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Hormone-Independent Repression of AP-1-Inducible Collagenase Promoter Activity by Glucocorticoid Receptors

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The role of the ligand in glucocorticoid receptor-mediated transactivation and transrepression of gene expression was investigated. Half-maximal transactivation of a mouse mammary tumor virus-chloramphenicol acetyltransferase reporter gene in transfected cells expressing the human glucocorticoid receptor mutant GRL753F, from which the rate of ligand dissociation is four to five times higher than the rate of dissociation from normal receptors, required a 200- to 300-fold-higher concentration of dexamethasone than was required in cells expressing the normal receptor. Immunocytochemical analysis demonstrated that this difference was not the result of a failure of the mutant receptor to accumulate in the nucleus after steroid treatment. In contrast, in cells cotransfected with a reporter gene containing the AP-1-inducible collagenase gene promoter, the concentration of dexamethasone required for 50% transrepression was the same for mutant and normal receptors. Efficient receptor-mediated transrepression was also observed with the double mutant GRL753F/C421Y, in which the first cysteine residue of the proximal zinc finger has been replaced by tyrosine, indicating that neither retention of the ligand nor direct binding of the receptor to DNA is required. RU38486 behaved as a full agonist with respect to transrepression. In addition, receptor-dependent transrepression, but not transactivation, was observed in transfected cells after heat shock in the absence of the ligand. Taken together, these results suggest that unlike transactivation, transrepression of AP-1 activity by the nuclear glucocorticoid receptor is ligand independent.

The glucocorticoid receptor (GR) is a member of a family of ligand-dependent transcription factors capable of both positive and negative regulation of gene expression (4, 51, 69). In its unactivated form, the GR is part of a large heteromeric complex which includes hsp90 (18, 35, 45, 61, 67) and hsp56 (FKBP52) (42, 57, 61, 63, 81). Binding of the agonist stimulates receptor activation, dissociation from hsp90 (17, 66), and nuclear translocation, prerequisites for both transactivation and transrepression. For transactivation, specific in vitro binding of the activated GR to the glucocorticoid response element does not appear to be ligand dependent (22, 79). However, in the absence of bound ligand, the nuclear form of the GR is a poor activator of target genes (52, 65). This suggests that a ligand-induced conformational change, comparable to that inferred to be necessary for activation of the progesterone (2) and estrogen (5) receptors, is required for efficient activation (or derepression) of the transcriptional activating function present in the GR ligand-binding domain (34, 78) and/or for interaction of the receptor with other components of the transcription apparatus. Direct evidence for such a change has been provided by Simons et al., who demonstrated that affinity-labeled GR was less sensitive to proteolytic digestion than the unoccupied GR (74).

Several mechanisms for GR-mediated transrepression have been described. These include GR binding to a sequence overlapping a *cis*-acting element for another *trans*-acting factor, thereby displacing it from, or preventing its binding to, its cognate element (1, 21, 49, 75). GR-mediated transrepression has also been attributed to direct or indirect interaction of the GR with other *trans*-acting factors, resulting in inhibition of their activity and/or ability to bind to DNA (9, 20, 24, 38, 40, 43, 60, 70, 77, 80). Both models require ligand binding to stimulate receptor activation, dissociation from hsp90, and nuclear translocation. However, it has not been determined whether either mechanism is dependent on the same ligand-induced conformational change presumably needed for transactivation.

In particular, the role of the bound ligand in GR-mediated repression of AP-1-responsive genes containing a tetradecanoyl phorbol acetate (TPA) response element has not been completely investigated. Repression of these genes has been proposed to be the result of the direct interaction of the GR with c-Jun (20, 43, 70, 76, 80) or c-Fos (39). The GR DNA-binding domain is necessary for this interaction, since most mutations in this domain result in the loss of repressor activity in vivo (20, 38, 43, 70, 80). The DNA-binding domain is also sufficient for inhibition of in vitro transcription from the collagenase promoter and inhibition of Jun-Fos heterodimer binding to the collagenase TPA response element (46). However, deletion or truncation of the ligand-binding domain also results in a significant loss of repressor activity (38, 70, 80), suggesting that the ligand-binding domain may contribute to, or modulate, the inhibition of AP-1 activity.

To examine the role of the ligand in GR-mediated transrepression of the collagenase promoter, we used the human GR (hGR) mutant GRL753F, cloned from the glucocorticoid-resistant human leukemic cell line ICR27TK.3 (56). It was previously shown that in extracts prepared from these cells, the rate of ligand dissociation from mutant receptors is four to five times higher than the rate of dissociation from normal receptors (50). Because activation results in the loss of the ability of even normal GR to effectively bind the ligand (47), activated GRL753F receptors rapidly become ligand free, and in intact ICR27TK.3 or COS-7 cells expressing this mutant receptor,

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only a small fraction of the receptors are occupied at steady state (50, 56). Thus, this mutant is ideally suited to examine the ability of ligand-free receptors to mediate transactivation and transrepression. In addition, the ability of the glucocorticoid antagonist RU38486 to repress AP-1 activity was examined. Finally, the ability of heat shock, which has been shown to promote nuclear accumulation of unoccupied GR (64), to induce transactivation and transrepression was examined. Our results indicate that GR-mediated transrepression of AP-1 activity is ligand independent.

MATERIALS AND METHODS

Plasmids. The hGR expression plasmids pRShGRα and pRShGR753F and the control plasmid pBluGR5.2 have been described previously (25, 56). The double mutant pRShGR753F/421Y was constructed by replacing the 1.0-kb *Sal*I-*Cla*I fragment of pRShGR753F with the corresponding fragment of pRShGR421Y (55). The presence of both mutations was confirmed by double-stranded sequencing. The glucocorticoid-inducible reporter plasmid pMSG-CAT and the β-galactosidase expression plasmid pCH110 were obtained from Pharmacia/LKB (Piscataway, N.J.). The reporter plasmid −73 COL-CAT (80) was a generous gift from Michael Karin. The β-galactosidase expression vector pTB.1 (6) was obtained from Daniel R. Schoenberg. For transfection, all plasmids were purified with reagents obtained from Qiagen (Chatsworth, Calif.).

Cell culture and transfection. CV-1 and COS-7 cells were maintained at 37°C in Dulbecco modified Eagle medium containing 10% fetal bovine serum (FBS) in a humidified atmosphere containing 5% CO_2. HeLa cells (CCL 2) were obtained from the American Type Culture Collection (Rockville, Md.) and were maintained under the same conditions. Cells were transfected by the calcium phosphate precipitation method of Chen and Okayama (11) and incubated in an atmosphere containing 3% CO_2 for 18 to 20 h. Transfected cells were washed in buffer A (25 mM Tris-HCl [pH 7.4], 137 mM NaCl, 5 mM KCl, 0.7 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.6 mM Na_2HPO_4) and calcium-free phosphate-buffered saline (PBS). Fresh medium containing 10% FBS was added, and the cells were returned to an atmosphere containing 5% CO_2. The efficiency of transfection, as measured by staining for β-galactosidase activity, was generally greater than 20%.

Determination of hGR-binding activity. For determination of [^{3}H]dexamethasone binding in intact cells, COS-7 cells ($\sim 3 \times 10^5$) grown in 100-mm-diameter plates were transfected with 20 μg of the hGR expression vector and 10 μg of the carrier plasmid pBluGR5.2. Transfected cells were incubated in fresh medium for 24 h and then in fresh medium containing 25 nM [^{3}H]dexamethasone (39 Ci $mmol^{-1}$; Amersham, Arlington Heights, Ill.) or fresh medium containing 25 nM [^{3}H]dexamethasone and 12.5 μM unlabeled dexamethasone for 90 min at 37°C. The cells were washed four times with 10 ml of PBS and broken by freeze-thaw lysis. The broken cells were extracted with 95% ethyl alcohol for 10 min, and the radioactivity in the extract was quantified by liquid scintillation counting. Specific binding was determined as the difference between binding activity in plates containing [^{3}H]dexamethasone alone and that in plates containing [^{3}H]dexamethasone and the unlabeled hormone. Transfected cells contained approximately 10^5 receptors per cell. For measurement of the rate of ligand dissociation, transfected cells were incubated for 48 h in fresh medium, removed by gentle scraping into 40 mM Tris-HCl buffer (pH 7.5) containing 10 mM EDTA and 150 mM NaCl, collected by centrifugation, and washed in ice-cold PBS. Extracts were prepared by three cycles of gentle freeze-thaw lysis in 15 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer (pH 8.0) containing 1 mM EDTA, 27 mM sodium molybdate, 3 mM dithiothreitol, 15% (vol/vol) glycerol, and the protease inhibitors leupeptin (20 μg ml^{-1}), aprotinin (20 μg ml^{-1}), and pepstatin A (5 μg ml^{-1}). After centrifugation of the extracts at 10,000 × *g* for 10 min, aliquots were incubated with 50 nM [^{3}H]dexamethasone in the absence or presence of 25 μM unlabeled dexamethasone for 2 h at 4°C. After the addition of 10 μM unlabeled dexamethasone to block the reassociation of [^{3}H]dexamethasone, dissociation of specifically bound [^{3}H]dexamethasone was determined as previously described (50).

