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Review

Redox Regulation of the Glucocorticoid Receptor

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ABSTRACT

Redox regulation is currently considered as a mode of signal transduction for coordinated regulation of a variety of cellular processes. The transcriptional regulation of gene expression is also influenced by cellular redox state, most possibly through the oxido-reductive modification of transcription factors. The glucocorticoid receptor belongs to a nuclear receptor superfamily and acts as a ligand-dependent transcription factor. We demonstrate that the glucocorticoid receptor function is regulated via redox-dependent mechanisms at multiple levels. Moreover, it is suggested that redox regulation of the receptor function is one of dynamic cellular responses to environmental stimuli and plays an important role in orchestrated crosstalk between central and peripheral stress responses. Antiox. Redox Signal. 1, 403–423.

MOLECULAR MECHANISM OF GLUCOCORTICOID HORMONE ACTION

Mammalian stress response and adrenal glucocorticoids

Glucocorticoids are secreted into the bloodstream from the adrenal glands in response to the activity of the hypothalamic-pituitary-adrenal axis, and indispensable for mammalian homeostatic regulation (Munck *et al.*, 1984). Indeed, targeted disruption of the glucocorticoid receptor (GR) gene results in serious maturation defects and early death after birth in mice (Cole *et al.*, 1995). On the other hand, glucocorticoids play an important role in immune regulation and have been widely used as a potent anti-inflammatory and immunosuppressive agent (Boumpas *et al.*, 1993; Cato and Wade, 1995).

In addition to central stress response, the adaptive responses are also operated against various intrinsic or extrinsic forces that disturb cellular homeostasis as a part of local host-defense mechanisms at a cellular level (Yu, 1994). It is still unknown whether the peripheral adaptive responses can be specific to each stressor, or 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. Holbrook *et al.* have shown that central stress induces hsp70 expression in rat adrenal cortex (Blake *et al.*, 1991), suggesting the presence of a communication between cen-

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tral stress response and peripheral adaptation mechanisms. However, it remains unknown how glucocorticoids interact with peripheral adaptation or cellular stress response systems.

Mechanism of glucocorticoid action

Glucocorticoids are believed to act via binding to ubiquitously distributed intracellular proteins, or GRs, which are ligand-dependent transcription factors and belong to the superfamily of the nuclear receptors (Evans, 1988; Beato *et al.*, 1995).

GR as a nuclear receptor: Nuclear receptors are still growing in number and more than 300 sequences have been reported. Many of them are important transcriptional regulators involved in widely diverse physiological functions such as control of embryonic development, cell differentiation, and metabolic homeostasis (Mangelsdorf *et al.*, 1995). Because of the complexity of the nomenclature of nuclear receptors, a unified nomenclature system has been formulated (Nuclear Receptor Nomenclature Committee, 1999). According to this system, GR, as NR3C1, belongs to the same subfamily with the receptors for mineral corticoid, androgen, progesterone, and estrogen (the member of this subfamily is sometimes classified as type I nuclear receptor). Concerning the human GR, two isoforms, GRα and GRβ, comprise 777 and 742 amino acids, respectively, and are splicing variants of the GR gene and differ at the carboxyl terminal (Hollenberg *et al.*, 1985). Although several investigators have indicated that GRβ acts as a dominant negative GR, others have presented contradictory results (Bamberger *et al.*, 1995, 1997; de Castro *et al.*, 1996; Oakley *et al.*, 1996, 1997; de Lange *et al.*, 1997; Hecht *et al.*, 1997; Leung *et al.*, 1997; Otto *et al.*, 1997; Hamid *et al.*, 1999; Shahidi *et al.*, 1999). The function of GRβ, therefore, should be further elucidated.

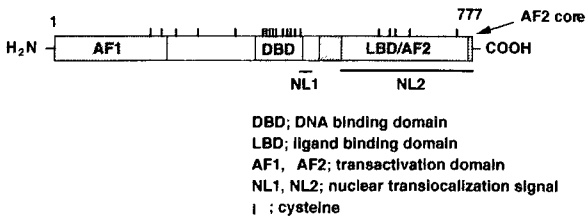

FIG. 1. Primary structure of the glucocorticoid receptor.

Structure and function of GR: Members of the nuclear receptor superfamily including the GR, share several structural features, *e.g.*, the ligand binding domain (LBD), DNA binding domain (DBD), and several transactivation domains (Fig. 1). The amino-terminal domain (AF-1) contains sequences responsible for activation of target genes and presumably interacts with components of the basal transcription machinery, and/or with cofactors and other transcription factors, largely in cell- or tissue-specific context. In the estrogen receptor (ER), this region is also known to be regulated by nonendocrine pathways, involving, for example, protein kinases that are often responsible for cell signaling (Kato *et al.*, 1995; Bunone *et al.*, 1996; Joel *et al.*, 1998a,b; Tremblay *et al.*, 1999; Rogatsky *et al.*, 1998). Recently, O'Malley's group has presented evidence showing that a particular RNA, termed "steroid receptor RNA activator" (SRA), selectively interacts with this AF-1 region and acts as a coactivator (Lanz *et al.*, 1999). Moreover, this region, as well as the LBD, is recently suggested to be targeted by a member of DRIP/TRAP cofactors. The central part of the receptor constitutes the DBD, which also participates in receptor dimelization, nuclear translocation, and transactivation. The structural motif of the DBD is two zinc fingers formed by the coordination of four cysteines to one zinc atom (Fig. 2). Site-directed mutagenesis demonstrated that the seven out of eight cysteines are absolutely required for receptor function (Severne *et al.*, 1988). The major grove of the DNA double helix has been shown to be a contact area (Scheidereit *et al.*, 1986). Particularly, the region spanning the carboxyl terminal of the first zinc finger, the P box, is considered to be involved in the specificity of the binding to DNA (Umesono and Evans, 1989). The second carboxy-terminal zinc finger is also required for DNA binding. Five amino acids at the amino terminal base of the second zinc finger comprise the D-box, which is involved in homodimerization of the GR by interacting with the equivalent part of the other DBD in a GR homodimer (Umesono and Evans, 1989). Adjacent to the second zinc finger, the amino acids responsible for the nuclear localization,

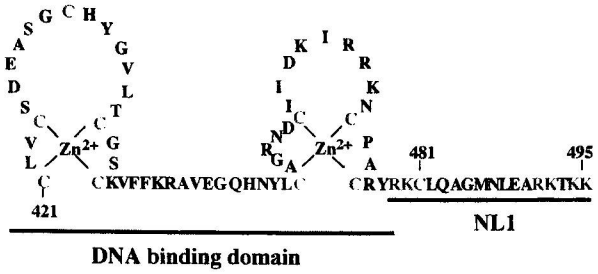

FIG. 2. Amino acid sequence of the DNA binding domain of the human GR.

the nuclear localization signal (NLS), exist (Picard and Yamamoto, 1987; Fig. 2). The carboxy-terminal portion of the receptor includes the sequences for specifically binding hormonal ligands, as well as those for heat shock protein binding, nuclear translocation, dimerization, and transactivation. The covalent affinity labeing of the GR by steroids led to the identification of the amino acid residues Met622, Cys656, and Cys754 of the rat GR (Carlstedt-Duke *et al.*, 1988). These amino acids are located in the hydrophobic segments within the LBD, indicating that ligand binds to the hydrophobic pocket-like structure (Carlstedt-Duke *et al.*, 1988). Recent crystallographic analysis of the LBD confirmed this hypothesis in, for example, the ER (Brzozowski *et al.*, 1997; Shiau *et al.*, 1998; Tanenbaum *et al.*, 1998; Wurtz *et al.*, 1998). The regions for the heat shock protein binding and the second nuclear localization signal overlap the LBD (Xu *et al.*, 1998). The carboxy-terminal transcriptional activation domain is hormone-dependent and termed AF-2. The very carboxy-terminal portion of the receptor, AF2-core, serves as a molecular switch that recruits coactivator proteins and activates the transcription of target genes when flipped into the active conformation by hormone binding (Feng *et al.*, 1998). The nuclear receptors contain distinct domains governing multiple functions, and their integration within the receptor may result in the exquisite specificity of the hormonal response.

