX may play an important role in response to tissue damage, for example. TRX is also transported to the nucleus by treatment with phorbol 12-myristate 13-acetate, and it potentiates AP-1 transcriptional activity via a redox-de-
pendent interaction with Ref-1 (17). Nuclear accumulation of TRX might thus be an important process in the function of certain transcription factors and regulation of gene expression. In addition to the alteration of expression levels induced by variety forms of cellular stress (51), such differential subcellular localization in response to the environmental stimuli may constitute a mechanism for the pleiotropic action of TRX.

Posttranslational modification of GR has been shown to be an important determinant of the complexity to the response to glucocorticoid hormonal signals. Ligand binding or association with heat shock protein 90 has been suggested to be modulated by a variety of cellular factors or stimuli, e.g. immunophilins (53), tyrosine kinases (54), heat shock (55), metal ions (31), and geldanamycin (56). Redox manipulation of the ligand binding function via modification of critical thiols in the LBD of the GR by oxidizing reagents or reducing reagents, including TRX systems (TRX and TRX reductase), has been previously described (27, 28, 33, 34). In addition to these observations, we have previously shown that ligand-independent transactivation by cellular luciferase activity was determined as described under "Experimental Procedures." Expressed luciferase activities when the cells were transfected with each reporter plasmid alone served as controls (columns 1 and 6). Results are plotted as mean ± S.D. of three experiments.

![Graph](image-url)

**Fig. 5.** A, schematic presentation of fusion proteins VP16-GR DBD and VP16-GR DBDΔNL1. B, subcellular localization of GFP-VP16-GR DBD and GFP-VP16-GR DBDΔNL1. COS7 cells were transfected with 5 μg of GFP-VP16-GR DBD or GFP-VP16-GR DBDΔNL1 expression plasmids. After 24 h of transfection, subcellular localization of expressed chimeric proteins was examined by fluorescence microscopy. C, VP16-GR DBD activates gene expression through sequence-specific interaction with GRE in a ligand-independent manner. COS7 cells were grown in 60-mm-diameter culture dishes and transfected with 2 μg of reporter plasmids pGRE-Luc or tk-GALpx3-Luc and 100 ng of the expression plasmids for VP16-GR DBD, VP16-GR DBDΔNL1, VP16-GAL4, and VP16. The cells were harvested at 24 h after transfection, and cellular luciferase activity was determined as described under "Experimental Procedures." Expressed luciferase activities when the cells were transfected with each reporter plasmid alone served as controls (columns 1 and 6). Results are plotted as mean ± S.D. of three experiments.

![Graph](image-url)

**Fig. 6.** Physical association of the GR DBD and TRX in living cells. Mammalian two-hybrid assay in CV-1 cells. CV-1 cells were grown in 60-mm-diameter culture dishes and transfected with 100 ng of reporter plasmid tk-GALpx3-Luc and expression plasmids for either GAL4-TRX and for either VP16, VP16-GR DBD, or VP16-GR DBDΔNL1, as indicated. After further 24-h culture of the cells, luciferase assay was performed as described under "Experimental Procedures." The luciferase activity in the cells that were transfected with reporter plasmid and each bait are served as controls (column 1), and results are expressed as fold induction compared with the controls. Means ± S.D. of three independent transfections are shown.
the LBD-truncated mutant GR (termed i550, see Ref. 47) is also suppressed by oxidative treatment of cells and rescued by overexpression of TRX (32), suggesting that the LBD of the GR is not a unique domain conveying redox/TRX-mediated signals. The present study further confirmed this issue by functional analysis of a chimeric protein in which isolated GR DBD was fused with the heterologous transactivation function of VP16 (VP16-GR DBD); VP 16-GR DBD-mediated gene expression was shown to be markedly influenced by oxidative conditions and TRX, indicating that the DBD of the GR also mediates the effect of oxidative reagents and/or communicates with TRX, because transactivation domain of VP16 does not contain any cysteine residues (48). Consistently, the mammalian two-hybrid assay employing the GR DBD and TRX as a bait and GST pull-down assay demonstrated the direct protein-protein interaction between TRX and the GR DBD both in vivo and in vitro. Redox signals including TRX systems, therefore, are suggested to communicate with the GR at two levels, the LBD and DBD, which may contribute to fine tuning of receptor function and/or glucocorticoid hormonal signal reception.