Intracellular localization of hGR. Transfected cells were incubated in fresh medium for 48 h, washed three times with ice-cold PBS, and fixed in 3% paraformaldehyde in buffer A for 1 h at 4°C. The cells were permeabilized for 30 min at 4°C in buffer A containing 0.5% Triton X-100 and 2% FBS. After three washes in the same buffer and incubation for 20 min at 23°C in PBS containing 5% FBS to block nonspecific antibody binding, the cells were incubated for 2 h at 23°C with a 1:300 dilution of the anti-hGR antiserum 710 (raised against the 28-amino-acid peptide Cys-245 to Thr-272 in the N-terminal region of the hGR) (56) in PBS containing 2% FBS, and this was followed by incubation for 45 min at 23°C with a 1:50 dilution of fluorescein-coupled donkey anti-rabbit immunoglobulin G (Amersham). After the cells were mounted in Perma Fluor (Lipshaw Immunon, Pittsburgh, Pa.), fluorescent staining was observed with a Leitz fluorescence microscope at a magnification of ×250 and photographed with ASA P1600 film (Eastman Kodak, Rochester, N.Y.).

CAT assays. For transactivation assays, CV-1 cells were transfected with 2 μg of hGR expression plasmid, 3 μg of pMSG-CAT, 3 μg of the β-galactosidase expression plasmid pCH110, and 12 μg of the carrier plasmid pBluGR5.2 as described above. The cells were incubated for 18 h in fresh medium and then for an additional 30 h in medium containing various concentrations of dexamethasone. Extracts were prepared as described by Gorman et al. (26). β-Galactosidase activity was assayed as described previously (56), and chloramphenicol acetyltransferase (CAT) activity was assayed with D-*threo*-[dichloroacetyl-1,2,-^{14}C]chloramphenicol (2.15 GBq/mmol; Amersham) and 5 mM *n*-butyryl coenzyme A (Pharmacia Biotechnology) as described previously by Seed and Sheen (71). All results were normalized for β-galactosidase activity and expressed as percentages of maximal CAT activity seen with the normal receptor in the presence of 1 μM dexamethasone. For transrepression assays, COS-7 cells were transfected with 7 μg of hGR expression plasmid, 2 to 9 μg of the reporter plasmid −73 COL-CAT (80), 2.5 μg of the β-galactosidase expression plasmid pTB.1, and 1 to 8 μg of pBluGR5.2. The cells were incubated in fresh medium for 24 h, and various concentrations of dexamethasone were added. TPA was added 12 h later, and the incubation continued for an additional 12 h. β-Galactosidase and CAT activities were determined as described above, and the results were expressed as the percentage of CAT activity obtained in cells treated with TPA alone. Typically, TPA treatment increased CAT activity by 9- to 10-fold.

RESULTS

Ligand-binding properties of normal and mutant receptors. Glucocorticoid-resistant ICR27TK.3 cells contain a single copy of the mutant hGR gene *GRL753F* (2, 56). When assayed under conditions which inhibit GR activation, receptors from these cells bind [^{3}H]dexamethasone to the same extent, and with the same affinity, as normal receptors (50, 56). However, when assayed under conditions which promote receptor activation, the same receptors exhibit markedly reduced ligand-binding activity (3, 29). In addition, the rate of [^{3}H]dexamethasone dissociation from receptors present in extracts prepared from ICR27TK.3 cells is four to six times higher than the rate of dissociation from normal receptors (50). On the basis of these results, we proposed that after activation, receptors in which leucine 753 has been replaced by phenylalanine cannot retain ligand (56). Because GR activation results in the loss of the ability of even normal receptors to bind ligand (47), activated GRL753F receptors rapidly become ligand free (50), and in intact cells at steady state, only a small fraction of the receptors are occupied (29, 50, 56).

To demonstrate that the altered phenotype of receptors in ICR27TK.3 cells is an intrinsic property of the GRL753F mutation, COS-7 cells were transfected with either the normal hGR expression vector pRShGRα or the mutant expression vector pRShGR753F or pRShGR753F/421Y. Intact cells were assayed at 37°C for [^{3}H]dexamethasone-binding activity, and cell extracts were used to measure the rate of [^{3}H]dexamethasone dissociation from normal and mutant receptors. The results of the whole-cell-binding experiments showed that cells expressing either of the mutant receptors had 14% of the [^{3}H]dexamethasone-binding activity present in cells expressing normal receptors. In addition, the rate of [^{3}H]dexamethasone dissociation from GRL753F receptors was 0.036 min^{-1}, compared with 0.008 min^{-1} for dissociation from normal receptors (Fig. 1). These results are comparable to those obtained with mutant receptors in ICR27TK.3 cells (44) and, combined with the results of immunoblot analysis showing that mutant GRL753F receptors are not more susceptible to proteolysis than normal receptors (56), indicate that after activation in intact cells, GRL753F receptors are largely ligand free.

Transactivation of MMTV-CAT by normal and mutant receptors. We previously showed that at 1 μM dexamethasone, mutant GRL753F receptors only partially activated the mouse mammary tumor virus (MMTV) promoter (56). To more fully examine the ability of ligand-free receptors to activate transcription, transactivation was assayed over a range of agonist concentrations. In cells transfected with pRShGRα, half-maximal induction of CAT activity was observed at approximately

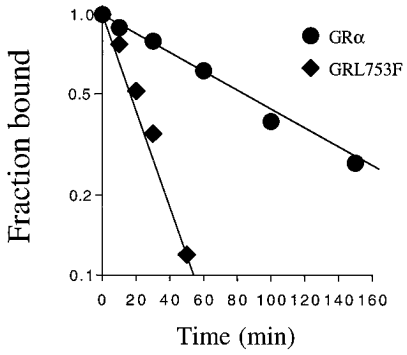

FIG. 1. Ligand dissociation from mutant GRL753F receptors is more rapid than dissociation from normal GR. Extracts prepared from COS-7 cells transfected with pRShGRα or pRShGR753F were incubated with 50 nM [^{3}H]dexamethasone in the absence or presence of 25 μM unlabeled dexamethasone for 2 h at 4°C. After the addition of 10 μM unlabeled dexamethasone to block reassociation of [^{3}H]dexamethasone, dissociation of specifically bound [^{3}H]dexamethasone was determined as described in Materials and Methods.

5 nM dexamethasone (Fig. 2). In contrast, the same level of induction in cells transfected with pRShGR753F required 1 μM hormone (Fig. 2). This is consistent with the decreased occupancy of the mutant receptor and provides direct evidence that bound ligand is required for efficient GR-mediated transactivation of target genes. As a control, induction of CAT activity was determined in cells transfected with the double mutant GRL753F/C421Y, in which the first cysteine residue of the proximal zinc finger is replaced by tyrosine. This mutant showed no activity, even at 1 μM dexamethasone.

A mutation comparable to GRL753F in the human estrogen receptor (L540Q) has been described (37) and was shown to be a powerful dominant-negative repressor of normal receptor activity. We therefore examined the ability of GRL753F to function as a dominant-negative repressor. At least at cotransfection ratios of 1:1, no such activity was found (Fig. 2). This is consistent with the observation that CEM-C7 cells, whose genotype is *GR$^+$/GRL753F* (56), are extremely sensitive to glucocorticoids (30, 48) and suggests that ligand-free GR do not compete with occupied GR for transactivation.