GR-mediated transcriptional regulation: Glucocorticoids, as lipophilic substances, are believed to cross the cell membrane readily and interact with the GR. On binding a hormone, the GR dissociates heat shock proteins and translocates to the nucleus (Pratt, 1993; Fig. 3). From the point of view of signal transduction, the glucocorticoid signal that finally influences gene expression is transmitted to the nucleus via this nuclear translocation of the ligand-bound receptor. Therefore, nuclear import of the GR is one of the key control points in regulation of glucocorticoid hormone action. In general, protein transport from the cytoplasm to the nucleus involves NLS, *i.e.*, short peptide sequences that are necessary and sufficient for nuclear localization of their respective proteins (Nigg, 1997). One of the best-characterized NLS motifs is that of simian virus 40 large tumor antigen (SV40 T-Ag) (Nigg, 1997). Nuclear import of the GR is mediated by NL1, a stretch of basic amino acids at the immediate carboxy-terminal end of the receptor DNA binding domain, and a second significantly less characterized NLS in the ligand binding domain, NL2 (Picard and Yamamoto, 1987). Whereas the NLS of SV40 T-Ag consists of a short domain of basic amino acids, NL1 of the GR is a bipartite domain and confers constitutive nuclear localization of the receptor (Picard and Yamamoto, 1987; Fig. 2). In contrast, NL2 acts as a dominant negative NLS in the absence of ligands (Picard and Yamamoto, 1987). It is be-

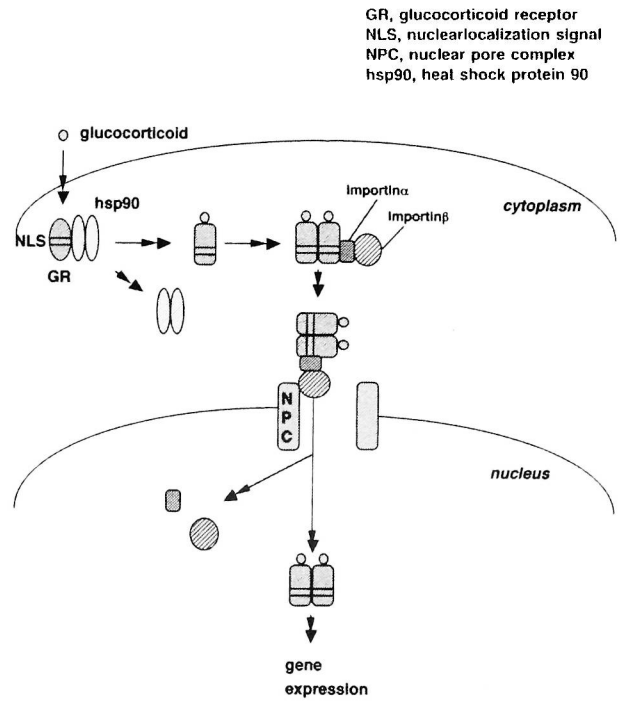

FIG. 3. Possible mechanism of nuclear translocation of the GR.

lieved that the GR shuttles between the cytoplasm and the nucleus and subcellular localization of the GR is determined by an equilibrium of both nuclear import and export. The GR translocates to the nucleus in a ligand- and energy-dependent manner, and nuclear export of the GR also requires ATP (Hsu *et al.*, 1992; Madan and DeFranco, 1993; Ogawa *et al.*, 1995; Carey *et al.*, 1996; Htun *et al.*, 1996; Sackey *et al.*, 1996; Tang and DeFranco, 1996; Yang and DeFranco, 1996, 1997; Tang *et al.*, 1997). Within the nucleus, the hormone-bound GR acts as a transcription factor binding to the palindromic DNA sequences, called glucocorticoid response elements (GREs), exclusively as homodimers (Evans, 1988; Glass, 1994; Beato *et al.*, 1995; Fig. 4). After binding to DNA, the GR is considered to communicate with basal transcription machinery, interacting with or without other transcription factors and coactivators, then regulates target gene expression (Evans, 1988; Beato *et al.*, 1995; Hörlein *et al.*, 1995; Mangelsdorf *et al.*, 1995; Onate *et al.*, 1995; Katzenellenbogen *et al.*, 1996; Fig. 4). A series of research for identification of proteins interacting with LBD converged on a family of related proteins that are collectively termed the p160 coactivators. They are represented by SRC-1/NCoA-1, TIF2/GRIP1/NCoA-2, and pCIP/ACTR/AIB1 (Ayer, 1999; Berk, 1999; Edwards, 1999; Xu *et al.*, 1999, and references therein). In addition to sequence homology, p160 proteins share an ability to stimulate ligand-dependent transactivation by a rather large number of nuclear receptors. A distinct structural feature of the p160 coactivators is the presence of multiple LXXLL signature motifs (also called LXDs, NR boxes, or NIDs), which comprise determinants for direct interactions with the nuclear receptor LBD (Xu *et al.*, 1999 and references therein). Although the amino acid context surrounding the LXXLL motif appears to influence the selectivity of the interaction, it is unclear at this point, what, if anything, influences the specificity of binding between nuclear receptor and p160 proteins. Recent studies for LBD crystal structures have established that upon ligand binding, the α-helix containing the AF2-core (helix 12) undergoes a major reorientation in the context of the overall LBD structure, forming part of a charged clamp that accommodates p160 coactivators within a hydrophobic cleft of the LBD; this occurs through direct contacts with the LXXLL motif (Xu *et al.*, 1999, and references therein). Of note, the estrogen antagonists tamoxifen and raloxifen appear to alter the position of AF-2 core such that helix 12 itself occupies the hydrophobic cleft in the LBD, thereby precluding coactivator binding (Brzozowski *et al.*, 1997; Shiau *et al.*, 1998). Insight into a potential mechanism of p160 coactivation came with the finding that SRC-1 is capable of interacting with the carboxy terminus of CBP/p300 and together they can coactivate transcription synergistically (Yao *et al.*, 1996). In addition, CBP/p300 itself interacts with nuclear receptors in a ligand-dependent manner, again through the AF-2 domain (Chakravarti *et al.*, 1996; Kamei *et al.*, 1996). CBP/p300 and p160 coactivators both possess intrinsic histone acetylase (HAT) activity and therefore may be acting in concert to remodel chromatin. Moreover, p/CAF, a mammalian homolog of the prototypical yeast HAT, GCN5, is a part of a 20 or so subunit complex containing TATA-box-associated factor (TAFs) and TAF-like proteins (Ogryzko *et al.*, 1996); it interacts with both CBP and some p160 coactivators, as well as directly with nuclear receptors (Chen *et al.*, 1997; Blanco *et al.*, 1998). Thus, one can imagine a growing HAT-containing, chromatin remodeling complex comprised of CBP/p300, p160, and p/CAF recruited to nuclear receptors to hormone-binding (Fig. 4). On the other

