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## The Biology of the Glucocorticoid Receptor: New Signaling Mechanisms in Health and Disease

Robert H. Oakley and John A. Cidlowski\*

Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, 111 TW Alexander Drive, Research Triangle Park, North Carolina, 27709

### Abstract

Glucocorticoids are primary stress hormones necessary for life that regulate numerous physiological processes in an effort to maintain homeostasis. Synthetic derivatives of these hormones have been mainstays in the clinic for treating inflammatory diseases, autoimmune disorders, and hematological cancers. The physiological and pharmacological actions of glucocorticoids are mediated by the glucocorticoid receptor (GR), a member of the nuclear receptor superfamily of ligand-dependent transcription factors. Ligand-occupied GR induces or represses the transcription of thousands of genes by direct binding to DNA response elements and/or by physically associating with other transcription factors. The traditional view that glucocorticoids act through a single GR protein has changed dramatically with the discovery of a large cohort of receptor isoforms with unique expression, gene-regulatory, and functional profiles. These GR subtypes are derived from a single gene by alternative splicing and alternative translation initiation mechanisms. Post-translational modification of these GR isoforms further expands the diversity of glucocorticoid responses. Here, we discuss the origin and molecular properties of the GR isoforms and their contribution to the specificity and sensitivity of glucocorticoid signaling in healthy and diseased tissues.

### INTRODUCTION

Glucocorticoids are hormones essential for life that are synthesized and released by the adrenal cortex in a circadian manner and in response to stress. The secretion of these hormones is controlled by the hypothalamic-pituitary-adrenal (HPA) axis (Figure 1). Internal and external signals trigger the hypothalamus to release corticotropin releasing hormone (CRH), which acts on the anterior pituitary to stimulate the synthesis and secretion of adrenocorticotropic hormone (ACTH). ACTH then acts on the adrenal cortex to stimulate the production and secretion of glucocorticoids. Acting on nearly every tissue and organ in the body, glucocorticoids function to maintain homeostasis both in response to normal diurnal changes in metabolism and in the face of stressful perturbations. Glucocorticoids regulate a plethora of physiological processes including intermediary metabolism, immune function, skeletal growth, cardiovascular function, reproduction, and cognition (1, 2). In a

\*Corresponding author. Mailing address: National Institute of Environmental Health Sciences, P.O. Box 12233, MD F3-07, Research Triangle Park, North Carolina, 27709, Phone: 919-541-1564. Fax: 919-541-1367. cidlows1@niehs.nih.gov.

classic negative feedback loop, glucocorticoids also target the hypothalamus and anterior pituitary to inhibit the production and release of CRH and ACTH and thereby limit both the magnitude and duration of the glucocorticoid increase (Figure 1).

Because of their powerful anti-inflammatory and immunosuppressive actions, glucocorticoids are one of the most widely prescribed drugs in the world today (3, 4). Synthetic glucocorticoids have been indispensable over the last half century for treating inflammatory and autoimmune diseases, such as asthma, allergy, sepsis, rheumatoid arthritis, ulcerative colitis, and multiple sclerosis. They are also commonly prescribed to prevent organ transplant rejection and to treat cancers of the lymphoid system such as leukemias, lymphomas, and myelomas. Unfortunately, the therapeutic benefits of glucocorticoids are limited by severe side effects that develop in patients chronically treated with these steroids (3, 5). Adverse effects include osteoporosis, skin atrophy, diabetes, abdominal obesity, glaucoma, growth retardation in children, and hypertension. In addition, patients on long term glucocorticoid therapy frequently develop tissue-specific glucocorticoid resistance. Understanding the factors at a molecular level that control the cellular response to glucocorticoids is a critical goal of current research as progress in this area will facilitate the development of novel glucocorticoids with improved benefit/risk ratios.

The physiological and pharmacological actions of glucocorticoids are mediated by the glucocorticoid receptor (GR, NR3C1), a member of the nuclear receptor superfamily of ligand-dependent transcription factors (6). Following glucocorticoid binding, GR induces or represses the transcription of target genes which can comprise up to 10-20% of the human genome (7-9). Consistent with the pleiotropic actions of glucocorticoids, GR is expressed in nearly every cell of the body and is necessary for life after birth (10). The cellular response to glucocorticoids is remarkably diverse, exhibiting profound variability in specificity and sensitivity (11, 12). For example, glucocorticoids induce the killing of thymocytes and osteoblasts but promote the survival of hepatocytes and cardiomyocytes. In addition, glucocorticoid sensitivity varies among individuals, within tissues of the same individual, and even within the same cell during different stages of the cell cycle (13, 14). While cell-type specific alterations in ligand bioavailability and cofactor expression can modulate the glucocorticoid response, recent studies have also identified a major role for an expanding array of GR isoforms (15, 16). GR is derived from a single gene; however, multiple GR proteins exist due to alternative splicing and alternative translation initiation. This large cohort of functionally distinct receptor subtypes are subject to various post-translational modifications that further regulate their signaling properties. Consequently, the cellular response to glucocorticoids is determined in large measure by the expressed complement and composite actions of the individual GR isoforms. In this review, we discuss the molecular heterogeneity of GR and its potential contribution to the regulation and dysregulation of glucocorticoid signaling.

