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Immunocytochemical analysis of Hormone Mediated Nuclear Translocation of Wild Type and Mutant Glucocorticoid Receptors

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We have analyzed structural and functional features of the human glucocorticoid receptor (hGR) for their effects on receptor subcellular distribution. COS 1 cells transiently transfected with wild type and mutant hGR cDNAs were assessed immunocytochemically using well-characterized antipeptide antibodies to the hGR. The effect of administration of steroid hormones (and the antiglucocorticoid RU486) on receptor localization was evaluated. Unliganded wild type receptors expressed in COS 1 cells were predominately cytoplasmic. Addition of glucocorticoids or the glucocorticoid receptor antagonist, RU486, resulted in complete translocation of these receptors into the nucleus whereas non-glucocorticoid steroids or dibutyryl cAMP were not effective in promoting nuclear translocation. Thus, nuclear translocation was specific for steroids capable of high affinity binding to the hGR. To elucidate the potential role of receptor domains in receptor localization, COS 1 cells transiently transfected with various receptor cDNA mutants were analyzed in a similar manner. Translocation of an hGR deletion mutant lacking the majority of the amino terminus (deletion of amino acids 77–262) was identical to the wild type receptor despite the absence of a transactivation domain. Receptors in which the DNA binding domain was either partially or totally deleted showed an impaired capacity to undergo hormone-inducible nuclear translocation. Deletion of the hinge region of the hGR (which also contains part of the nuclear localization signal, NL1) resulted in receptor localization in the cytoplasm. Mutants in the ligand binding domain exhibited two localization phenotypes, exclusively nuclear or cytoplasmic. Receptor mutants truncated after amino acid 550 were found in the nucleus in the presence and absence of hormone consistent with the existence of nuclear localization inhibitory sequences in the ligand binding domain of the receptor. However, a linker insertion mutant (at amino acid 582) which results in a receptor deficient in ligand binding did not undergo nuclear translocation indicating that nuclear localization inhibitory sequences were intact in this mutant. The role of receptor phosphorylation on hormone induced nuclear translocation was also examined. Mouse glucocorticoid receptors which contained mutations of certain hormone inducible phosphorylation sites exhibited translocation properties similar to wild type mGR indicating that these phosphorylation sites on the receptor do not play a major role in hormone inducible nuclear translocation.

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INTRODUCTION

Glucocorticoid receptors (GRs) belong to a large family of gene regulatory proteins that includes receptors for all steroid hormones. In response to glucocorticoid hormone binding, these receptors interact with specific DNA sequences termed glucocorticoid response elements (GREs) and modulate the transcription of specific genes. Members of this receptor superfamily share a common organization of conserved structural and functional domains. The GR consists of a variable

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amino terminal region that contains a trans-activation domain that is necessary for regulation of gene transcription. The central region of the receptor contains a highly conserved DNA binding domain composed of two zinc finger motifs which are responsible for receptor binding to GREs. The carboxyl domain of GR is responsible for steroid hormone binding and is involved in regulating the activation of the receptor to a specific DNA binding conformation. Studies on the subcellular distribution of GR have revealed that in most tissues and cells expressing GR, the receptor is located predominately in the cytoplasm when the steroid ligand is absent. Biochemical studies suggest that upon hormone binding, the receptor has a high affinity for the nuclear compartment. In the rat and human GR, two nuclear translocation signals (NL1 and NL2) located in the carboxyl terminal part of the DNA binding domain (NL1) and the hormone binding domain (NL2) have been identified [1, 2]. In these studies, both NL1 and NL2 were shown to be essential for hormone-dependent nuclear translocation of GR [1, 2]. Recently more controversy has arisen concerning the subcellular distribution of GR [3, 4]. Using a well characterized epitope purified polyclonal antibody to human GR [5], we have extensively studied the subcellular distribution of human GR. Furthermore, using various mutants of the human GR we have confirmed and extended the previous studies on hormone dependent nuclear translocation of GR. Moreover, Bodwell *et al.* have shown that GR is a phosphoprotein [6] and that several phosphorylation sites on the mouse GR become hyper-phosphorylated upon exposure to hormone [7]. Interestingly, when rat fibroblasts were treated with dexamethasone and okadaic acid, a protein phosphatase inhibitor, the receptor became hyperphosphorylated and translocated to the nucleus. When hormone was withdrawn, the receptors recycled back to the cytoplasm. However, if okadaic acid treatment was maintained, the receptor could not re-enter the nucleus even in the presence of dexamethasone [8]. These results suggest that the phosphorylation status of the receptor may be important in its subcellular distribution. Therefore, we evaluated mouse glucocorticoid receptors mutated at defined phosphorylation sites [6, 8] for their ability to undergo hormone induced nuclear translocation.

MATERIALS AND METHODS

Hormones and chemicals

Steroid hormones were obtained from Steraloids (Wilton, NH) and RU38486 (RU486) was kindly provided by Dr R. Deraedt, Roussel UCLAF (Romainville, France). Dibutyryl cAMP was from Sigma Chemical Co. (St Louis, MO).

Experimental approach

We assessed the distribution of hGR and hGR mutants overexpressed in COS 1 cells transfected with wild type and mutant receptor cDNAs (kindly provided by R. Evans). The distribution of receptors was examined in the presence and absence of steroid hormone treatment. Cells were transfected in serum-free media and cultured in media containing serum that was stripped of endogenous steroids. Receptors were visualized immunohistochemically using well-characterized, polyclonal epitope-purified, antipeptide antibodies specific for GR [5]. These antibodies (1859 and 1857) recognize both liganded and unliganded receptors in their unactivated (with hsp) and activated (DNA binding) conformations. Both antibodies are directed towards peptides within the amino-terminal portion of the hGR (amino acids 245–259, 346–367 designated 1859 and 1857, respectively). Cells which expressed the transfected receptor cDNAs were intensely stained and were easily distinguished from untransfected cells present in the same culture. All experiments were conducted such that the immunohistochemist was not aware of which experimental or treatment groups he processed.