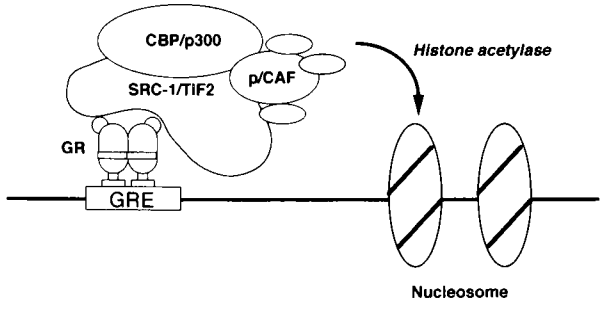

FIG. 4. Model of the GR-coactivator complex–DNA interactions. On binding ligands, the GR binds to the glucocorticoid response element (GRE) in the promoter region of target genes. p160 coactivators such as SRC-1, then anchor a complex comprised of CBP/p300 and p/CAF to the GR in a ligand-dependent manner. The net of these interactions would provide histone acetylase activity, resulting in remodeling of chromatin.

hand, corepressors have been shown to deacetylate histones in combination with other nuclear proteins such as Sim3. Kang *et al.* have reported that in the case of the GR, hsp90 behaves as a corepressor in the nucleus (Kang *et al.*, 1999). As well as coactivators and corepressors, another class of cofactors for nuclear receptors, termed DRIP/TRAP, have been isolated (Rachez *et al.*, 1998, 1999; Yuan *et al.*, 1998; Fondell *et al.*, 1999). Moreover, other chromatin remodeling machineries such as ATP-dependent chromatin remodeling complexes have been found; BAF, for example, is a mammalian homolog of yeast SWI/SNF (Wang *et al.*, 1996; Fryer and Archer, 1998; Zhao *et al.*, 1998). Thus, the fields of transcriptional regulation and chromatin structure and function have now merged, and regulation of gene expression by nuclear receptors is now being described in the context of chromatin. Indeed, disruption of interdomain and coactivator interactions are recently indicated in the pathophysiology of patients with oligospermic infertility associated with AR mutation (Ghadessy *et al.*, 1999).

In addition to ligand-dependent activation, extracellular signaling molecules, such as peptide hormones, growth factors, and cytokines, communicate with their intracellular targets through surface receptors, which activate signal transduction pathways that finally lead to regulation of gene expression mediated by transcription factors such as c-Fos, c-Jun, cAMP-responsive element binding protein, and others. The mechanism usually involves phosphorylation of the transcription factor by a kinase that is activated as a result of the ligand–receptor interaction at the cell surface. Nuclear receptors are also indicated to be targets of kinases involved in such signal transduction (Freedman, 1999; Shao and Lazar, 1999), and phosphorylation of nuclear receptors provides an important mechanism for crosstalk between signaling pathways. Already multiple kinase pathways have been implicated in modulation of nuclear receptor-mediated gene regulation—cAMP-dependent protein kinase, casein kinase, glycogen synthase kinase (GSK-3), c-Jun kinase, cyclin-dependent kinases (CDKs), and MAP kinases (MAPKs) (Freedman, 1999; Shao and Lazar, 1999). All aspects of receptor function can be regulated, including DNA binding and dimerization, transcriptional activity, interaction with cofactors, and ligand binding. In the case of the GR, Ser246 (Krstic *et al.*, 1997), Ser224 and 232 (Krstic *et al.*, 1997), and Thr171 (Rogatsky *et al.*, 1998), all of which are located in AF-1 region, are indicated to be a target of MAPK, CDKs, and GSK-3, respectively.

In a physiological and/or pharmacological context, it should be notified that many effects of glucocorticoids are achieved by not only activation but also inhibition of target gene expression (Reichardt *et al.*, 1998; Fig. 5). This is particularly true for the antiinflammatory/immunosuppressive effects of glucocorticoids that involve negative transcriptional regulation of proinflammatory genes (Cato and Wade, 1996). This mode of regulation is distinct from previously described positive regulation and does not necessarily involve the interaction of the GR with GRE; instead, it is achieved by interaction between the GR and so-called negative GRE (nGRE) (Drouin *et al.*, 1993; Fig. 5). On the other hand, the expression of many proinflammatory genes is positively regulated by a certain class of transcription factors, for example, AP-1 and NF-κB (Cato and Wade,

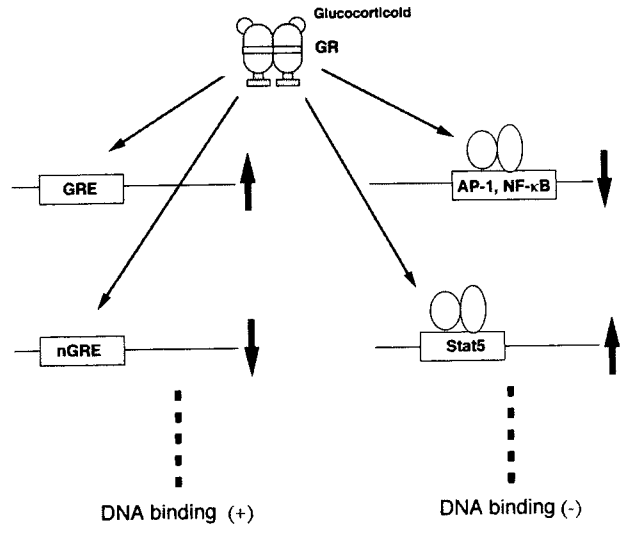

FIG. 5. Multiple mechanisms of GR-mediated transcriptional regulation. The GR, after binding ligands, regulates gene expression in a variety of fashions. Interaction with GRE and negative GRE (nGRE) requires DNA binding of the GR. In contrast, interaction with AP-1, NF-κB, and STAT5 does not require GR DNA binding, although requirement of the DNA binding domain of the GR is suggested in some cases.

1996). Negative regulation of these genes by the GR is sometimes referred as "cross-talk" between GR and these transcription factors (Cato and Wade, 1996; Fig. 5). Already numerous molecular mechanisms have been presented to account for such mutually exclusive interaction between transcription factors, *e.g.*, direct protein–protein interaction, squelching of coactivators, and inhibition of catalytic activity of such enzymes that modulate the transcription factors (Cato and Wade, 1996). Moreover, it has recently been presented that the GRα could heterodimerize with the other members of nuclear receptor superfamily, including GRβ (Oakley *et al.*, 1996), MR (Liu *et al.*, 1995), and AR (Chen *et al.*, 1997).

Together, the GR, after activation by ligand, elicits pleiotropic and conditional regulation of gene expression, which may enable fine tuning of cellular metabolic processes and the stress response.

REDOX REGULATION OF GENE EXPRESSION

Cellular system for redox regulation

Mammalian cells continuously produce reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide (O_2^-), and the hydroxyl radical (•OH) as side products of electron transfer reactions; above-normal levels of ROS are referred to as oxidative stress. This condition occurs frequently in cells exposed to UV light, X rays, or low concentrations of H_2O_2, but also upon stimulation of cells with cytokines in inflammatory tissues. The intracellular concentrations of ROS, or cellular redox state, seem to be finely tuned to preserve cellular homeostasis through the expression and regulation of many enzymes (Halliwell and Gutteridge, 1989; Demple and Amabile-Cuevas, 1991; Frei, 1994; Yu, 1994).