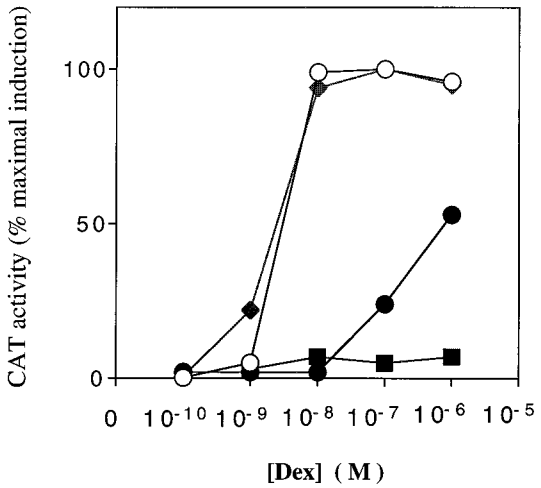

FIG. 2. Mutant GRL753F receptors are poor activators of an MMTV-CAT reporter gene and do not function as dominant-negative repressors. CV-1 cells were transfected with pRShGRα (○), pRShGR753F (●), pRShGR753F/421Y (■) or pRShGRα and pRShGR753F (◆), the reporter plasmid pMSG-CAT, and the β-galactosidase expression plasmid pCH110. Transfected cells were treated with various concentrations of dexamethasone (Dex) for 30 h and assayed for CAT and β-galactosidase activity as described in Materials and Methods. The results represent the averages of at least two independent experiments.

Mutant GRL753F receptors translocate to the nucleus. One possible explanation for the weak transactivating activity of mutant GRL753F receptors is that after exposure to ligand and activation, they fail to accumulate in the nucleus. This could also explain the absence of dominant-negative repressor activity. To determine the intracellular localization of GRL753F receptors after steroid treatment, CV-1 cells transfected with the mutant or wild-type receptor were treated with 10 nM dexamethasone and the receptors were visualized by indirect immunofluorescence with monospecific anti-GR antibodies (56). In the absence of ligand, both the normal and the mutant receptors were largely cytoplasmic (Fig. 3a and c). In contrast, after treatment with 10 nM dexamethasone, both receptors were clearly localized in the nucleus (Fig. 3b and d). Almost no receptor staining was seen in cells transfected with the control vector (Fig. 3e) or in preparations in which the anti-GR antibodies were preincubated with the synthetic peptide against which they were raised (Fig. 3f). These results are consistent with the observation that unoccupied GR can translocate to the nucleus (64) and indirectly suggest that even limited occupancy of GRL753F receptors is sufficient to promote dissociation from hsp90. In addition, they demonstrate that the weak transactivating activity of GRL753F receptors cannot be attributed to inefficient nuclear accumulation and provide additional support for the hypothesis that a specific ligand-induced conformational change is necessary for efficient transactivation.

Transrepression by GRL753F receptors. Direct protein-protein interactions between the GR and other nuclear transcription factors are responsible, at least in part, for the repression of some negatively regulated glucocorticoid-responsive genes (9, 20, 24, 38, 40, 43, 60, 62, 70, 77, 80). In particular, the repression of AP-1-inducible collagenase promoter activity involves the direct interaction of the GR with the Jun-Fos complex (20, 39, 43, 70, 76, 80). To evaluate the role of the ligand in modulating this interaction, the ability of mutant GRL753F receptors to repress TPA induction of the collagenase promoter was determined. Cells transfected with the mutant or wild-type receptor and the negatively regulated reporter gene −73 COL-CAT were treated with various concentrations of dexamethasone and then assayed for CAT activity. The results showed that in cells transfected with the normal receptor, half-maximal inhibition of collagenase promoter activity was observed at 50 pM dexamethasone, and maximum inhibition was seen between 0.1 and 1.0 nM hormone (Fig. 4). These concentrations are approximately 100-fold less than those required for half-maximal and maximal induction of the MMTV promoter (Fig. 3). In addition, they are well below those required for half-maximal and full occupancy of the hGR (31), suggesting that, at steady state, inhibition of the collagenase promoter requires that only a small fraction of the receptors be occupied. More importantly, unlike induction of MSG-CAT, the dose-response curve for GRL753F-mediated repression of AP-1-inducible collagenase promoter activity was indistinguishable from that obtained with the normal receptor (Fig. 4). Thus, in contrast to transactivation of the MMTV promoter, GR-mediated repression of AP-1 activity does not appear to require the continuous presence of bound ligand.

The addition of hormone to cells which had not been transfected with either the normal or mutant receptor failed to block the TPA-induced increase in CAT activity (data not shown), demonstrating that inhibition of AP-1 responsive promoter activity is hGR dependent. In addition, the double mutant GRL753F/C421Y was as effective as either the single

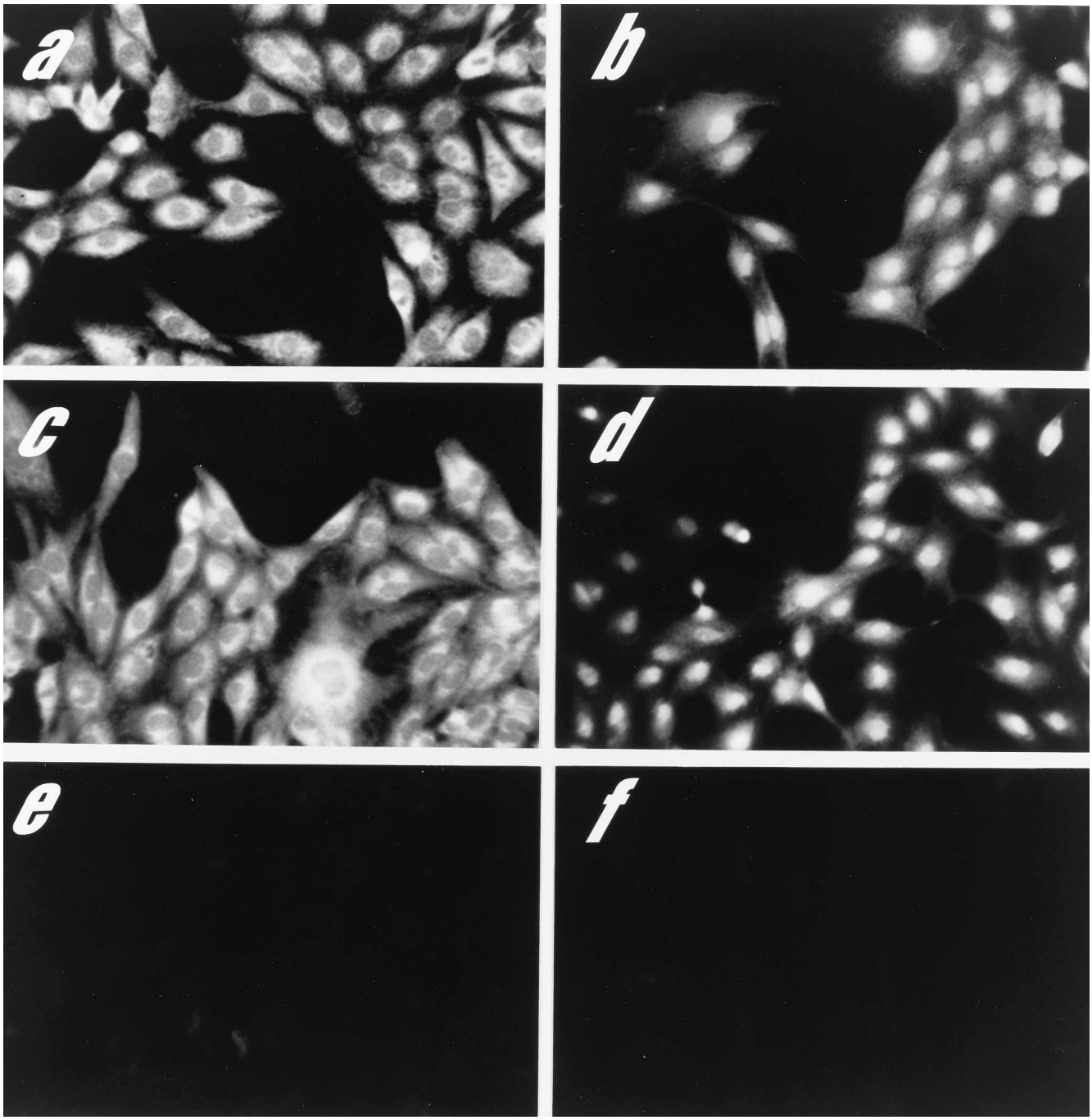

FIG. 3. Mutant GRL753F receptors translocate to the nucleus after steroid treatment. CV-1 cells transfected with pRShGRα (a, b, and f), pRShGR753F (c and d), or a control plasmid (e) were incubated in the absence (a, c, and e) or presence (b, d, and f) of 10 nM dexamethasone for 24 h. GR was visualized with the anti-hGR antibody 710 (a, b, c, d, and e) or the same antibody preincubated with the peptide against which it was raised (f).

mutant GRL753F or the normal receptor (Fig. 5). This is consistent with other reports that although portions of the DNA-binding domain are required for transrepression, specific binding of the GR to its cognate response element is not required (39, 70). Thus, GR-mediated transrepression is independent of both the bound ligand and specific DNA binding.