## GR SIGNALING

### Classic GR Signaling Pathway

GR is a modular protein composed of three major domains: an N-terminal transactivation domain (NTD), a central DNA-binding domain (DBD), and a C-terminal ligand binding domain (LBD) (Figure 2) (17). The DBD and LBD are separated by a flexible region of the protein termed the hinge region. The DBD is the most conserved domain across the nuclear receptor family and contains two zinc finger motifs that recognize and bind target DNA sequences called glucocorticoid-responsive elements (GREs). The NTD houses a powerful transcriptional activation function (AF1) that interacts with coregulators and the basal transcription machinery and is the primary site for post-translational modifications (Figure 2). The LBD, consisting of 12  $\alpha$ -helices and four  $\beta$ -sheets, forms a hydrophobic pocket for binding glucocorticoids and also contains an activation function (AF2) that interacts with coregulators in a ligand-dependent manner (18). Two nuclear localization signals, NL1 and NL2, are located at the DBD/hinge region junction and within the LBD, respectively.

In the absence of hormone, GR resides predominantly in the cytoplasm of cells as part of a large multi-protein complex that includes chaperone proteins (hsp90, hsp70, and p23) and immunophilins of the FK506 family (FKBP51 and FKBP52) (Figure 3) (19, 20). These proteins maintain the receptor in a conformation that is transcriptionally inactive but favors high affinity ligand binding. Cortisol, the most abundant endogenous glucocorticoid in man, is transported in the blood predominantly bound to corticosteroid-binding globulin (CBG). CBG not only facilitates cortisol distribution but also plays a role in its release to tissues. CBG-free cortisol passively diffuses across the plasma membrane; however, its bioavailability within the cell is controlled by two enzymes working in an opposing manner (21).  $11\beta$ -Hydroxysteroid dehydrogenase type 2 ( $11\beta$ -HSD2) oxidizes cortisol into the inactive metabolite cortisone; whereas  $11\beta$ -hydroxysteroid dehydrogenase type 1 ( $11\beta$ -HSD1) converts cortisone to cortisol (Figure 3). Changes in the level and/or activity of these enzymes can contribute to cellular differences in glucocorticoid sensitivity. In fact, inhibitors of  $11\beta$ -HSD1 have been designed to limit the adverse metabolic side effects of elevated endogenous glucocorticoids (22). In contrast to cortisol, most synthetic glucocorticoids do not bind CBG and are not metabolized by  $11\beta$ -HSD2. Upon binding glucocorticoids, GR undergoes a conformational change resulting in the dissociation of the associated proteins. This structural rearrangement exposes the two nuclear localization signals, and GR is rapidly translocated into the nucleus through nuclear pores.

Once inside the nucleus, GR binds directly to GREs and regulates the expression of target genes (Figure 3) (23, 24). The consensus GRE sequence, GGAACAnnnTGTTCT, is an imperfect palindrome that is comprised of two 6-base pair half sites. GR binds this element as a homodimer, with each half site occupied by one receptor subunit. The three nucleotide spacing between the two half sites is strictly required for GR to dimerize on this element. The GRE has been shown to mediate the glucocorticoid-dependent induction of many genes and therefore is often referred to as an activating or positive GRE. However, recent genome-wide analyses have revealed that GR occupancy of the canonical GREs can also lead to the repression of target genes (25). These findings suggest a critical role for factors outside the

GRE sequence, such as epigenetic regulators and chromatin context, in determining the polarity of the transcriptional response. A negative GRE (nGRE) has also been recently described that mediates glucocorticoid-dependent repression of specific genes (26). The consensus nGRE sequence, CTCC(n)<sub>0-2</sub>GGAGA, is palindromic but differs from the classic GRE in sequence, in having a variable spacer that ranges from 0 to 2 nucleotides, and in being occupied by two GR monomers that do not homodimerize (27). These nGREs are abundant throughout the genome, however more work is needed to clarify the extent this motif is utilized by GR for directly repressing target genes and whether this element can also be employed in the activation of gene expression.