Cellular redox state is regulated by balancing production of ROS and sequestration of ROS. In this regard, glutathione (GSH) and thioredoxin systems play an important role in fine tuning of cellular homeostatic control of oxygen. Cellular H_2O_2 and other peroxides are eliminated by selenoenzyme GSH-peroxidase-catalyzed reduction, with GSH as substrates. As a result of this reaction, oxidized GSH (GSSG) is formed, and then the GSSG is restored to GSH by GSH reductase (Prinz *et al.*, 1997; Sies, 1997). Thioredoxin (TRX) is a small protein (m.w. ~12 kDa) found in all living cells, and, together with the FAD-containing enzyme TRX reductase and NADPH, serves as a hydrogen donor for ribonucleotide reductase essential for DNA synthesis and a general protein disulfide reductase involved in redox regulation (TRX system) (Holmgren, 1985, 1995; Prinz *et al.*, 1997). These redox-controlling systems are known to have multifaceted physiological functions, including antioxidant defense, detoxification of electrophilic xenobiotics, modulation of redox-dependent signal transduction, regulation of cell proliferation, storage and transport of cysteines, regulation of immune response, and regulation of prostaglandin and leukotriene metabolism (Gamaley and Klyubin, 1999). Moreover, TRX has been reported to be involved in numerous biological processes—anti-apoptotic function, stimulation of cytokine expression, tissue protection from ischemia-reperfusion injury, cytoprotection from cytotoxic and DNA-damaging agents, proliferation, and drug resistance in certain malignancies (Nakamura *et al.*, 1997; Schulze-Osthoff *et al.*, 1997; Sen, 1998; Gamaley and Klyubin, 1999; Rhee, 1999; Kamata and Hirata, 1999). A recent gene targeting experiment has revealed that TRX expression is essential for early differentiation and morphogenesis in the mouse embryo because early embryonic lethality is caused by targeted disruption of the mouse TRX gene (Matsui *et al.*, 1996).

Redox regulation of gene expression in mammalian cells

Currently, the cellular redox state is considered to influence not only intracellular signals but also gene expression (Korthuis and Granger, 1986; Halliwell and Gutteridge, 1989; Demple and Amabile-Cuevas, 1991; Frei, 1994; Yu, 1994; Dalton *et al.*, 1999; Tanaka *et al.*, 1999). Hypoxic and hyperoxic stresses can activate or repress the transcription of certain genes by pathways that probably involve protein kinases (Bauskin *et al.*, 1991). On the other hand, the response to severe oxidative stress may in-

volve an additional effect in which redox-sensitive factors can be directly activated or inactivated through the oxidation of sulfhydryl residues. Already, the DNA binding activity of a number of transcription factors, *e.g.*, AP-1 (Abate *et al.*, 1990; Xanthoudakis *et al.*, 1992, 1994), Sp1 (Ammendola *et al.*, 1994; Wu *et al.*, 1996), Egr-1 (Huang and Adamson, 1993), NF-κB (Toledano and Leonard, 1991; Schreck *et al.*, 1991, 1992; Meyer *et al.*, 1993; Anderson *et al.*, 1994; Schenk *et al.*, 1994), c-Myb (Guehmann *et al.*, 1992; Myrset *et al.*, 1993), E2 (McBridge *et al.*, 1992), p53 (Hainaut and Milner, 1993; Rainwater *et al.*, 1995; Jayaraman *et al.*, 1997; Verhaegh *et al.*, 1997), NFI (Bandyopadhyay and Gronostajski, 1994), PEBP2 (Akamatsu *et al.*, 1997), Pax-8 (Arnone *et al.*, 1995), TTF-1 (Kambe *et al.*, 1996), NF-Y (Nakshatri *et al.*, 1996), Ets (Wasylyk and Wasylyk, 1993) arylhydrocarbon receptor (Ireland *et al.*, 1995), and USF (Pognonec *et al.*, 1992) have been shown to be reduced or lost when critical cysteine residues are oxidized *in vitro*. In contrast to these suppressive effects, it has recently been reported that oxidative stress activates the transcription factor STAT3 (Carballo *et al.*, 1999) via promotion of their nuclear translocation. We, thus, may consider that redox signal may play a regulatory role in transcriptional regulation of gene expression. A cellular buffering system against oxidative stress, therefore, may also be important for the homeostatic control of gene expression.

The role of redox regulating enzymes for mammalian gene expression has been initially described by Curran and associates. They proposed that the transcription factor AP-1 is physiologically regulated by direct redox regulation of its DNA binding activity and Ref-1 was identified as an endogenous reducing catalyst of AP-1 (Abate *et al.*, 1990; Xanthoudakis *et al.*, 1992, 1994). TRX is also known to be involved in gene regulation via interaction with a number of transcriptional factors. For example, intracellular ROS induces NF-κB activation in various cells, and reducing reagents, including TRX, titrate this oxidant-induced activation of NF-κB (Schreck *et al.*, 1991, 1992; Schenk *et al.*, 1994). Recently, it is suggested that AP-1 transcriptional activity is regulated by a direct association between TRX and Ref-1; TRX modulates Ref-1 activity via direct protein–protein interaction, and Ref-1 modulates AP-1 activity in a similar fashion, presenting an example of a cascade in cellular redox regulation (Hirota *et al.*, 1997).

REDOX REGULATION OF GLUCOCORTICOID HORMONE ACTION—CROSSTALK BETWEEN HORMONAL STRESS RESPONSE AND PERIPHERAL ANTIOXIDANT SYSTEM

Redox regulation of glucocorticoid hormone action

The human GR contains 20 cysteine residues, concentrated in the central region spanning the DBD and LBD (Evans, 1988; Fig. 1). Previous biochemical studies have shown that the cysteine residues in these subdomains are crucial for maintaining the structure and function of these domains (Bodwell *et al.*, 1984; Silva and Cidlowski, 1989; Esposito *et al.*, 1995; Hutchison *et al.*, 1991; Simons *et al.*, 1995). For example, the ligand-binding activity of the GR is determined by the absence or presence of an intramolecular disulfide between a vicinally spaced pair of cysteine thiol groups lying in the LBD (between Cys656 and Cys661 in rat GR; Chakraborti *et al.*, 1992; Simons *et al.*, 1995). Moreover, it was shown that the ligand binding activity of GR is strongly impaired by thiol-oxidizing reagents and rescued by the TRX system *in vitro* (Simons and Prah, 1995). Concerning the DBD, crystallographic analysis of the DBD–DNA complex has demonstrated that the cysteine residues in the DBD coordinate zinc atoms and have a critical and direct role in the receptor–DNA interaction (Luisi *et al.*, 1991; Glass, 1994). It has also been shown that conversion of sulfhydryls in the DBD to disulfides blocks receptor binding to DNA cellulose (Bodwell *et al.*, 1984). Despite this biochemical evidence of redox regulation of the GR, it remains unknown whether cellular glucocorticoid action is modulated in a redox-dependent manner. Therefore, we addressed whether redox-dependent modulation of GR function operates in live cells.

Ligand binding activity of the GR: Using GR-expressing CHO-pMTGR cells (Alksnis *et al.*,

1991), we first showed that ligand binding activity of cellular GR is similarly affected under oxidative or reducing conditions as shown by biochemical experiments. Moreover, transient transfection of the TRX expression plasmid into the cells, oxidative repression of ligand binding was canceled. Because GR immunoreactivity was unaffected by either treatment with oxidants such as H_2O_2 or transfection of TRX expression plasmid, these results indicated 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 (Makino *et al.*, 1996a).