Cell culture and transfection

COS 1 cells (African Green Monkey kidney) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 9 mg/ml glucose, 100 IU/ml penicillin, 100 μg/ml streptomycin and supplemented with 2 mM glutamine and 10% (v/v) of a mixture (1:1) of fetal calf/calf serum. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2. COS 1 cells were passed every 3–4 days and were maintained in culture for no more than 15 passages. Subconfluent cell monolayers were transfected by the DEAE–dextran method of Sompayrac and Danna [9] as modified by Gorman [10]. Cells were placed in steroid stripped FCS:CS supplemented DMEM after a 3 h incubation with the DNA/DEAE–dextran mixture and further incubated at 37°C for 24 h.

Immunocytochemistry

COS 1 cells were transfected and incubated as described above and then plated in two-chamber glass slides. After an additional 24 h incubation, transfected cells were treated for 1–2 h with the steroid hormones indicated in the text or with control vehicle and were processed for immunocytochemical staining as previously described [5]. Briefly, cells were fixed in 2% paraformaldehyde, washed in PBS and permeabilized in 0.2% Triton X-100. Cells were again washed in PBS, treated with 2% normal goat serum, washed in PBS and incubated with epitope-purified anti-glucocorticoid receptor antiserum 1857 (1:7500) [5] for 20 h at 2°C. The cells were washed in PBS and incubated with biotinylated goat anti-rabbit IgG (1:400) for 1 h at room temperature. Immunoreactivity was visualized by staining with avidin–biotin–peroxidase.

RESULTS

Subcellular distribution of wild type hGR in transfected COS 1 cells

To determine if the intracellular localization of hGR transiently expressed in COS 1 cells resembled the distribution reported for endogenous hGR [5] and for receptor in other transfected cell systems [2, 3, 5], COS 1 cells transiently transfected with a wild type hGR cDNA were examined immunocytochemically in the presence and absence of dexamethasone. In preliminary experiments, we found that localization of transfected hGR was influenced by endogenous steroids in the serum component of the media since higher levels of nuclear staining were apparent in cells cultured in serum-containing media (data not shown). To eliminate this problem, cells were cultured in media containing serum that had been depleted of steroids using dextran-coated charcoal. In Fig. 1, receptor was processed using a peroxidase staining technique. This staining method was used throughout our study. In cells that were not treated with dexamethasone, hGR was predominately cytoplasmic with some diffuse and variable nuclear staining (Fig. 1, left panel, CON). Following treatment (30 min–2 h) with dexamethasone (100 nM), hGR was completely translocated to the nuleus (Fig. 1, right panel, DEX). The primarily cytoplasmic localization of transfected hGR in the absence of hormone and the nuclear translocation of receptor after hormone treatment was indistinguishable from the behavior of native GR protein in HeLa [5] and other GR-containing cells [1, 2].

The ligand specificity of ligand induced hGR nuclear translocation was next examined. As expected, treatment of transfected COS 1 cells with the glucocorticoids (100 nM each) dexamethasone, cortisol and corticosterone resulted in nuclear translocation of hGR (Fig. 2). In contrast, estradiol (100 nM, data not shown), progesterone (100 nM) and the androgen agonist R1881 (100 nM) did not promote nuclear translocation of GR (Fig. 2). This specificity in nuclear translocation correlates with affinity to those hormones which are bound by GR and suggests that COS 1 cells overexpressing a transfected hGR cDNA provide a good model for identifying factors which affect glucocorticoid receptor localization.

It has been shown that cAMP can synergize transcriptional activation by ligand induced GR [11]. This synergy could be the result of a direct effect on GR or through an indirect mechanism. One possible effect by cAMP could be to alter the subcellular distribution of hGR. To determine whether cAMP alone could influence the subcellular distribution of hGR, COS 1 cells transfected with hGR were treated with the cell permeable cAMP analog dibutyryl cAMP and analyzed immunohistochemically. Dibutyryl cAMP (1 μM) had no effect on the intracellular distribution of hGR (Fig. 2, bottom panel). This result indicates that the effects by cAMP may be occurring after the nuclear translocation process.

To further evaluate the steroid specificity of nuclear translocation, we have investigated the effects of the GR antagonist RU486 [12]. Different models have been proposed to explain the antagonistic mechanism of RU486 action. Some studies have suggested that RU486 inhibits receptor activation to a DNA binding form. However, *in vitro* studies have shown that like agonist–GR complexes, RU486–GR complexes selectively interact with GRE-containing DNA [13]. However, the conformation of GR differs depending on whether receptor is bound to an agonist or antagonist [14, 15]. These investigators have proposed that this alteration in receptor structure may account for the inability of RU486 to promote gene transcription. We show in our experiments that RU486 promoted

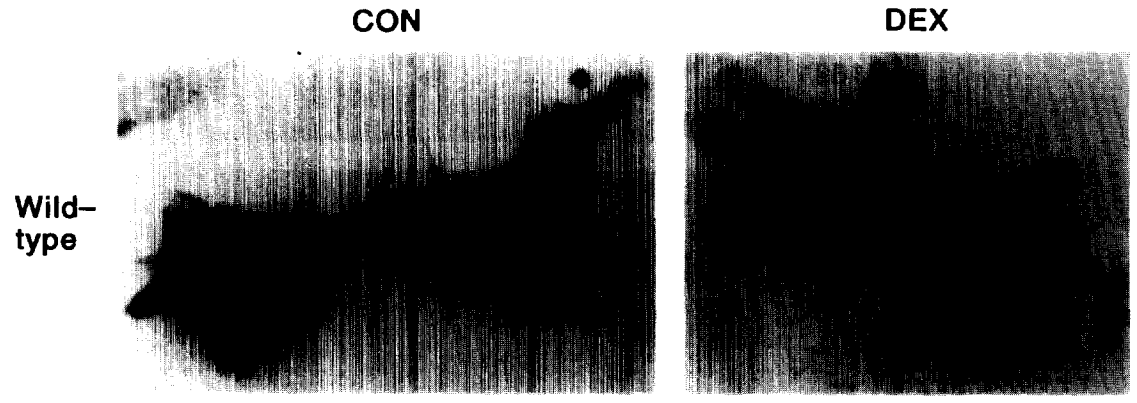

Fig. 1. Ligand-dependent translocation of human glucocorticoid receptors (hGR) expressed in COS 1 cells. COS 1 cells were transfected with the wild type hGR cDNA expression vector pRShGR [11]. Cells were treated for 1–2 h with 100 nM dexamethasone (DEX) or were not treated (CON) 2 days after transfection. To detect the presence of immunoreactive hGR, cells were incubated with antipeptide antibody 1857 followed by peroxidase-conjugated secondary antibody as described in Materials and Methods.