GR-dependent transrepression is stimulated by RU38486. The results presented above suggest that bound ligand is not required for inhibition of TPA-induced AP-1 activity. If this is the case, then other ligands which promote receptor activation and dissociation from hsp90 but which do not appear to induce a ligand-binding domain conformation suitable for GR-mediated transactivation might also stimulate transrepression. To test this hypothesis, the ability of RU38486 to inhibit TPA induction of the collagenase promoter was determined. Although RU38486 is somewhat less efficient than dexamethasone or other agonists in promoting receptor activation (27, 28, 72), it can efficiently mediate in vitro activation and in vivo

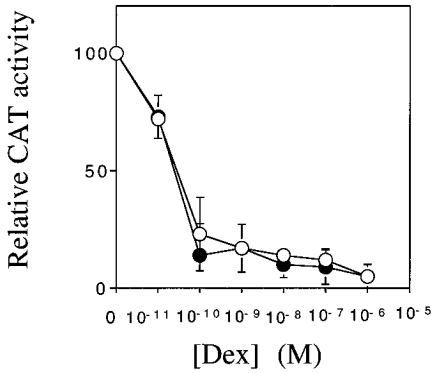

FIG. 4. Mutant GRL753F receptors repress TPA-inducible collagenase promoter activity. COS-7 cells were transfected with pRShGRα (○) or pRShGR 753F (●), the reporter plasmid −73 COL-CAT, and the β-galactosidase expression plasmid pTB.1. Transfected cells were treated with various concentrations of dexamethasone (Dex) for 24 h, during which, for the last 12 h, TPA (60 ng ml^{-1}) was present. CAT and β-galactosidase activities were assayed as described in Materials and Methods. The results represent the averages of three independent experiments ± the standard deviations.

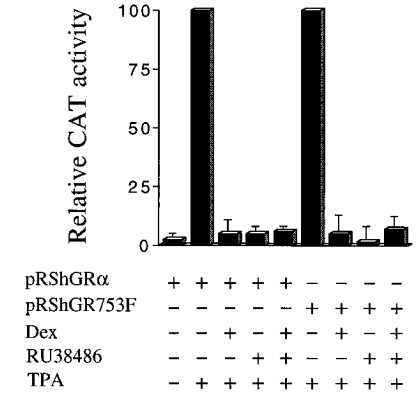

FIG. 6. Inhibition of TPA-induced COL-CAT activity by the glucocorticoid "antagonist" RU38486. COS-7 cells were transfected with pRShGRα or pRShGR753F, the reporter plasmid −73 COL-CAT, and the β-galactosidase expression plasmid pTB.1. Transfected cells were treated with 60 ng of TPA ml^{-1}, 100 nM dexamethasone (Dex), and/or 100 nM RU38486 as indicated. CAT and β-galactosidase activities were assayed as described in the legend to Fig. 4. The results represent the averages of four or five independent determinations ± the standard deviations.

nuclear translocation of the GR (22, 59, 68). Indeed, in cells transfected with either the wild-type or mutant receptor, RU38486 efficiently induced nuclear translocation (data not shown). More importantly, 100 nM RU38486 failed to antagonize and in fact was as effective as 100 nM dexamethasone in blocking TPA-induced COL-CAT activity in cells transfected with normal receptor (Fig. 6). The same result was obtained in cells transfected with mutant GRL753F receptors (Fig. 6). Hence, it appears that any ligand which promotes receptor dissociation from hsp90 and nuclear translocation can function as an agonist with respect to transrepression of AP-1 activity.

Heat shock-induced transrepression of AP-1 activity. In addition to ligand-induced activation, other, nonhormonal stimuli have been shown to promote nuclear accumulation of GR (64, 73). In particular, heat shock induces nuclear GR accumulation and weak transactivation of the MMTV promoter (64). To examine further the role of the ligand in GR-mediated transrepression of AP-1 activity, we therefore compared the effects of thermal stress on transactivation of the MMTV promoter and transrepression of the collagenase promoter. The results of these experiments show that incubation of COS-7 cells transfected with pRShGRα at 43°C for 1 h was sufficient to induce nuclear accumulation of the hGR protein (Fig. 7). More importantly, heat shock had little or no effect on MMTV promoter activity in the absence or presence of transfected GR (Fig. 8A). However, incubation of cells at 43°C prior to the addition of TPA resulted in an 85% reduction in CAT activity (Fig. 8B). This inhibition was completely dependent upon the presence of transfected hGR, since in cells transfected with the control plasmid, heat shock had no effect on TPA-induced CAT activity (Fig. 8B). Heat shock-induced transrepression of AP-1 activity was also seen in HeLa cells transfected with −73 COL-CAT (Fig. 9), indicating that the repression seen in COS-7 cells was not the result of overexpression of GR. Thus, it appears that any stimulus which promotes GR activation and nuclear localization is sufficient to repress AP-1-responsive collagenase gene promoter activity.

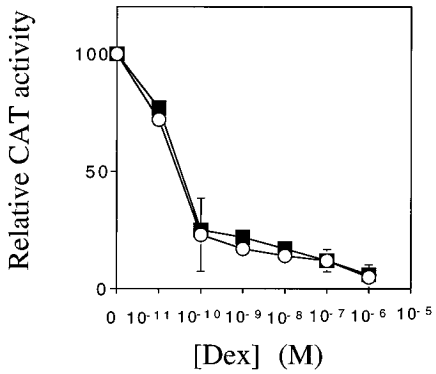

FIG. 5. The double mutant GRL753F/C421Y containing mutations in both the DNA- and ligand-binding domains represses TPA-inducible collagenase promoter activity. COS-7 cells were transfected with pRShGRα (○) or pRShGR 753F/421Y (■), the reporter plasmid −73 COL-CAT, and the β-galactosidase expression plasmid pTB.1. Transfected cells were treated with various concentrations of dexamethasone (Dex) for 24 h, during which, for the last 12 h, TPA (60 ng ml^{-1}) was present. CAT and β-galactosidase activities were assayed as described in the legend to Fig. 4. The results represent the averages of three independent experiments ± the standard deviations.

DISCUSSION

Several lines of evidence indicate that bound agonist is required for the efficient, in vivo, GR-mediated transactivation of genes containing canonical glucocorticoid response elements (52, 65). Presumably, this reflects the need to maintain the ligand-binding domain in a conformation suitable for productive interaction of the receptor with other components of the transcription apparatus. However, the role of the ligand in negative regulation of gene expression is less clearly defined. This is due, in part, to the multiplicity of mechanisms which have been described for GR-mediated repression of different genes (1, 20, 21, 38, 39, 51, 62, 70, 80). In addition, it is often difficult to distinguish between the role of the ligand in stimulating receptor dissociation from the unactivated oligomeric complex to which it is tethered in the cytoplasm and its role in regulating the various functional activities which have been mapped to the ligand-binding domain (10, 14, 23, 34, 36, 58, 74, 78). Specifically, while there have been experiments in which receptors lacking the ligand-binding domain eliminate the hsp90 binding site (14, 36, 58), thereby leaving the receptor free to bind to DNA or interact with other transcription fac-