Dissociation of hsp90 and nuclear translocation: Inhibition of ligand binding may affect subsequent dissociation of hsp90 and receptor movement into the nucleus. To address these issues, we decided to use green fluorescent protein (GFP)-tagged hGR, since GFP is known to be a powerful tool for seeing the intracellular localization of protein molecules in living cells (Chalfie, 1995; Tsien, 1998), and this chimeric GR mimics endogenous GR in terms of ligand-dependent nuclear translocation (Ogawa *et al.*, 1995; Okamoto *et al.*, 1999). After transient transfection of pGFP-hGR into COS-7 cells, 20–30% of the cells showed cytoplasmic green fluorescence, indicating expression of the GFP–hGR fusion protein in those cells; they also expressed GFP–hGR chimeras that were localized exclusively in the cytoplasm in the absence of hormone, whereas GFP-hGR translocated into the nucleus in a time-dependent manner after hormone treatment (Fig. 6). However, having GFP in the amino-terminal end of the human GR, GFP-hGR possesses transactivation properties similar to wild-type GR (Ogawa *et al.*, 1995; Okamoto *et al.*, 1999). Using this system, we examined the effect of oxidative treatment on nuclear translocation of GFP-hGR in COS-7 cells. As shown in Fig. 6, the rate of ligand-dependent nuclear translocation of GFP-hGR was markedly delayed upon addition of H_2O_2. This suppressive effect of H_2O_2 was dose-dependent. Indeed, in the presence of 2 m*M* H_2O_2, nuclear translocation was severely compromised even after treatment with 100 n*M* dexamethasone. *N*-Acetyl-L-cysteine partially, but efficiently, reversed the negative effects of 2 m*M* H_2O_2, resulting in the

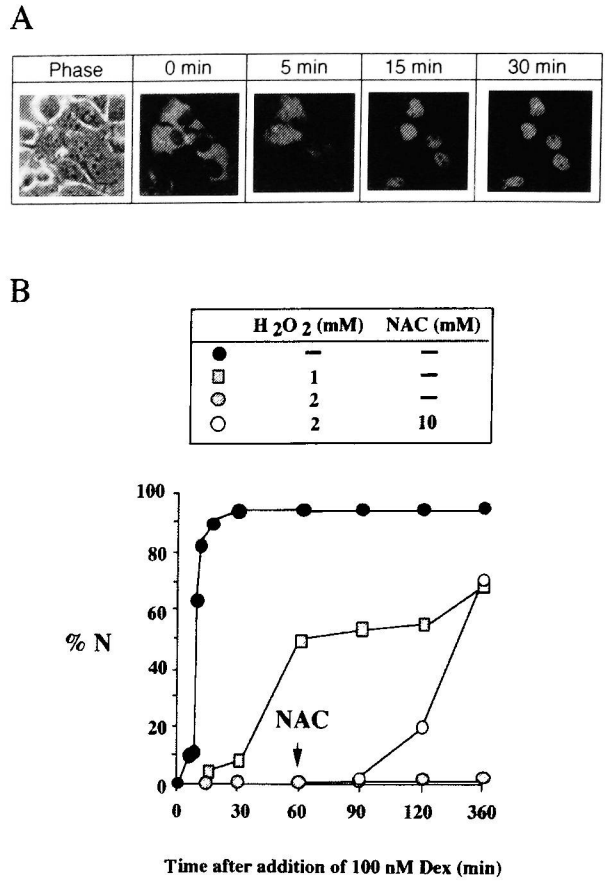

FIG. 6. Redox regulation of nuclear translocation of the GR—green fluorescent protein study. A. GFP-hGR-expressing COS-7 cells were cultured in the presence of 100 n*M* dexamethasone and GFP fluorescence was serially monitored using an inverted fluorescent microscopy. **B.** GFP-hGR-expressing COS-7 cells were incubated for 2 hr in the absence (●) or presence of various concentrations of H_2O_2: 1 m*M* (▩), and 2 m*M* H_2O_2 (◉), then, 100 *nM* dexamethasone was added. As indicated by arrow, *N*-acetyl-L-cysteine (NAC) was added after 1 hr of incubation with dexamethasone (○). At the indicated time points after addition of dexamethasone, subcellular localization of GFP-hGR was assessed quantitatively, and the percentages of the category N (GFP fluorescence is exclusively nuclear) are shown.

nuclear translocation of GFP-hGR in the presence of 100 n*M* dexamethasone (Fig. 6). This oxidative inhibition of the nuclear import of the GR is observed not only in COS-7 cells, but also in CHO-K1 and HeLa cells as well (Okamoto, *et al.*, unpublished observation).

Ligand-dependent dissociation of hsp90 was also studied using GFP-hGR. As in the case of the native GR, dissociation of hsp90 from GFP-hGR requires a ligand (Okamoto *et al.*, 1999). Treatment of cells with H_2O_2 suppressed this ligand-dependent dissociation of hsp90 only

partially, even at high concentrations of H_2O_2. This dissociation between the effect of oxidative treatment on hsp90 release and those on nuclear translocation of the GR suggests that the effects of oxidative treatment on these two phenomena are separable. Indeed, we showed that NL1 function is negatively regulated under oxidative condition. To eliminate the involvement of the effect on ligand–receptor interaction, we constructed the expression plasmid for the fusion protein of GFP, the VP16 transactivation domain, and the DNA binding domain of the GR. We have already shown that this plasmid contains NL1 of the GR and constitutively localizes in the nucleus (Okamoto *et al.*, 1999). After transient expression of GFP-VP16-GR DBD in COS-7 cells, the cells were cultured in the presence or absence of H_2O_2 for 12 hr, and subcellular localization of this fusion protein was analyzed. In the absence of H_2O_2, GFP-VP16-GR DBD was constitutively localized in the nucleus. However, in the presence of 1 m*M* H_2O_2, part of cells showed cytoplasmic retention of GFP fluorescence. Moreover, almost all cells having GFP fluorescence revealed a significant cytoplasmic fluorescent signal at 2 m*M* H_2O_2, indicating that GFP-VP16-GR DBD, at least in part, docks in the cytoplasm under oxidative conditions (Fig. 7). Thus, we may conclude that the GR nuclear translocation process is negatively modulated in a redox-dependent mechanism even after ligand-dependent dissociation of hsp90. Because the trafficking of the GR between the cytoplasm and the nucleus is dynamic and bidirectional (Madan and DeFranco, 1993), the equilibrium of distribution of the GR is determined by the relationship between nuclear import and export rates. Compared with nuclear protein import, it is generally believed that the export kinetics are relatively slow. Therefore, nuclear protein transport appears to be strictly unidirectional in the short term (Nigg, 1997). Predominantly cytoplasmic localization of the GR in the absence of hormone strongly suggests that nuclear import is a rate-limiting step. Indeed, treatment with H_2O_2 largely affects nuclear import of GFP-hGR. Moreover, the function of SV40 T-Ag NLS, which does not contain cysteine residues, is not impaired under oxidative condition (Okamoto *et al.*, 1999), indicating that NLS of the GR, especially NL1, could be a target of redox-dependent modulation. Interestingly, the cysteine residue within NL1 (position 481 in the human GR) is highly conserved among nuclear receptors (Okamoto *et al.*, 1999). To test the hypothesis that this conserved cysteine residue is a target of redox regulation, we substituted Cys for Ser to generate GFP-VP16-GR DBD/C481S, and examined nuclear import of this fusion protein. This chimeric protein was extremely resistant to oxidative treatment and localized in the nucleus, even at high concentration of H_2O_2 (Fig. 7).