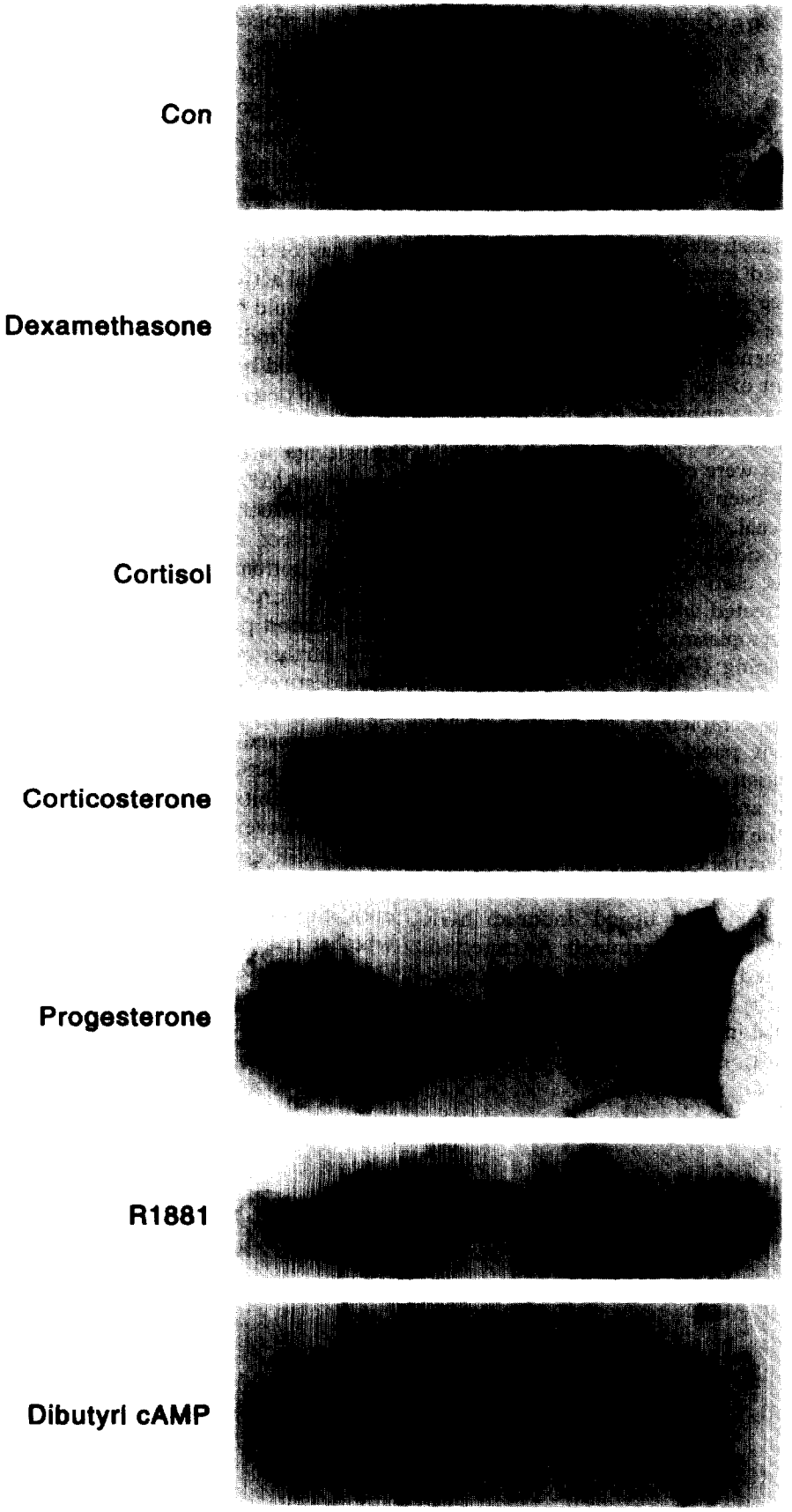

Fig. 2. Specificity of hormone-inducible hGR nuclear translocation. COS 1 cells were processed as described in Fig. 1 using the peroxidase-conjugated secondary antibody and cells were untreated (Con) or treated with 100 nM each of the steroid hormones indicated or with 1 μM dibutyryl cAMP.

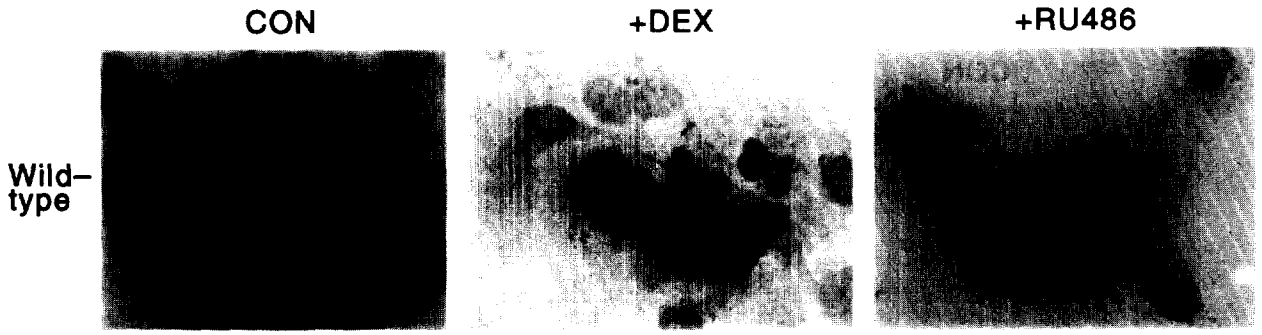

Fig. 3. RU486-induced nuclear translocation of hGR. COS 1 cells were processed as described in Fig. 1 and were untreated (CON), or treated with 100 nM dexamethasone (+DEX) or 100 nM RU486 (+RU486).

nuclear translocation as efficiently and completely as dexamethasone (Fig. 3). This finding confirms and extends to hGR the earlier observation of Picard and Yamamoto [1] who found that RU486 induces nuclear translocation of the rat GR.

Role of the domains of the hGR in nuclear translocation

To evaluate how the functional domains of the hGR may influence intracellular receptor distribution, we performed immunohistochemical analysis of COS 1 cells transfected with a variety of receptor cDNA deletion and point mutants. All mutant hGR cDNAs were obtained from Dr R. Evans and were expressed in the same vector driven by the Rous Sarcoma Virus (RSV) promoter to ensure that the expression levels of each receptor were relatively constant [16].