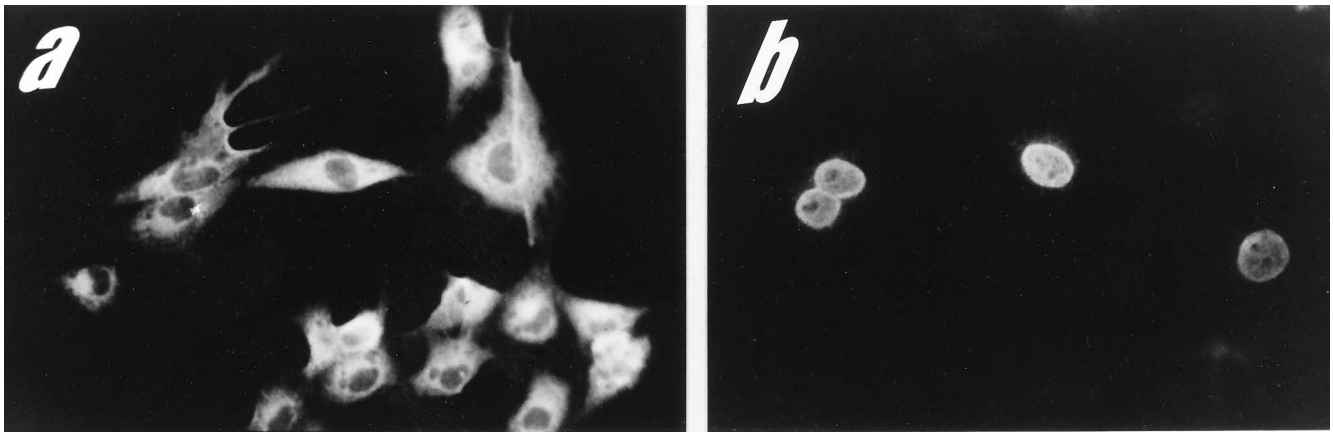

FIG. 7. Heat shock induces GR nuclear translocation. COS-7 cells transfected with pRShGRα were incubated at 37°C (a) or 43°C (b) for 1 h, and GR was visualized by indirect immunofluorescence as described in the legend to Fig. 3.

tors, they cannot address the potential contribution of the ligand-binding domain to these interactions.

Two approaches were used to examine the role of the ligand in facilitating holoreceptor-mediated transrepression of the collagenase promoter. The first used the hGR mutant L753F, from which the rate of ligand dissociation is abnormally high. Because, after dissociation from hsp90, the GR cannot rebind ligand (47), this mutant is effectively ligand free in its activated form, as demonstrated by the results of whole-cell-binding assays performed at 37°C (data not shown). In addition, despite the relative inability of mutant GRL753F receptors to retain bound ligand, they accumulate in the nucleus after exposure to hormone to the same extent as normal, occupied receptors do, thereby providing a novel system to evaluate the role of the ligand in transactivation and transrepression.

Mutant GRL753F receptors were only weak activators of transcription from the MMTV promoter, confirming the notion that bound ligand is required for efficient transactivation. This is consistent with the effects of other mutations in the carboxyl-terminal region of the GR encoded by exon 9. These mutations commonly result in the alteration or complete loss of ligand-binding activity and/or decreased transactivating activity (12, 13, 15, 16, 41, 44). Interestingly, an analogous mutation in the human estrogen receptor results in a receptor with a potent dominant-negative phenotype (37). This was not the case for L753F in the hGR (Fig. 2) or for the double mutant M758A/L759A in the mouse GR (15), suggesting that despite the sequence homology between the carboxyl-terminal regions of the GR and estrogen receptor in the region surrounding L753, these regions do not share complete functional homology.

Despite the lack of transactivating activity, GRL753F receptors were potent negative regulators of TPA-induced collagenase promoter activity. The dose-response curves for mutant and normal receptors were indistinguishable, suggesting that retention of bound ligand is not required for transrepression and, by extension, that transrepression of AP-1 activity does

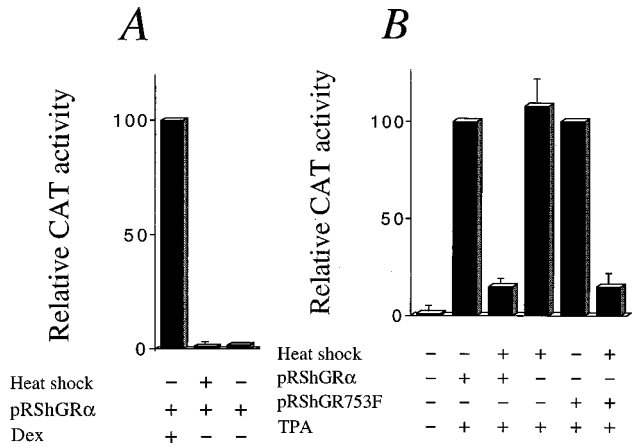

FIG. 8. Heat shock selectively induces GR-mediated transrepression. (A) Cells transfected with pRShGRα, MSG-CAT, and pCH110 were incubated for 1 h at 43°C and were returned to 37°C for an additional 14 h or treated with 10 nM dexamethasone (Dex) for 30 h. CAT and β-galactosidase activities were assayed as described in the legend to Fig. 2. The results represent the averages of three independent determinations ± the standard deviations. (B) Cells transfected with pRShGRα, pRShGR753F, or control plasmid and with −73 COL-CAT and pTB.1 were incubated for 1 h at 37°C or 43°C as indicated. The cells were incubated for 6 h at 37°C and then treated with TPA for an additional 12 h. CAT and β-galactosidase activities were determined as described in the legend to Fig. 4. The results represent the averages of three to six independent determinations ± the standard deviations.

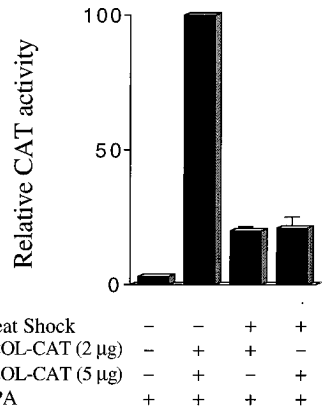

FIG. 9. Heat shock induces transrepression of AP-1 activity in HeLa cells containing endogenous hGR. HeLa cells transfected with −73 COL-CAT were incubated for 1 h at 37°C or 43°C as indicated. The cells were incubated for 17 h at 37°C and then treated with TPA for an additional 12 h. CAT and β-galactosidase activities were determined as described in the legend to Fig. 4. The results represent the averages of three independent determinations ± the standard deviations.

not require the same ligand-binding domain conformation necessary for transactivation. Interestingly, the concentration of dexamethasone required for a half-maximal response in cells transfected with either receptor was 50 pM, nearly 100-fold less than the concentration required for half-maximal induction of the MMTV promoter by normal GR and well below the K_d of the hGR for dexamethasone (31). Transfection of smaller amounts of either the normal or mutant receptor shifted the respective dose-response curves to the right (data not shown), indicating that at the concentrations of GR present in transfected cells, there are a large number of spare receptors. These data also raise the possibility that at subsaturating concentrations of ligand, the balance between transactivation and transrepression of different genes is dependent on the level of receptor expression.

The second approach used to examine the role of bound ligand in transrepression of AP-1 activity utilized stimuli which promote GR nuclear accumulation but not transactivation. In cells transfected with either normal or GRL753F receptors and treated with the glucocorticoid "antagonist" RU38486, treatment resulted in an almost complete inhibition of TPA-induced CAT activity. This result confirms the recent report of Heck et al. (32) and suggests not only that a specific, agonist-induced conformation of the ligand-binding domain is unnecessary for repression of AP-1 activity but that other conformations unsuited for transactivation may be allowed as well. There are a few other examples in which RU38486 behaves as an agonist. RU38486 elicited the same posttranscriptional down-regulation of GR mRNA expression induced by agonists in both transfected cells in which GR mRNA expression is under the control of the Rous sarcoma virus promoter (7) and in cells in which GR expression was under the control of its own promoter (33). In addition, RU38486 behaved as a partial agonist with respect to repression of a promoter containing the negative glucocorticoid response element from the rat prolactin gene in transfected COS-7 cells (8). While it has not been demonstrated that repression of these genes involves protein-protein interactions of the GR with AP-1 or other transcription factors, these results indicate that ligand-independent, GR-mediated transrepression is not unique to the collagenase gene.