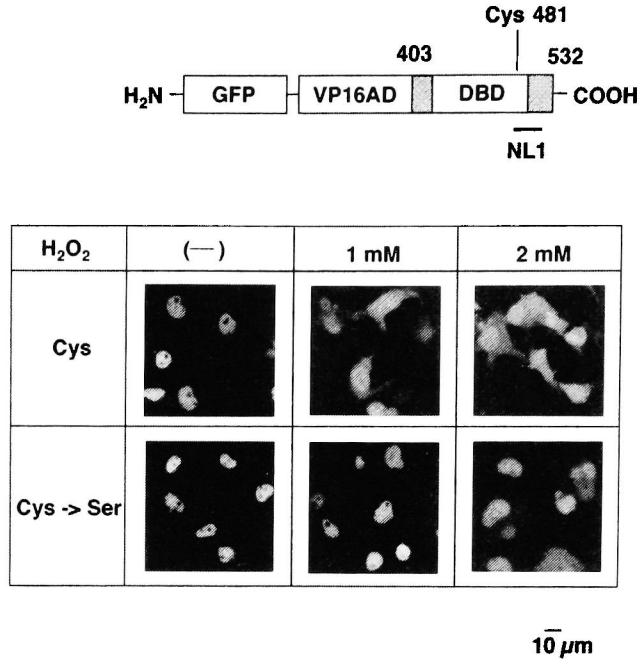

FIG. 7. Redox regulation of the ligand-independent nuclear localization of the GR: role of Cys481. COS-7 cells were transfected with the expression plasmid for GFP-VP16-GR DBD or GFP-VP16-GR DBD/C481S, and cultured for 12 hr in the absence or presence of H_2O_2. Photographs were taken using inverted fluorescent microscopy.

Together, oxidative stress-mediated expression of GR nuclear import may not only be related to decreased ligand binding activity and subsequent impairment of the release of hsp90, but also related to dysfunction of nuclear translocation process itself. Moreover, our present data argue that the conserved Cys481 might confer redox-dependency of the nuclear import of the GR. The fact that Cys481 for Ser substitution almost completely abolished redox sensitivity of NL1 function strongly indicates

that Cys481 is one of the regulatory amino acids involved in redox-dependent intramolecular disulfide bond formation in the GR (Okamoto *et al.*, 1999). For example, disulfide bond formation involving this cysteine residue may result in a protein conformation that hampers efficient interaction of the GR with NLS receptor proteins, *e.g.*, importin-α/karyopherin-α even in the presence of ligands. Under reducing conditions, NL1 may interact with NLS receptor proteins in a ligand-dependent fashion, or alternatively, this may occur after liberation from hsp90, resulting in nuclear translocation of the GR. Although this cysteine residue is well conserved among nuclear receptors, the functional significance has not yet been characterized for other receptor proteins. Previously, it has been shown that amino acid substitution of Cys481 to Ser, contrary to amino acid substitution of Cys481 to Arg (Zilliacus *et al.*, 1992), does not affect either DNA binding or transactivation functions of the GR (Schena *et al.*, 1989). Therefore, it will be interesting to test whether this cysteine residue generally defines redox-dependent subcellular localization of nuclear receptors.

We have also observed that sensitivity to H_2O_2 of nuclear translocation of GFP-hGR is extremely variable among distinct cell types (see also Schreck and Baeuerle, 1994, for NF-κB activation) and steroid hormone-inducible gene expression is suppressed at lower concentrations of H_2O_2 in certain cells (Makino *et al.*, 1996a; Hayashi *et al.*, 1997; Okamoto *et al.*, 1998). Among the cells that we have studied, human mammary tumor cells ZR-75-1 were most sensitive to treatment with H_2O_2, and expression of reporter genes for either the ER or GR was affected under physiological concentrations of H_2O_2 (Okamoto *et al.*, unpublished observation).

Although involvement of cysteine residues is also suggested, nuclear translocation of the transcription factor yAP-1, in clear contrast to the GR, is rather promoted in response to oxidative stress (Kuge *et al.*, 1997). Moreover, the multicopy suppressor of SNF1 protein 2, Msn2p, which contains two zinc finger motifs, translocates into the nucleus in response to a broad variety of stresses, *e.g.*, exposure to heat shock, oxidative stress, ethanol, sorbate, or osmostress (Görner *et al.*, 1998). The activation and nuclear translocation of STAT3 is also promoted under oxidative conditions, most possibly via oxidative stress-mediated activation of kinases and phosphorylation of STAT3 (Carballo *et al.*, 1999). Thus, redox-dependent modification of cysteine residues is considered to be one of the key regulatory mechanisms for conditional protein localization within the cells and subsequent gene expression. From a mechanistic point of view, it should be emphasized that not only oxidative stress but hypoxic conditions also influence subcellular compartmentalization of transcription factors. Notably, nuclear translocation of the hypoxia-inducible factor 1-α is promoted under hypoxic conditions (Kallio *et al.*, 1998). Therefore, cells may respond to alteration in oxygen tension via variable mechanisms including segregation of distinct transcription factors.

DNA binding: Already a number of studies, including ours, have indicated that the DBD of the GR is a target of redox regulation *in vitro*. Moreover, *in vitro* DNA binding experiments suggested that the reducing catalyst TRX may modulate DNA binding activity of the GR (Makino *et al.*, 1996a). Because TRX translocates into the nucleus under oxidative conditions, redox regulation of DNA binding of the GR *in vivo* have to be analyzed also in context of the effect of TRX. The GR mutant I550, which lacks the ligand binding domain, is constitutively present in the nucleus even in the absence of hormone (Rangarajan *et al.*, 1992; Jewell *et al.*, 1995) and acts as a ligand-independent transcriptional activator (Giguere *et al.*, 1986), was shown to be sensitive to oxidative stress (Makino *et al.*, 1996a). Indeed, suppression of I550-mediated gene expression either by treament with H_2O_2 or antisense TRX expression is similar to that seen for wild-type GR (Makino *et al.*, 1996a)—further evidence for the nuclear location of the GR-TRX interaction. VP16-GR DBD can then be used for monitoring the influence of TRX on the GR DBD and its interaction with GRE in the nucleus. VP16-GR DBD induced reporter gene expression by 105-fold in the absence of H_2O_2, indicating the productive interaction between the DBD and GRE in the nucleus. This transcriptional activity of VP16-GR DBD was lowered by five-fold after

treatment with H_2O_2, and restored by overexpression of TRX (Makino *et al.*, 1999). These results strongly indicate that TRX, at least functionally, communicates with the GR DBD in the nucleus. Because TRX has been shown to associate directly with target proteins in exerting its reducing action (Qin *et al.*, 1995; Hirota *et al.*, 1997), we postulated that TRX may physically associate with the GR DBD. This possibility was verified by using the mammalian two-hybrid assay, in which a cDNA of the GR DBD or TRX was subcloned downstream of the GAL4 DBD (harboring an NLS capable of taking fusion proteins to the nucleus; Ma *et al.*, 1987) or the transactivation domain of VP16 in frame (Makino *et al.*, 1999; Fig. 8). VP16-GR DBD constitutively localizes in the nucleus, thus the 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 a GST pull-down assay in combination with diamide cross-linking (Hirota *et al.*, 1997). 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, 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 analyzed by Western immunoblot assay. When either Sepharose or GST-bound Sepharose was added, TRX was not detected, 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, strongly suggesting direct protein–protein interaction between TRX and the GR DBD under oxidative conditions *in vitro* (Makino *et al.*, 1999).