The amino terminus of the receptor is known to harbor a transactivation domain Tau 1 [16, 17]. The potential contribution of this portion of the hGR in receptor localization has been largely ignored. We examined the localization of an hGR mutant which is lacking amino acids 77–262. This region encompasses a large portion of the transactivation domain of the receptor. Figure 4 shows that both mutant and wild type receptors are present primarily in the cytoplasm prior to hormone stimulation and are found within the nucleus after hormone administration. Therefore, the hormone-inducible nuclear localization of this deletion mutant is indistinguishable from that of the wild type receptor suggesting that sequences located between amino acids 77–262 have no role in nuclear localization.

We next evaluated the role of the DNA binding domain in receptor localization. The DNA binding domain of the hGR extends from approximately amino acids 421 to 486. Contained within this region are two zinc finger motifs which are essential for DNA binding

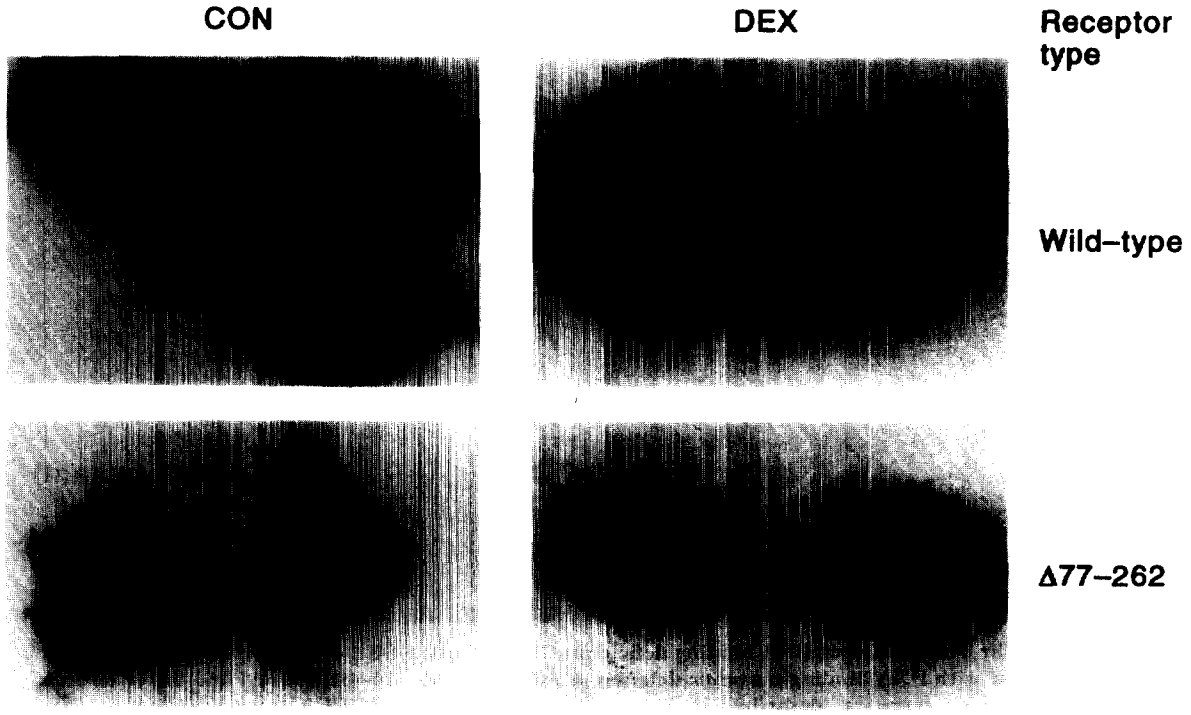

Fig. 4. Subcellular distribution of an amino-terminal deletion mutant of hGR. COS 1 cells were transfected with the wild type hGR, or with deletion mutant 77–262 and were left untreated (CON) or treated with 100 nM dexamethasone (DEX) and processed as described in Fig. 1.