In contrast with the LBD, the DBD is the most highly conserved region between members of the steroid hormone receptor family (21). TRX may thus interact with other members via the DBD as well. We have previously demonstrated that TRX also augments estrogen receptor function, which is negatively modulated under oxidative conditions (57), indicating that TRX may be a general factor that allows cross-talk between redox signal and steroid hormone actions. Recently, a small nuclear RING finger protein SNURF, which directly binds to the DBD of the androgen receptor and can coactivate receptor function, was identified (58). In addition to a panel of coactivators or corepressors for steroid receptors that have been shown to associate with the LBD or the transactivation domains (59, 60), DBD-associating proteins, such as TRX or SNURF, might play an important role in regulation of steroid receptor-mediated gene expression.

In addition to the steroid hormone receptors, the DBD structure is widely shared as a DNA binding motif by the other nuclear receptors (25). Some nuclear receptors are considered to be ligand-independent transcription factors, activity of which is modulated by posttranslational modification or cross-coupling with the other protein factors (25). Such ligand-independent receptors, compared with the receptors for known ligands, have been shown to be ancient in evolutionary terms (61) and thus have been suggested as potential regulators of development, differentiation, and other fundamental physiological processes. TRX, via its interaction with the conserved DBD, might play a role in regulation of such nuclear receptors or their target gene expression and thus influence those biological processes. Correspondingly, TRX is a ubiquitous protein and widely conserved from prokaryote to eukaryote (14), and targeted disruption of the mouse TRX gene causes early embryonic lethality (62).

Recent studies on the redox regulation of gene expression suggest that in some but not all cases, direct or indirect association between the catalysts of cellular reduction and the transcription factors is essential. For example, in addition to the present case of interaction between TRX and the GR, direct physical association of TRX and oligopeptides from the DNA binding loop of p50 subunit of NFκB has been demonstrated by nuclear magnetic resonance (49). Ref-1 has also been shown to be directly targeted by TRX (17), which in turn restores the DNA binding activity of transcription factor AP-1 (19, 20). Similarly, Ref-1 has been shown to augment DNA binding and transcriptional activities of p53 (7). Besides the transcription factors, direct interaction between apoptosis signal-regulating kinase and TRX has been suggested to be a possible mechanism for the redox-dependent regulation of apoptosis (63). Probably on structural grounds, there seems to be distinctions in the interplay between transcription factors and reducing catalysts; for example, whereas Ref-1 acts on the DNA binding activity of AP-1 but not on that of the GR, AP-1 is not itself a direct substrate of TRX (19). Therefore redox signals initially generated as broad intracellular reactive oxygen species might converge onto or be directed toward target molecules via specific interactions with reducing catalysts, such as TRX and Ref-1.

Elucidation of the coupling partner catalysts of the transcription factors, therefore, may be extremely important for understanding the mechanism of redox regulation of gene expression.

In the present study, we also showed that the GR can communicate with redox/TRX-mediated signals in the nucleus independent from a cytoplasmic interaction; the biological mean-
ing this two-compartment interaction between GR and TRX remains to be explored. In the case of the redox regulation of the NF-κB function, the action of TRX in the cytoplasm and in the nucleus has been suggested to be distinct, with TRX inhibiting prooxidant-mediated activation of NF-κB in the cytoplasm (64) and potentiating DNA binding in the nucleus (4). Moreover, some reducing factors vary in their subcellular localization; TRX and glutathione can translocate from the cytoplasm to the nucleus, whereas Ref-1 and nucleoredoxin are resident in the nucleus (19, 50). Consideration of redox regulation of the transcription factors with respect to cellular compartments, therefore, might be an important issue, especially in attempting to modulate transcription factor function in a redox-dependent manner.

In summary, we have shown that a direct interaction between the GR DBD and TRX in the nucleus, distinct from the GR LBD-TRX interaction in the cytoplasm, might participate in redox regulation of GR function. Such an interaction affects the complexity of redox-mediated modification of GR function and, moreover, may provide a possible model for the redox regulation of nuclear receptor function and target gene expression.

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REFERENCES

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