Heat shock, which also elicited nuclear accumulation of GR, was a poor activator of the MMTV promoter, confirming previous results (65, 73). However, a brief exposure to an elevated temperature was sufficient to completely block TPA induction of collagenase promoter activity. Thus, by several different criteria, we conclude that in contrast to GR-mediated transactivation, nuclear translocation of the receptor, regardless of its state of occupancy, is both necessary and sufficient for transrepression of the collagenase promoter. It is therefore possible that the GR could act as a second messenger for nonsteroid-hormone-mediated signal transduction pathways whose target is repression of AP-1-modulated transcription (Fig. 10). In some ways, this would be analogous to ligand-independent activation of the estrogen, progesterone, vitamin D, and thyroid hormone β receptors (55) and the orphan receptor COUP (54). However, in these cases, phosphorylation of the receptor, or a receptor cofactor, appears to substitute for or obviate the need for a ligand-induced conformational change (19, 53), whereas there is no evidence that phosphorylation is required for ligand-independent GR activity. More importantly, ligand-free (or RU38486-occupied) nuclear GR did not activate MMTV transcription, suggesting that unlike the ligand-independent activation of other steroid hormone receptors, GR-mediated activation of genes containing a cognate hormone response element is still hormone dependent. Thus, only a subset of genes may be affected by ligand-independent activation of the GR, thereby providing a potential mechanism for the differential regulation of glucocorticoid-responsive genes. Indeed, cells which express only GRL753F receptors show none of the typical glucocorticoid responses seen in parental cells expressing normal GR (50, 56).

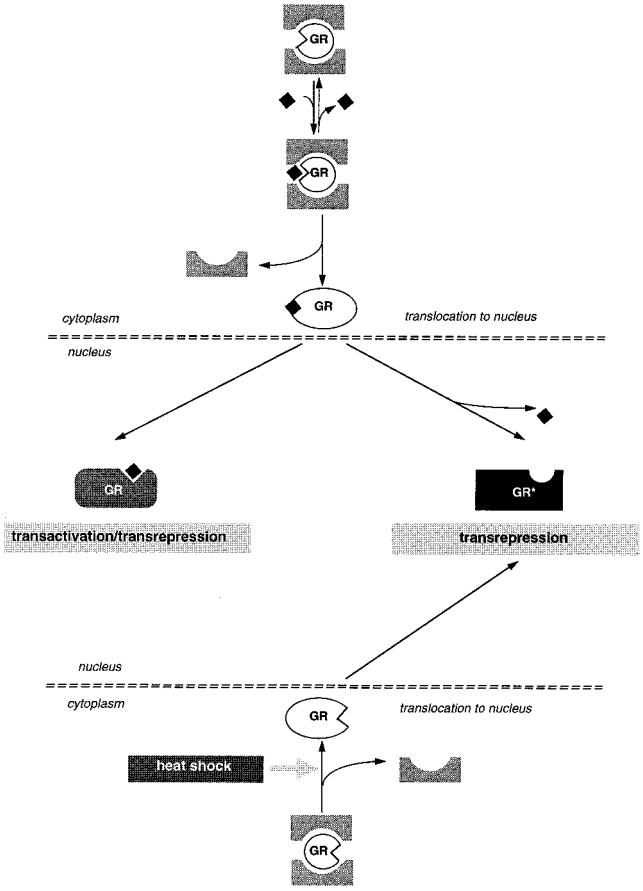

FIG. 10. A mechanism for ligand-independent transrepression of the collagenase promoter. The binding of agonists such as dexamethasone (◆) induces the dissociation of the receptor from the GR-hsp90 complex and translocation to the nucleus and induces a conformational change necessary for efficient transactivation. This form of the receptor is also an efficient repressor of AP-1 activity. The dissociation of the agonist produces a different conformation that is an efficient transrepressor but not a transactivator. Selective repressor activity can also result from the binding of antagonists of transactivation which promote nuclear translocation but induce a different receptor conformation (not shown) or from nonhormonal stimuli, such as heat shock, which induce nuclear accumulation of the unoccupied receptor.

The results presented here do not address the nature of the interaction between the GR and AP-1. However, they provide strong direct evidence that ligand-induced conformational changes are not involved in the transrepression of the collagenase promoter. In addition, the observation that the double mutant GRL753F/C421Y was also an effective transrepressor suggests that specific DNA binding is not required. This is in apparent conflict with the observations of others that disruption of the GR DNA-binding domain markedly decreases transrepression (20, 38, 76, 80). However, these studies examined the transrepression of promoters containing multiple TPA response elements in various configurations. In contrast, when only a single TPA response element such as that in the −73 COL-CAT gene used in this study was present, specific DNA binding was not necessary (70). In addition, in vitro GR inhi-

bition of AP-1 binding did not require an intact DNA-binding domain structure (39). Thus, at least for repression of the −73 COL-CAT promoter, a specific conformation of the DNA-binding domain is not required. For other genes, whose repression may require DNA binding and/or interaction with other transcription factors, the conformation of the DNA-binding domain could be more critical. In addition, GR-mediated inhibition of other transcription factors may require specific ligand-induced conformational changes not necessary for the inhibition of AP-1 activity. If this is the case, then it may be possible to identify specific ligands or other stimuli which differentially regulate their expression.