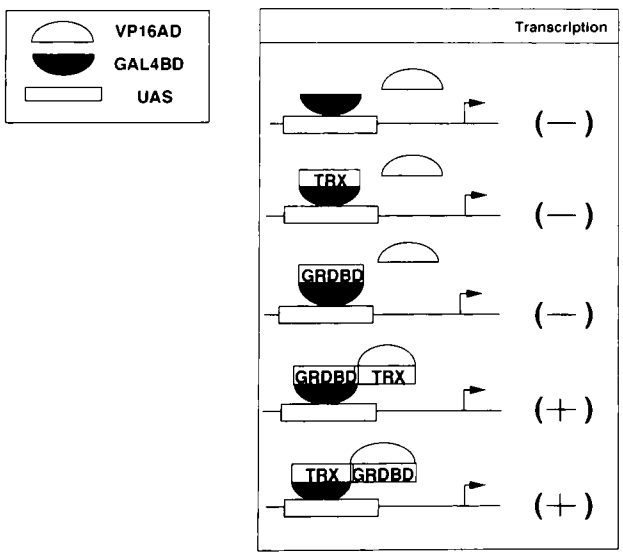

FIG. 8. Summary of two-hybrid assay demonstrating direct protein–protein interaction between the GR and thioredoxin (TRX). Results of mammalian two-hybrid assay in CV-1 cells are summarized. CV-1 cells were grown in 60-mm-diameter culture dishes and transfected with 100 ng of reporter plasmid tk-GALpx3-Luc and expression plasmids for VP16, GAL4, and for chimeric proteins. VP16-GR DBD, GAL4-TRX, VP16-GR DBD, and GAL4-GR DBD as indicated. After a further 24 hr of culture, a luciferase assay was performed.

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 (Masutani *et al.*, 1996). These results suggest 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 potentiates AP-1 transcriptional activity via a redox-dependent interaction with Ref-1 (Hirota *et al.*, 1997). 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 (Masutani *et al.*, 1996), such differential subcellular localization in response to the environmental stimuli may constitute a mechanism for the pleiotropic action of TRX.

In contrast with the LBD, the DBD is the most highly conserved region between members of the steroid hormone receptor family (Beato *et al.*, 1995). Not only oxidative stress but also TRX may thus interact with other members via the DBD as well. We have previously demonstrated that TRX also augments ER function, which is negatively modulated under oxidative conditions (Hayashi *et al.*, 1997), indicating that

TRX may be a general factor that allows crosstalk between redox signal and steroid hormone actions. Recently, a small nuclear RING finger protein, SNURF, which binds directly to the DBD of the AR and can coactivate the receptor function, was identified (Moilanen *et al.*, 1998). 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 (Xu *et al.*, 1999), DBD-associating proteins like TRX or SNURF might also act as a cofactor for 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 as well (Mangelsdorf *et al.*, 1995). Some nuclear receptors are considered to be ligand-independent transcription factors, the activity of which is modulated by posttranslational modification or cross coupling with the other protein factors (Mangelsdorf *et al.*, 1995). Such ligand-independent receptors, compared with the receptors for known ligands, have been shown to be ancient in evolutionary terms (Escriva *et al.*, 1997), 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 (Holmgren, 1985), and targeted disruption of the mouse TRX gene causes early embryonic lethality (Matsui *et al.*, 1996).

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 protein with concomitant formation of oxidized TRX (Holmgren, 1985, 1995). The reactivity of a thiol group is determined by its accessibility and the microenvironment within the protein, including adjacent amino acids (Snyder *et al.*, 1981; Holmgren, 1985, 1995; Cappel and Gilbert, 1988). Thiol groups with metal associations are among the strongest nucleophiles in the cell (Snyder *et al.*, 1981; Cappel and Gilbert, 1988). The zinc finger motifs of the GR, when oxidized, may have a structure that permits efficient access of TRX. Together with the recent report of the redox regulation of the Sp1 transcription factor (Wu *et al.*, 1996), sulfhydryl groups of some zinc finger motifs may have relatively high thiol-disulfide oxidation potentials. Several reports have presented evidence showing direct protein–protein interaction with TRX and transcription factors (Qin *et al.*, 1995, 1996; Hirota *et al.*, 1997). Three-dimensional structural analysis of the DBD of the GR suggested that the cysteine residue is involved in the shell of a hydrophobic core which surrounds the aromatic cluster mainly composed of Phe and Tyr, stabilizes the globular fold, fixes the relative orientation of these substructures (Luisi *et al.*, 1991). Oxidative conditions may, via involvement of intra- or intermolecular disulfide bond formation, hamper the conservation of such structure and impair GR function.

Together with the recent report showing that CBP/p300 cofactor recruitment for particular transcription factor (Ema *et al.*, 1999), redox signals including TRX systems may communicate with the GR at multiple levels, which may contribute to fine tuning of receptor function and/or glucocorticoid hormonal signal reception.

Biological implication and pharmacological application: During the evolution and the accompanying genetic divergence of the nuclear receptor superfamily, the GR might become a unique molecular switch that docks in the cytoplasm in unstimulated cells (Mangelsdorf *et al.*, 1995). Therefore, the GR could convey not only hormonal signals but also redox signals into the nucleus as an integrator of extracellular environments. Considering that the DBD is postulated to be sterically hindered in untransformed unliganded GR (Simons and Pratt, 1995), the interaction between the LBD and TRX appears to be predominant in the cytoplasm. Thus, TRX might modulate the ligand binding activity of the GR, keeping the LBD conformation in a reduced state, especially when cells are under oxidative stress. The ligand binding, which is the initial receptor function, is rapidly repressed by oxidative stress to

cells, and unliganded GR is harbored in the cytoplasm even in the presence of the agonistic ligands. This phenomenon appears to be reasonable, for example, at the inflammatory tissues where cells are believed to be exposed to severe oxidative stress (Korthuis and Granger, 1986; McCord, 1987; Halliwell and Gutteridge, 1989), because suppression of glucocorticoid action in inflammatory cells may potentiate endogenous defense mechanisms and prevent premature termination of the cascade of inflammatory reactions. The delayed induction of TRX after oxidative stress (Leppa *et al.*, 1997), then, may restore the ligand binding activity via reducing the LBD and permit the GR to translocate efficiently to the nucleus. Moreover, part of TRX is translocated into the nucleus under oxidative conditions and then restores GR function, including DNA binding activity. The subsequent activation of anti-inflammatory genes or repression of inflammatory genes may prevent overshoot of inflammation (Fig. 9). This multistep regulation of GR function by cellular redox mechanisms would be relevant as one of the molecular explanations for the "general adaptation syndrome" that was originally postulated by H. Selye (Selye, 1946; Munck *et al.*, 1984). Because macrophages are known to produce ROS as a result of oxygen burst after phagocytosis (Cohn, 1978), it is particularly interesting to test whether such oxidative stress suppresses GR function and commits macrophages in a "proinflammatory status," allowing them to act as self-defense machinery.