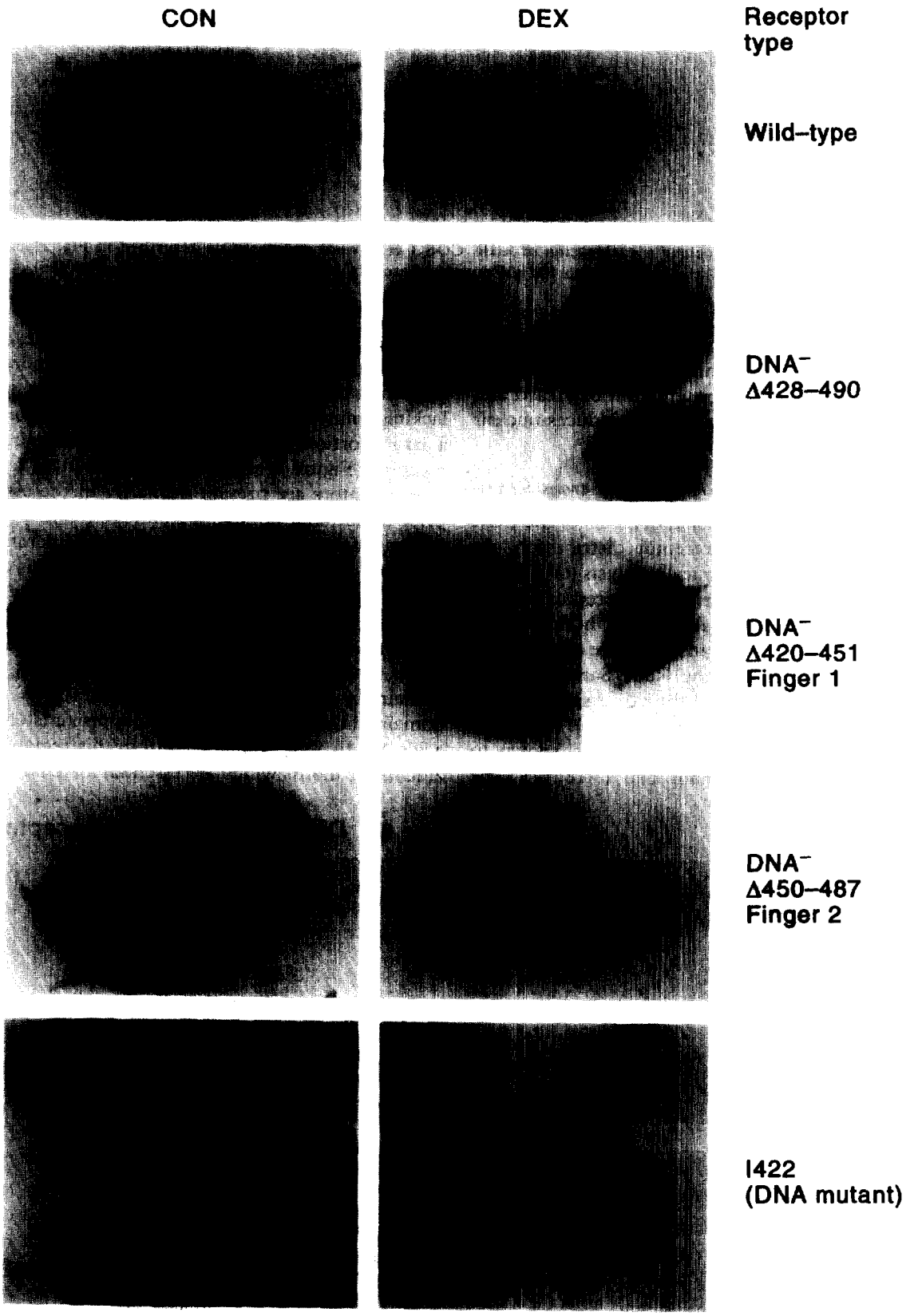

Fig. 5. Subcellular distribution of DNA binding deficient hGR mutants. COS 1 cells were transfected with the wild type hGR or with DNA binding mutants: deletion mutants 428–490, 420–451, 450–487 and linker insertion mutant I422, and processed as described in Fig. 1. Cells were untreated (CON) or treated with 100 nM dexamethasone (DEX).

by the hGR. We first tested nuclear translocation of mutants which lacked either the entire DNA binding domain (including both zinc fingers, 428–490), or lacked zinc finger 1 (420–451) or zinc finger 2 (450–487). In the absence of steroid each of these mutants was located in the cytoplasm (Fig. 5, left panel). Following hormone addition, the mutants were found predominately in the nucleus but did not completely translocate like the wild type receptor. The DNA binding domain mutant that lacked zinc finger 2 (450–487) was the most deficient in hormone-inducible nuclear translocation. The inefficient nuclear translocation exhibited by this mutant was not surprising based on the identification of a bipartite nuclear localization signal between amino acids 478–487 and 491–498 (NL1) in hGR [2] which is partially deleted in the mutant that lacks zinc finger 2. However, despite the deletion of part of NL1, this mutant was not completely deficient in hormone-inducible nuclear translocation.

To further evaluate the relationship between DNA binding and nuclear translocation, we made use of the linker insertion mutant I422. This mutant cannot bind DNA or activate gene transcription due to the insertion of 3 amino acids beginning at amino acid 422 [17]. The I422 mutant displayed wild type capacity to undergo nuclear translocation following either dexamethasone (Fig. 5) or RU486 administration (data not shown), suggesting that binding DNA is not a necessary component of the translocation process.

As discussed above, based on the findings of Picard and Yamamoto [1] and Cadepond *et al.* [2] that a nuclear localization signal (NL1) is present between the carboxyl terminal end of the DNA binding domain and extends into the hinge region of the GR, we examined an hGR mutant which lacks amino acid sequences 491–582 for nuclear translocation. This mutant did not translocate into the nucleus following hormonal stimulation (Fig. 6). This mutant lacks the second part of NL1, and is also missing the transactivation domain Tau 2 and most of the heat shock protein 90 binding site [18].

The requirement for steroid binding in hGR nuclear translocation was demonstrated using an insertion mutant I582 which is incapable of binding cognate ligands. This mutant was found predominately in the cytoplasm in the presence and absence of hormone (Fig. 6). This result is inconsistent with the studies by Cadepond *et al.* [2] who show that I582 is mainly nuclear. Interestingly, a ligand binding mutant which is truncated after amino acid 550 (I550*) was constitutively located in the nucleus (Fig. 6). These results are consistent with the proposal of Cadepond *et al.* [2] that NL1 activity can be repressed by sequences in the ligand binding domain. In the absence of this NL1 repressing activity, the receptor was constitutively localized in the nucleus. Our data indicate that putative sequences which can repress NL1 activity are located between amino acid 550 and the carboxyl terminus of the hGR.

To examine the contribution of the hinge region more precisely, two additional mutants 488–532 and 490–515 were examined. The 488–532 mutant displayed essentially no hormone inducible nuclear translocation (Fig. 7). Mutant 490–515 showed a low level of nuclear staining following hormone addition. These results are interesting since both mutants as well as deletion mutant 491–582 are lacking the second half of the bipartite NL1 signal (491–498) and suggest that additional sequences between 515 and 532 may be important in the translocation process.

The role of phosphorylation in mouse GR nuclear translocation

With the finding that hyper-phosphorylation of mouse GR occurs upon exposure to hormone, we assessed the ability of various single substitution mutants to undergo hormone mediated nuclear translocation. Initially, we noted that the mouse GR showed greater variability in its subcellular distribution than we had observed with human hGR. However, it should be noted that upon hormone administration, the mouse GR was found to be exclusively nuclear (Fig. 8, Wild-type CON and DEX). We next tested phosphorylation mutants which had compensatory charge alterations, serine 212 to aspartic acid and serine 220 to aspartic acid [8]. The top panels of Fig. 8 show that upon hormone administration, the two substituted mutants translocated to the nucleus. Mutants which had the more drastic substitution of serine to alanine [8] were also examined. Figure 8 (bottom panels) shows that substitution of amino acids 212 or 220 or 234 had no effect on hormone mediated nuclear translocation.