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REFERENCES

1. **Akerblom, I. W., E. P. Slater, M. Beato, J. D. Baxter, and P. L. Mellon.** 1988. Negative regulation by glucocorticoids through interference with a cAMP responsive enhancer. Science **241:**350–353.
2. **Allan, G. F., S. Y. Tsai, M.-J. Tsai, and B. W. O'Malley.** 1992. Ligand-dependent conformational changes in the progesterone receptor are necessary for events that follow DNA binding. Proc. Natl. Acad. Sci. USA **89:** 11750–11754.
3. **Ashraf, J., and E. B. Thompson.** 1993. Identification of the activation-labile gene—a single point mutation in the human glucocorticoid receptor presents as 2 distinct receptor phenotypes. Mol. Endocrinol. **7:**631–642.
4. **Beato, M.** 1991. Transcriptional control by nuclear receptors. FASEB J. **5:**2044–2051.
5. **Beekman, J. M., G. F. Allan, S. Y. Tsai, M.-J. Tsai, and B. W. O'Malley.** 1993. Transcriptional activation by the estrogen receptor requires a conformational change in the ligand binding domain. Mol. Endocrinol. **7:**1266–1274.
6. **Borras, T., C. A. Peterson, and J. Piatigorsky.** 1988. Evidence for positive and negative regulation in the promoter of the chicken delta 1-crystallin gene. Dev. Biol. **127:**209–219.
7. **Burnstein, K. L., C. M. Jewell, and J. A. Cidlowski.** 1990. Human glucocorticoid receptor cDNA contains sequences sufficient for receptor down-regulation. J. Biol. Chem. **265:**7284–7291.
8. **Cairns, C., W. Cairns, and S. Okret.** 1993. Inhibition of gene expression by steroid hormone receptors via a negative glucocorticoid response element—evidence for the involvement of DNA-binding and agonistic effects of the antiglucocorticoid antiprogestin RU486. DNA Cell Biol. **12:**695–702.
9. **Celada, A., S. McKercher, and R. A. Maki.** 1993. Repression of major histocompatibility complex-IA expression by glucocorticoids: the glucocorticoid receptor inhibits the DNA binding of the X-box DNA binding protein. J. Exp. Med. **177:**691–698.
10. **Chakraborti, P. K., and S. S. Simons, Jr.** 1991. Association of heat shock protein-90 with the 16kDa steroid binding core fragment of rat glucocorticoid receptors. Biochem. Biophys. Res. Commun. **176:**1338–1344.
11. **Chen, C., and H. Okayama.** 1987. High-efficiency transformation of mammalian cells by plasmid DNA. Mol. Cell. Biol. **7:**2745–2752.
12. **Chen, D., K. Kohli, S. Zhang, M. Danielsen, and M. R. Stallcup.** 1994. Phenylalanine-780 near the C-terminus of the mouse glucocorticoid receptor is important for ligand binding affinity and specificity. Mol. Endocrinol. **8:**422–430.
13. **Chen, D. G., and M. R. Stallcup.** 1994. The hormone-binding role of 2 cysteines near the C terminus of the mouse glucocorticoid receptor. J. Biol. Chem. **269:**7914–7918.
14. **Dalman, F. C., L. C. Scherrer, L. P. Taylor, H. Akil, and W. B. Pratt.** 1991. Localization of the 90-kDa heat shock protein-binding site within the hormone-binding domain of the glucocorticoid receptor by peptide competition. J. Biol. Chem. **266:**3482–3490.
15. **Danielian, P. S., R. White, J. A. Lees, and M. G. Parker.** 1992. Identification of a conserved region required for hormone dependent transcriptional activation by steroid hormone receptors. EMBO J. **11:**1025–1033.
16. **Danielsen, M., J. P. Northrop, and G. M. Ringold.** 1986. The mouse glucocorticoid receptor: mapping of functional domains by cloning, sequencing and expression of wild-type and mutant receptor proteins. EMBO J. **5:**2513–2522.
17. **Denis, M., L. Poellinger, A.-C. Wikström, and J.-Å. Gustafsson.** 1988. Requirement of hormone for thermal conversion of the glucocorticoid receptor to a DNA-binding state. Nature (London) **333:**686–688.
18. **Denis, M., A.-C. Wikström, and J.-Å. Gustafsson.** 1987. The molybdate-stabilized nonactivated glucocorticoid receptor contains a dimer of Mr 90,000 non-hormone binding protein. J. Biol. Chem. **262:**11803–11806.
19. **Denner, L. A., N. L. Weigel, B. L. Maxwell, W. T. Schrader, and B. W. O'Malley.** 1990. Regulation of progesterone receptor-mediated transcription by phosphorylation. Science **250:**1740–1743.
20. **Diamond, M. I., J. N. Miner, S. K. Yoshinaga, and K. R. Yamamoto.** 1990. Transcription factor interactions: selectors of positive or negative regulation from a single DNA element. Science **249:**1266–1272.
21. **Drouin, J., M. A. Trifiro, R. K. Plante, M. Nemer, P. Eriksson, and Ö. Wrange.** 1989. Glucocorticoid receptor binding to a specific DNA sequence is required for hormone-dependent repression of pro-opiomelanocortin gene transcription. Mol. Cell. Biol. **9:**5305–5314.
22. **Elsasser, M. S., L. P. Eisen, A. T. Riegel, and J. M. Harmon.** 1991. Stability and sequence-specific DNA binding of activation-labile mutants of the human glucocorticoid receptor. Biochemistry **30:**11140–11146.
23. **Fawell, S. E., J. A. Lees, R. White, and M. G. Parker.** 1990. Characterization and colocalization of steroid binding and dimerization activities in the mouse estrogen receptor. Cell **60:**953–962.
24. **Gauthier, J. M., B. Bourachot, V. Doucas, M. Yaniv, and F. Moreau-Gachelin.** 1993. Functional interference between the Spi-1/PU.1 oncoprotein and steroid hormone or vitamin receptors. EMBO J. **12:**5089–5096.
25. **Giguere, V., S. M. Hollenberg, M. G. Rosenfeld, and R. M. Evans.** 1986. Functional domains of the glucocorticoid receptor. Cell **46:**645–652.
26. **Gorman, C. M., G. T. Merlino, M. C. Willingham, I. Pastan, and B. H. Howard.** 1982. The Rous sarcoma virus long terminal repeat is a strong promoter when introduced into a variety of eukaryotic cells by DNA-mediated transfection. Proc. Natl. Acad. Sci. USA **79:**6777–6781.
27. **Groyer, A., G. Schweizer-Groyer, F. Cadepond, M. Mariller, and E.-E. Baulieu.** 1987. Antiglucocorticosteroid effects suggest why steroid is required to bind DNA in vivo but not in vitro. Nature (London) **328:**624–626.
28. **Gruol, D. J., and K. A. Wolfe.** 1990. Transformation of glucocorticoid receptors bound to the antagonist RU-486: effects of alkaline phosphatase. Biochemistry **29:**7958–7966.
29. **Harmon, J. M., M. S. Elsasser, L. P. Eisen, L. A. Urda, J. Ashraf, and E. B. Thompson.** 1989. Glucocorticoid receptor expression in receptorless mutants isolated from the human leukemic cell line CEM-C7. Mol. Endocrinol. **3:**734–743.
30. **Harmon, J. M., M. R. Norman, B. J. Fowlkes, and E. B. Thompson.** 1979. Dexamethasone induces irreversible G1 arrest and death of a human lymphoid cell line. J. Cell. Physiol. **98:**267–278.
31. **Harmon, J. M., and E. B. Thompson.** 1981. Isolation and characterization of dexamethasone-resistant mutants from human lymphoid cell line CEM-C7. Mol. Cell. Biol. **1:**512–521.
32. **Heck, S., M. Kullmann, A. Gast, H. Ponta, H. J. Rahmsdorf, P. Herrlich, and A. C. B. Cato.** 1994. A distinct modulating domain in glucocorticoid receptor monomers in the repression of activity of the transcription factor AP-1. EMBO J. **13:**4087–4095.
33. **Hoeck, W., S. Rusconi, and B. Groner.** 1989. Down-regulation and phosphorylation of glucocorticoid receptors in cultured cells: investigations with a monospecific antiserum against a bacterially expressed receptor fragment. J. Biol. Chem. **264:**14396–14402.
34. **Hollenberg, S. M., and R. M. Evans.** 1988. Multiple and cooperative transactivation domains of the human glucocorticoid receptor. Cell **55:**899–906.
35. **Howard, K. J., and C. W. Distelhorst.** 1988. Evidence for intracellular association of the glucocorticoid receptor with the 90-kDa heat shock protein. J. Biol. Chem. **263:**3474–3481.
36. **Howard, K. J., S. J. Holley, K. R. Yamamoto, and C. W. Distelhorst.** 1990. Mapping the hsp90 binding region of the glucocorticoid receptor. J. Biol. Chem. **265:**11928–11935.
37. **Ince, B. A., Y. Zhuang, C. K. Wrenn, D. J. Shapiro, and B. S. Katzenellenbogen.** 1993. Powerful dominant negative mutants of the human estrogen receptor. J. Biol. Chem. **268:**14026–14032.
38. **Jonat, C., H. J. Rahmsdorf, K. K. Park, A. C. B. Cato, S. Gebel, H. Ponta, and P. Herrlich.** 1990. Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. Cell **62:** 1189–1204.
39. **Kerppola, T. K., D. Luk, and T. Curran.** 1993. Fos is a preferential target of glucocorticoid receptor inhibition of AP-1 activity in vitro. Mol. Cell. Biol. **13:**3782–3791.
40. **Kutoh, E., P.-E. Strömstedt, and L. Poellinger.** 1992. Functional interference between the ubiquitous and constitutive octamer transcription factor-1 (OTF-1) and the glucocorticoid receptor by direct protein-protein interaction involving the homeo subdomain of OTF-1. Mol. Cell. Biol. **12:**4960–4969.
41. **Lanz, R. B., M. Hug, M. Gola, T. Tallone, S. Wieland, and S. Rusconi.** 1994. Active, interactive, and inactive steroid receptor mutants. Steroids **59:**148–152.
42. **Lebeau, M. C., N. Massol, J. Herrick, L. E. Faber, J. M. Renoir, C. Radanyi, and E.-E. Baulieu.** 1992. P59, an hsp 90-binding protein: cloning and sequencing of its cDNA and preparation of a peptide-directed polyclonal antibody. J. Biol. Chem. **267:**4281–4284.