Cellular antioxidant defense systems involve many substances—antioxidants, free radical scavengers, chain terminators, or reductants. The antioxidant systems responsible for cellular

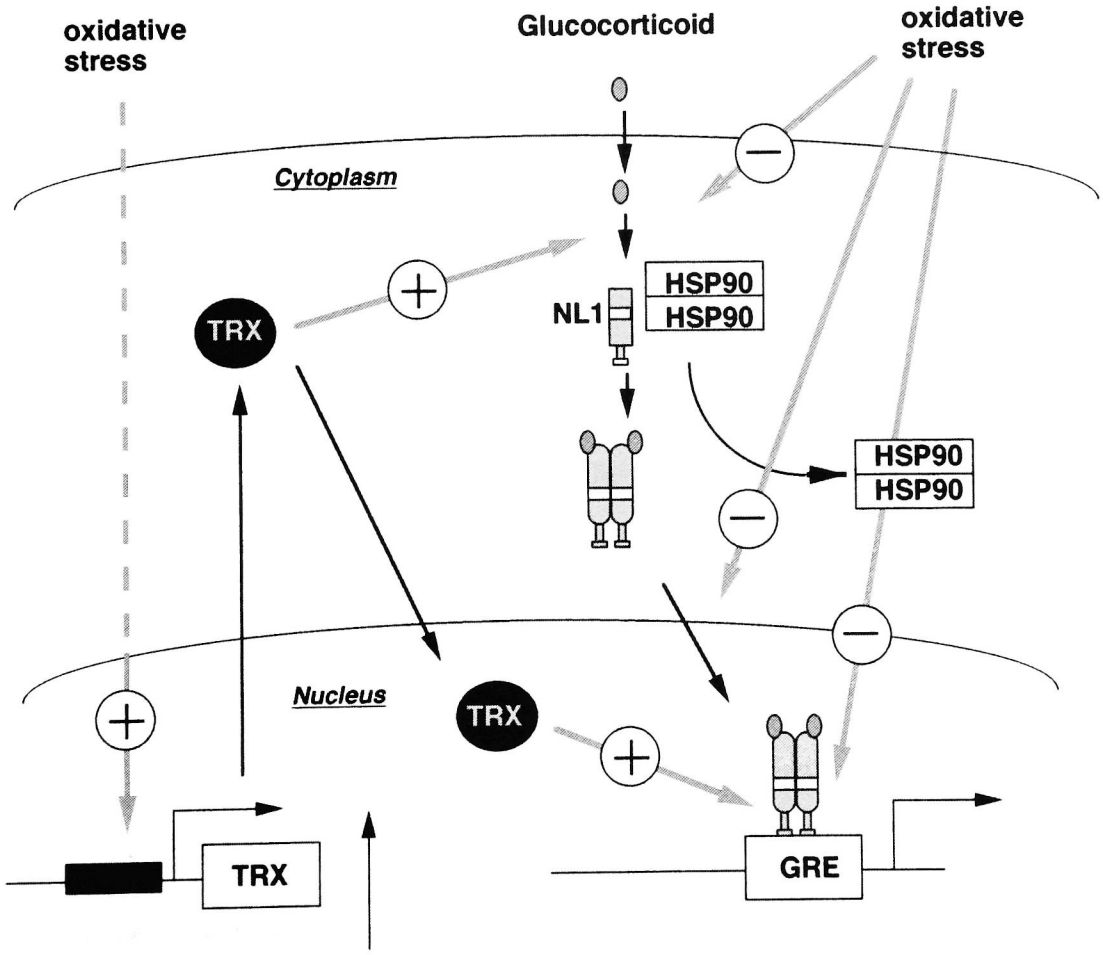

FIG. 9. Model of redox regulation of the GR in mammalian cells. Oxidative stress, either directly or indirectly, negatively modulates GR function at multiple levels. In contrast, thioredoxin (TRX) gene expression is upregulated under oxidative conditions. Thus, GR-mediated gene expression is coordinatedly regulated by oxidative stress and cellular TRX.

protection against oxidative stress are considered to be as diversified as ROS themselves, and these molecules are coordinatedly compartmentalized in cells (Yu, 1994). Recent studies on the redox regulation of gene expression suggest that in some, but not all, cases direct or indirect association between the catalysis of cellular reduction and the transcription factors is essential. GR-TRX interaction might be one of many partnerships occurring *in situ*, and as yet undetermined factors may also participate in the redox regulation of the GR in the distinct compartments of cells. Because the GR is present in almost every tissue and oxidative treatment impairs glucocorticoid-inducible gene expression, we speculate that the TRX system operates as an endogenous defense machinery for the GR, or glucocorticoid-mediated stress responses, against oxidative stress. It is of interest that the TRX system has recently been shown to be communicated with other endocrine systems including the thyroid gland (Kambe *et al.*, 1996) and mammary gland (Hayashi *et al.*, 1997). Therefore, role of TRX in endocrine control of homeostasis might not be unique to glucocorticoid-GR system (Tanaka *et al.*, 1999). In addition to the interaction between TRX and the GR, direct physical association of TRX and oligopeptides from the DNA-binding loop of p50 subunit of NF-κB has been demonstrated by nuclear magnetic resonance (Qin *et al.*, 1995). Ref-1 has also been shown to be directly targeted by TRX (Hirota *et al.*, 1997), which in turn restores the DNA binding activity of transcription factor AP-1 (Xanthoudakis *et al.*, 1992, 1994). Similarly, Ref-1 has been shown to augment the DNA binding and transcriptional activities of p53 (Jayaraman *et al.*, 1997). 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 (Saitoh *et al.*, 1998). Probably on structural grounds, there seem to be distinctions in the interplay between transcription factors and reducing catalysts—for example, although 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 (Xanthoudakis *et al.*, 1992). Redox signals, thus, initially generated as broad intracellular ROS 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 cellular factors, including transcription factors, therefore, may be extremely important for understanding the mechanism of redox regulation of cellular physiology.

Our present results also suggest that radical scavengers/antioxidants may serve as a potentiator of glucocorticoid action, especially in oxidative stress conditions. In contrast, it is well known that oxidative stress activates expression of, for example, a transcription factor NF-κB (Schreck *et al.*, 1991, 1992; Meyer *et al.*, 1993; Schenk *et al.*, 1994), and that various antioxidant chemicals suppress NF-κB activation (Schreck *et al.*, 1991, 1992; Meyer *et al.*, 1993; Schenk *et al.*, 1994). In clear contrast, glucocorticoid-inducible gene expression is suppressed under oxidative conditions and restored by reducing reagents (Okamoto *et al.*, 1998). Considering that expression of a number of proinflammatory genes are regulated by NF-κB and the GR in mutually opposite directions (Cato and Wade, 1996), the redox regulatory mechanisms of the GR may be important in pharmacological modulation of inflammation (Sen, 1998).

CONCLUDING REMARKS

Conditional regulation of protein function is essential for cells to respond to changing extra- and intracellular environments. Protein–protein interactions, allosteric changes generated by ligand binding, and chemical modification such as phosphorylation are well-known mechanisms involved in such regulation. Recent technical advance in assessing the disulfide-bonded states of proteins *in situ* is now going to unveil physiological significance of redox regulation of transcription factors in bacteria (Åslund and Beckwith, 1999). Growing evidence strongly indicates that redox state variably modulates functional activity of many, but not all, transcription factors in mammalian cells. Therefore, as represented in the case of the GR, redox regulation of transcription may be one of the dynamic processes of cells in response to environmental signals. Further mechanical and structural analysis would defi-

nitely clarify this issue and contribute to improvement of, for example, treatment of various diseases.

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ABBREVIATIONS

AR, andorogen receptor; CDK, cyclin-dependent kinase; DBD, DNA binding domain; ER, estrogen receptor; GFP, green fluorescent protein; GR, glucocorticoid receptor; GRE, glucocorticoid response element; GSH, glutathione; GSK, glycogen synthase kinase; GSSG, oxidized glutathione; GST, glutathione-S-transferase; H_2O_2, hydrogen peroxide; hsp, heat shock protein; LBD, ligand binding domain; MAPK, mitogen-activated protein kinase; MR, mineralocorticoid receptor; NLS, nuclear localization signal; •OH, hydroxy radical; ROS, reactive oxygen species; SRA, steroid receptor RNA activator; SV40, simian virus 40; TAF, TATA box-associated factor; TRX, thioredoxin.

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