DISCUSSION

In the transfected cell model system, using epitope purified antibodies which recognize both non-hormone as well as hormone bound hGR in both untransformed and DNA binding states, nuclear translocation was dependent upon hormone binding. In the absence of steroid, hGR was predominately cytoplasmic even in the presence of media components such as phenol red which other investigators have suggested can trigger GR nuclear localization [1]. Addition of glucocorticoids resulted in complete hGR nuclear translocation. These results are in agreement with our earlier findings in cells stably transfected with a wild type hGR cDNA [5] and with the findings of others in transiently transfected cells [1, 2] as well as in cells which contain endogenous receptor proteins [5, 19]. Even though antibodies and fixation techniques were different from those used in previous studies [1, 2] the complete nuclear translocation of transiently transfected hGR was remarkably consistent. However, these results do not agree with studies that have shown GR localization

in the nucleus in the apparent absence of hormone [20, 21]. It is not clear if this discrepancy is due to technical differences or tissue specific differences such as the basal level of GR expression [4].

Consistent with the capacity of the GR antagonist RU486 to promote specific DNA binding by GR *in vitro*, this antagonist stimulated hGR nuclear translocation as effectively as dexamethasone. Non-glucocorticoid steroid hormones were not effective in promoting nuclear translocation. Nuclear translocation may therefore represent an additional control mechanism for inhibiting promiscuous activation by GR.

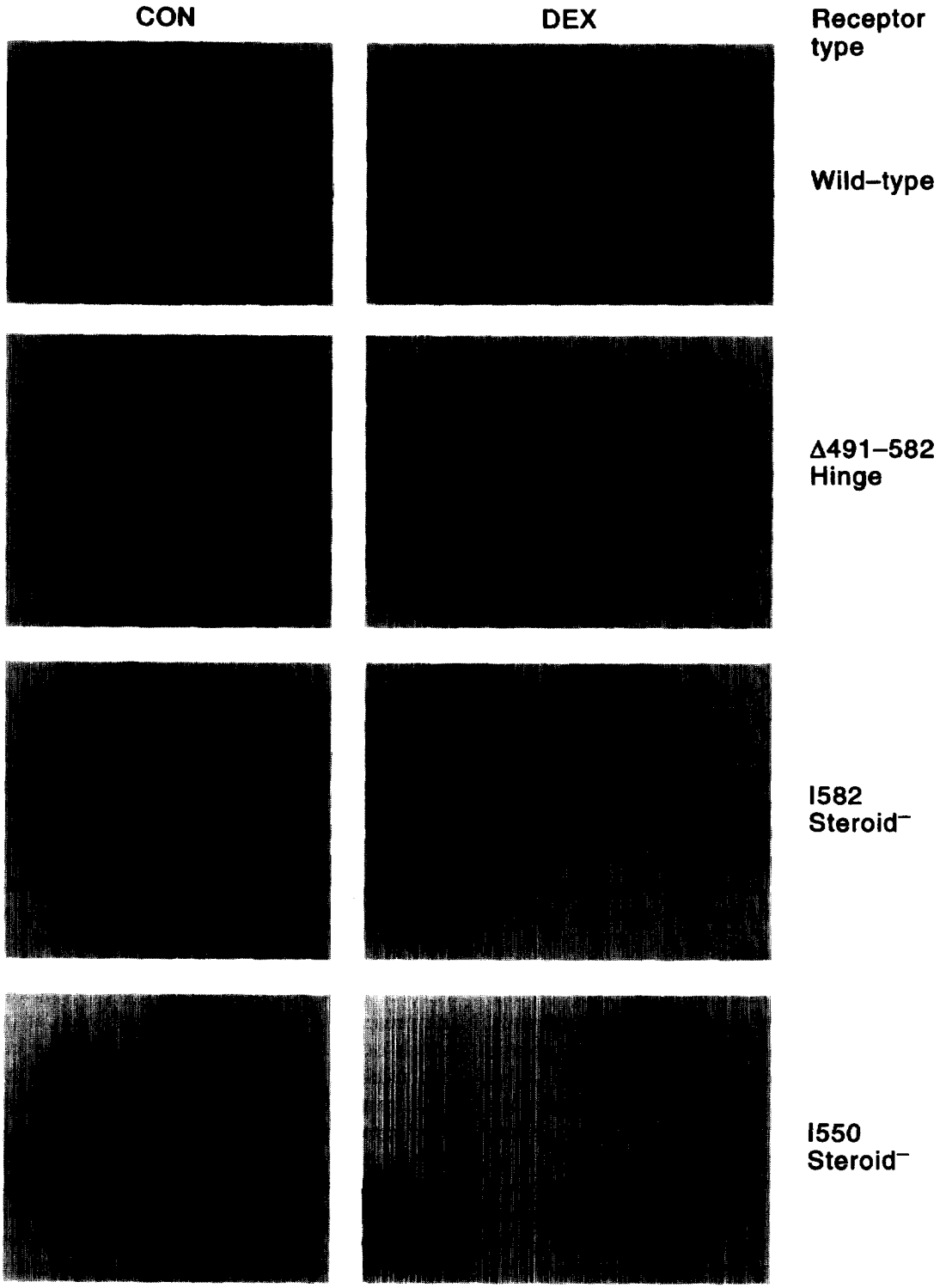

Fig. 6. Subcellular distribution of hinge region deletion mutant and steroid binding domain mutants. COS 1 cells were transfected with wild type hGR; deletion mutants 491–582 (hinge region), and two ligand binding deficient mutants: linker insertion I582 and truncation I550*. Cells were prepared as described in Fig. 1 and cultured in the absence (CON) or presence of 100 nM dexamethasone (DEX).

Although cAMP has been shown to facilitate GR-mediated transcription, our work suggests that GR is not sensitive to nuclear translocation by this intracellular signal. Our finding that dibutyryl cAMP did not promote nuclear translocation of hGR suggests a possible reason for the failure of cAMP alone to stimulate transactivation by GR.

We found that deletion of a portion of the amino-terminal region of the hGR had no effect on nuclear translocation. The intracellular distribution of this mutant (77–262) was identical to that of the wild type hGR both in the presence and absence of dexamethasone. This result indicates that this region does not appear to play a role in hormone-dependent nuclear translocation of hGR. The mutant 77–262 lacks Tau 1, a region of the GR involved in transcriptional activation. Clearly, this Tau 1 domain does not influence intracellular partitioning of hGR. Despite the capacity of this mutant to translocate into the nucleus in response to glucocorticoid, this mutant receptor exhibits less than 10% of the transactivation potential of the wild type receptor suggesting that 77–262 is deficient in a step that occurs following nuclear translocation [22].