43. **Lucibello, F. C., E. P. Slater, K. U. Jooss, M. Beato, and R. Müller.** 1990. Mutual transrepression of Fos and the glucocorticoid receptor: involvement of a functional domain in Fos which is absent in FosB. EMBO J. **9:**2827–2834.
44. **Malchoff, D. M., A. Brufsky, G. Reardon, P. McDermott, E. C. Javier, C. H. Bergh, D. Rowe, and C. D. Malchoff.** 1993. A mutation of the glucocorticoid receptor in primary cortisol resistance. J. Clin. Invest. **91:**1918–1925.
45. **Mendel, D. B., J. E. Bodwell, B. Gametchu, R. W. Harrison, and A. Munck.** 1986. Molybdate-stabilized nonactivated glucocorticoid-receptor complexes contain a 90-kDa non-steroid-binding phosphoprotein that is lost on activation. J. Biol. Chem. **261:**3758–3763.
46. **Mordacq, J. C., and D. I. H. Linzer.** 1989. Co-localization of elements required for phorbol ester stimulation and glucocorticoid repression of proliferin gene expression. Genes Dev. **3:**760–769.
47. **Nemoto, T., Y. Ohara-Nemoto, M. Denis, and J.-Å. Gustafsson.** 1990. The transformed glucocorticoid receptor has a lower steroid-binding affinity than the nontransformed receptor. Biochemistry **29:**1880–1886.
48. **Norman, M. R., and E. B. Thompson.** 1977. Characterization of a glucocorticoid-sensitive human lymphoid cell line. Cancer Res. **37:**3785–3791.
49. **Oro, A. E., S. M. Hollenberg, and R. M. Evans.** 1988. Transcriptional inhibition by a glucocorticoid receptor-β-galactosidase fusion protein. Cell **55:**1109–1114.
50. **Palmer, L. A., and J. M. Harmon.** 1991. Biochemical evidence that glucocorticoid-sensitive cell lines derived from the human leukemic cell line CCRF-CEM express a normal and a mutant glucocorticoid receptor gene. Cancer Res. **51:**5224–5231.
51. **Pfahl, M.** 1993. Nuclear receptor/AP-1 interaction. Endocr. Rev. **14:**651–658.
52. **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 (London) **348:**166–168.
53. **Power, R. F., O. M. Conneely, and B. W. O'Malley.** 1992. New insights into activation of the steroid hormone receptor superfamily. Trends Pharmacol. Sci. **13:**318–323.
54. **Power, R. F., J. P. Lydon, O. M. Conneely, and B. W. O'Malley.** 1991. Dopamine activation of an orphan of the steroid receptor superfamily. Science **252:**1546–1548.
55. **Power, R. F., S. K. Mani, J. Codina, O. M. Conneely, and B. W. O'Malley.** 1991. Dopaminergic and ligand-independent activation of steroid hormone receptors. Science **254:**1636–1639.
56. **Powers, J. H., A. G. Hillmann, D. C. Tang, and J. M. Harmon.** 1993. Cloning and expression of mutant glucocorticoid receptors from glucocorticoid-sensitive and glucocorticoid-resistant human leukemic cells. Cancer Res. **53:**4059–4065.
57. **Pratt, W. B., M. J. Czar, L. F. Stancato, and J. K. Owens.** 1993. The hsp56 immunophilin component of steroid receptor heterocomplexes: could this be the elusive nuclear localization signal-binding protein? J. Steroid Biochem. Mol. Biol. **46:**269–279.
58. **Pratt, W. B., D. J. Jolly, D. V. Pratt, S. M. Hollenberg, V. Giguere, F. M. Cadepond, G. Schweizer-Groyer, M. G. Catelli, R. M. Evans, and E.-E. Baulieu.** 1988. A region in the steroid binding domain determines formation of the non-DNA binding, 9 S glucocorticoid receptor complex. J. Biol. Chem. **263:**267–273.
59. **Qi, M., L. J. Stasenko, and D. B. DeFranco.** 1990. Recycling and desensitization of glucocorticoid receptors in v-mos transformed cells depend on the ability of nuclear receptors to modulate gene expression. Mol. Endocrinol. **4:**455–464.
60. **Ray, A., and K. E. Prefontaine.** 1994. Physical association and functional antagonism between the p65 subunit of transcription factor NF-kappaB and the glucocorticoid receptor. Proc. Natl. Acad. Sci. USA **91:**752–756.
61. **Rexin, M., W. Busch, B. Segnitz, and U. Gehring.** 1992. Structure of the glucocorticoid receptor in intact cells in the absence of hormone. J. Biol. Chem. **267:**9619–9621.
62. **Sakai, D. D., S. Helms, J. Carlstedt-Duke, J. Å. Gustafsson, F. M. Rottman, and K. R. Yamamoto.** 1988. Hormone-mediated repression: a negative glucocorticoid response element from the bovine prolactin gene. Genes Dev. **2:**1144–1154.
63. **Sanchez, E. R.** 1990. Hsp56—a novel heat shock protein associated with untransformed steroid receptor complexes. J. Biol. Chem. **265:**22067–22070.
64. **Sanchez, E. R.** 1992. Heat shock induces translocation to the nucleus of the unliganded glucocorticoid receptor. J. Biol. Chem. **267:**17–20.
65. **Sanchez, E. R., J. L. Hu, S. J. Zhong, P. Shen, M. J. Greene, and P. R. Housley.** 1994. Potentiation of glucocorticoid receptor-mediated gene expression by heat and chemical shock. Mol. Endocrinol. **8:**408–421.
66. **Sanchez, E. R., S. Meschinchi, W. Tienrungroj, M. J. Schlesinger, D. O. Toft, and W. B. Pratt.** 1987. Relationship of the 90-kDa murine heat shock protein to the untransformed and transformed states of the L cell glucocorticoid receptor. J. Biol. Chem. **262:**6986–6991.
67. **Sanchez, E. R., D. O. Toft, M. J. Schlesinger, and W. B. Pratt.** 1985. Evidence that the 90-kDa phosphoprotein associated with the untransformed L-cell glucocorticoid receptor is a murine heat shock protein. J. Biol. Chem. **260:**12398–12401.
68. **Schmidt, T. J.** 1989. Comparison of in vivo activation of triamcinolone acetonide- and RU38486-receptor complexes in the CEM-C7 and IM-9 human leukemic cell lines. Cancer Res. **49:**4390–4395.
69. **Schüle, R., and R. M. Evans.** 1991. Cross-coupling of signal transduction pathways: zinc finger meets leucine zipper. Trends Genet. **7:**377–381.
70. **Schüle, R., P. Rangarajan, S. Kliewer, L. J. Ransone, J. Bolado, N. Yang, I. M. Verma, and R. M. Evans.** 1990. Functional antagonism between oncoprotein c-jun and the glucocorticoid receptor. Cell **62:**1217–1226.
71. **Seed, B., and J. Y. Sheen.** 1988. A simple phase-extraction assay for chloramphenicol acetyltransferase activity. Gene **67:**271–277.
72. **Segnitz, B., and U. Gehring.** 1990. Mechanism of action of a steroidal antiglucocorticoid in lymphoid cells. J. Biol. Chem. **265:**2789–2796.
73. **Shen, P., Z. J. Xie, H. Li, and E. R. Sanchez.** 1993. Glucocorticoid receptor conversion to high affinity nuclear binding and transcription enhancement activity in Chinese hamster ovary cells subjected to heat and chemical stress. J. Steroid Biochem. Mol. Biol. **47:**55–64.
74. **Simons, S. S., Jr., F. D. Sistare, and P. Chakraborti.** 1989. Steroid binding activity is retained in a 16-kDa fragment of the steroid binding domain of rat glucocorticoid receptors. J. Biol. Chem. **264:**14493–14497.
75. **Strömstedt, P.-E., L. Poellinger, J.-Å. Gustafsson, and J. Carlstedt-Duke.** 1991. The glucocorticoid receptor binds to a sequence overlapping the TATA box of the human osteocalcin promoter: a potential mechanism for negative regulation. Mol. Cell. Biol. **11:**3379–3383.
76. **Touray, M., F. Ryan, R. Jaggi, and F. Martin.** 1991. Characterisation of functional inhibition of the glucocorticoid receptor by fos/jun. Oncogene **6:**1227–1234.
77. **Tverberg, L. A., and A. F. Russo.** 1992. Cell-specific glucocorticoid repression of calcitonin/calcitonin gene-related peptide transcription: localization to an 18-base pair basal enhancer element. J. Biol. Chem. **267:**17567–17573.
78. **Webster, N. J. G., S. Green, J. R. Jin, and P. Chambon.** 1988. The hormone-binding domains of the estrogen and glucocorticoid receptors contain an inducible transactivation function. Cell **54:**199–207.
79. **Willmann, T., and M. Beato.** 1986. Steroid-free glucocorticoid receptor binds specifically to mouse mammary virus tumor DNA. Nature (London) **324:**688–691.
80. **Yang-Yen, H. F., J. C. Chambard, Y. L. Sun, T. Smeal, T. J. Schmidt, J. Drouin, and M. Karin.** 1990. Transcriptional interference between c-jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein-protein interaction. Cell **62:**1205–1215.
81. **Yem, A. W., A. G. Tomasselli, R. L. Heinrikson, H. Zurcher-Neely, V. A. Ruff, R. A. Johnson, and M. R. Deibel.** 1992. The hsp56 component of steroid receptor complexes binds to immobilized FK506 and shows homology to FKBP-12 and FKBP-13. J. Biol. Chem. **267:**2868–2871.