Immunocytochemical examination of a DNA binding linker insertion mutant (I422) revealed that in the absence of DNA binding, nuclear translocation occurred normally after hormonal stimulation. Although the DNA binding function was not involved in nuclear translocation, regions within the DNA binding domain influenced the extent of nuclear translocation. The DNA binding deletion mutants 428–490, 420–451 and 450–487 were not completely translocated into the nuclei after the addition of dexamethasone to transfected cell cultures. In particular,

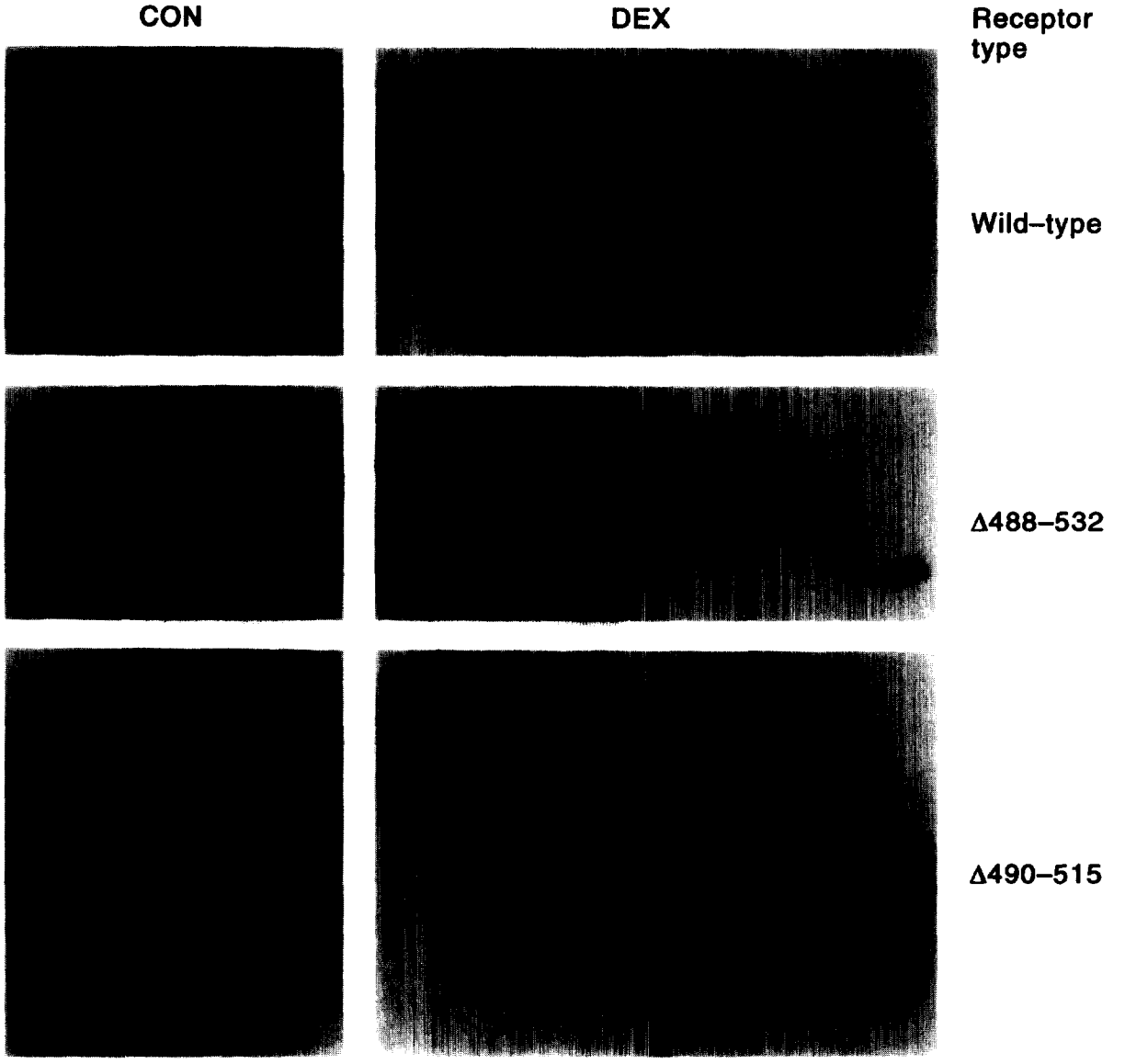

Fig. 7. Subcellular distribution of hinge region deletion mutants. Wild type hGR and hinge region deletion mutants, 488–532 and 490–515 were transfected into COS 1 cells and processed as described in Fig. 1. Cells were either untreated (CON) or treated with 100 nM dexamethasone (DEX).

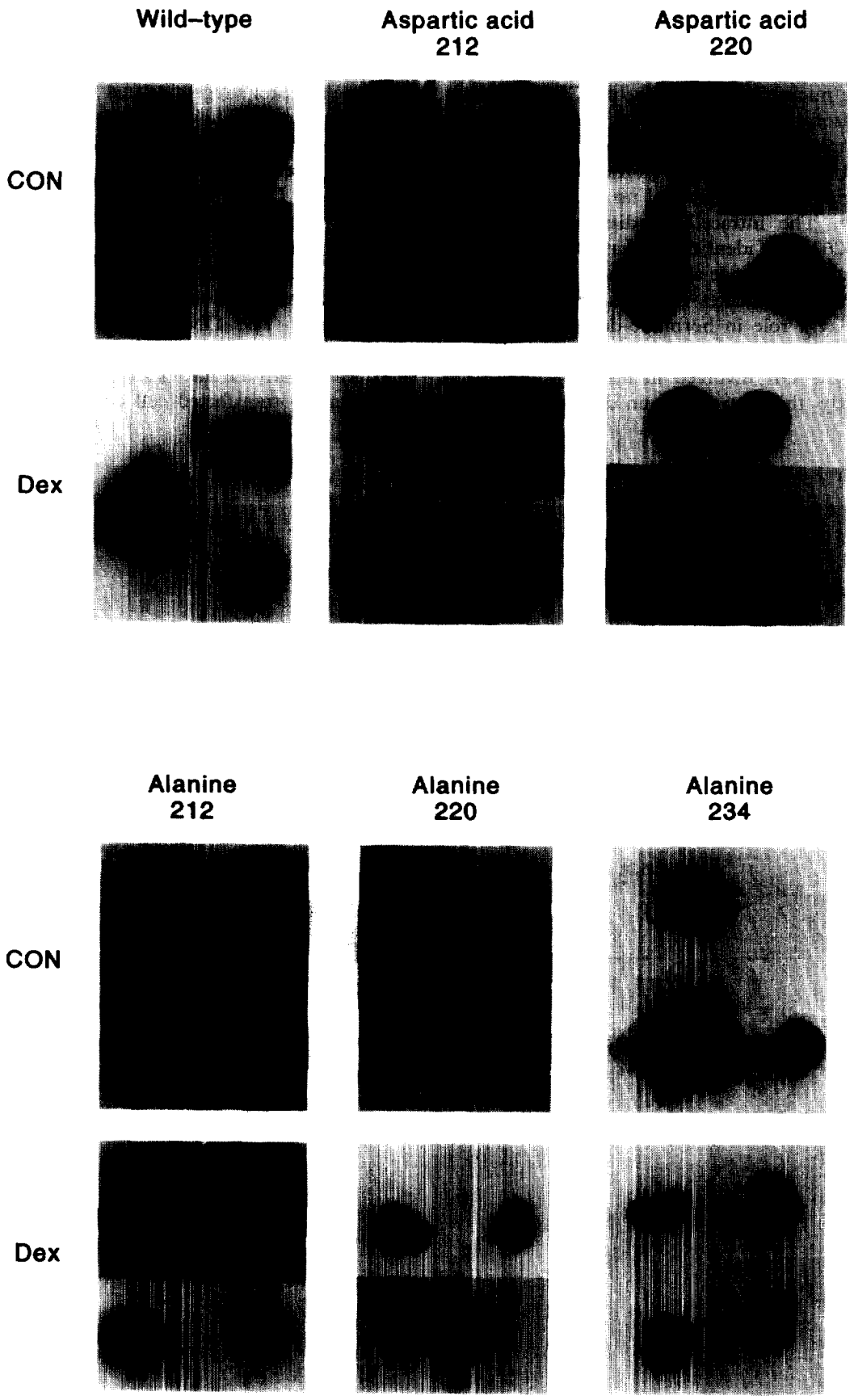

Fig. 8. Subcellular distribution of mouse phosphorylation mutants. Wild type and phosphorylation mutant receptors were transfected into COS 1 cells and processed as described in Fig. 1. Cells were either untreated (CON) or treated with 100 nM dexamethasone (DEX).

nuclear translocation of 450–487 and 428–490 appeared to be the most compromised (Fig. 5). Since these mutants lack the first half of the bipartite nuclear localization signal (NL1) identified by Cadepond *et al.* [2] in hGR, the decrease in nuclear localization is likely due to a flaw in this signal and not from a loss of DNA binding function in these mutants. Nevertheless, the partial deletion of NL1 in these mutants had only a temperate effect on nuclear translocation which supports the existence of the second half of NL1 in the hinge/ligand binding domain region of hGR [2]. Interestingly, when the putative second half of NL1 [2] was deleted (488–532 and 490–515), two different results were observed. The 488–532 deletion showed little nuclear staining compared to the 490–515 deletion which showed moderate nuclear staining. These results indicate that sequences between 515 and 532 may also be important in the translocation process. Therefore, NL1 may consist of three regions where a loss of any two of these regions totally abrogates the translocation process while a loss of only one of the regions causes a moderate decrease in translocation but does not totally disrupt nuclear translocation. Furthermore, we are in disagreement on the translocation ability of I582. We have found that I582 is constitutively cytoplasmic while Cadepond *et al.* [2] showed nuclear localization of this truncated receptor. It is interesting that the truncation mutant I582 was observed in equal concentrations as both 8S and 4S forms by Cadepond *et al.* [2]. It should be noted that a larger hGR truncation mutant I550* which lacks NL2 and potential NL1 repression sequences was constitutively expressed in the nucleus as a 4S form [our studies and 2]. Therefore, we argue that potential repression sequences are also located between amino acids 550 and 582. Additional repression sequences may be located after amino acid 696 as the work by Cadepond *et al.* suggests [2]. Steroid receptors which have only a single NL signal are constitutively located in the nucleus even in the absence of ligand, for example, the estrogen receptor [23, 24]. Other steroid receptors such as the progesterone receptor (PR) have two NLS [25], however, other studies suggest that the PR is located in the nucleus independent of hormone binding [26]. In addition, the androgen receptor (AR) which translocates to the nucleus in a hormone dependent manner similar to GR also contains two NLS [27]. Therefore, we believe that for hGR there are three NLS which contribute to hormone mediated nuclear translocation. There also seems to be additional sequences located in the ligand binding domain of hGR which have a repressive function in the translocation process. Recently, DeFranco *et al.* [28] have also suggested that rat GR has three NLS which agrees with our findings on hGR.

With the recent finding that certain phosphorylation sites on the mouse GR become hyper-phosphorylated (up to 3-fold on serine 220, [7]), we assessed the ability of conservative and non-conservative substitution mutants to undergo hormone mediated nuclear translocation. None of the substitution mutants we have studied of the mouse GR had any effect on hormone mediated nuclear translocation. This result is not particularly surprising as the human deletion mutant 77–262 also translocated in the presence of hormone. Therefore, we believe that the phosphorylation status of the receptor has no effect on the initial hormone inducible nuclear translocation of GR. Again, we would like to point out that there was greater variability in the subcellular distribution of mouse GR than of human GR. Occasional nuclear staining was observed in the absence of hormone which indicates that there are some minor differences between these two species being studied in this system.

The nucleocytoplasmic shuttling of steroid receptors remains ill-defined. However, DeFranco *et al.* [28] have recently presented what is currently understood about steroid receptor shuttling. Previously, Guiochon-Mantel *et al.* [29] determined that the NLS are also involved in the shuttling of receptor out of the nucleus. Therefore, the studies presented in this paper give us an insight to regions of the hGR which may be important for shuttling and efforts using these defined regions to examine shuttling are underway.

In summary, using antibodies which recognize unactivated as well as hormone activated hGR with equal affinity, we have given additional evidence to the argument that unoccupied GR resides in the cytoplasm with an obligate translocation to the nucleus upon exposure to cognate ligands. Future studies on the exact control mechanisms of this translocation process should enlighten researchers on this all important initial step in glucocorticoid induced signal transduction.

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