Global GR recruitment assays indicate that only a small fraction of GREs are actually occupied by the receptor, and the specific sites of GR binding vary in a tissue-specific manner due to differences in chromatin accessibility and exposure of the GRE (28). These findings suggest that the widely varying effects of glucocorticoids on different tissues can be attributed in part to cell-type specific differences in the chromatin landscape that influence which GREs are accessible for GR binding. In addition, the concentration of glucocorticoids at which GR binds GREs and regulates gene expression varies throughout the genome (29). Some GREs are occupied by GR at very low concentrations of glucocorticoids (hypersensitive) whereas others require high doses of ligand for GR binding to occur. Both chromatin accessibility and other DNA binding proteins appear to govern the sensitivity of specific GREs. The identification of hypersensitive GREs suggests that low-dose glucocorticoid therapy may provide a novel treatment option for regulating specific sets of genes that avoids the deleterious side effects of high exogenous glucocorticoids.

Genome-wide analyses have also found that the majority of GR binding sites are located outside the promoter of glucocorticoid-responsive genes in intergenic or intragenic regions often far removed from the transcription start site (30). For example, glucocorticoid induction of  $\beta$ arrestin1 and repression of  $\beta$ arrestin2 occur via an intron1 GRE and intron11 nGRE, respectively (31). Both  $\beta$ arrestin isoforms play critical roles in the termination and transduction of GPCR signals. By altering the ratio of  $\beta$ arrestin1 and  $\beta$ arrestin2, glucocorticoids shift the balance of G protein and  $\beta$ arrestin-dependent signaling responses for a given GPCR (31). This redirection of the GPCR signaling profile may account for the superior clinical efficacy of glucocorticoid/ $\beta$ 2 adrenergic receptor ( $\beta$ 2AR) agonist combination therapies currently used for treating asthma and COPD. The  $\beta$ 2AR is preferentially desensitized by  $\beta$ arrestin2. Therefore, the glucocorticoid-dependent reduction in  $\beta$ arrestin2 may counteract  $\beta$ 2AR desensitization and allow agonist bound  $\beta$ 2AR to promote a greater and/or more sustained relaxation response in airway smooth muscle cells. In addition, lower levels of  $\beta$ arrestin2 in lung epithelial cells may impair the pro-inflammatory effects of  $\beta$ 2AR agonists that depend on the signaling function of  $\beta$ arrestin2. Another example of a GR binding site located a great distance from the transcription start site is the intragenic nGRE recently identified in exon 6 of the GR gene that mediates homologous down regulation of GR expression, a mechanism that may limit therapeutic responses to glucocorticoids (32). The location of GREs and nGREs at great distances from the transcription start site suggests that these response elements can loop to the promoter areas of target genes to regulate transcription.

The interaction of GR with DNA is highly dynamic, with GR cycling between bound and unbound states every few seconds (33). Once bound to the GRE, the receptor undergoes additional conformational changes that lead to the recruitment of coregulators and chromatin-remodeling complexes that modulate gene transcription rates by affecting the activity of RNA polymerase II (34-36). Cofactors that mediate transcriptional activation include steroid receptor coactivators (SRC1-3), the histone acetyltransferases CBP/p300, and the nuclear methylase coactivator-associated arginine methyltransferase (CARM1). NCoR and SMRT are established corepressors that are recruited to GR bound to nGREs. The specific cofactors assembled and their subsequent activity are dictated by both the nature of the glucocorticoid ligand and the specific GRE sequence bound by the receptor (37, 38).

GR can also regulate the transcription of target genes by physically interacting with other transcription factors (Figure 3). The association of GR with specific members of the STAT family, either apart from or in conjunction with GRE binding, has been shown to enhance the transcription of responsive genes (39). In contrast, the interaction of GR with the proinflammatory transcription factors, AP1 and NF- $\kappa$ B, antagonizes their activity and is considered to be a primary mechanism by which glucocorticoids suppress inflammation. GR directly binds the Jun subunit of AP1 and the p65 subunit of NF- $\kappa$ B and interferes with the transcriptional activation function of these two proteins (40, 41). For some genes, the repression is accomplished by GR tethering itself to these DNA-bound proteins without itself directly interacting with the DNA. For other genes, however, GR functions in a composite manner, binding directly to a GRE and physically associating with AP1 or NF- $\kappa$ B bound to a neighboring site on the DNA. GR-dependent recruitment of the GR interacting protein 1 (GRIP1), a transcriptional coregulator of the p160 family, is important for this inhibition (42). Interestingly, recent work has demonstrated that the anti-inflammatory actions of glucocorticoids differ in a sex-specific manner (43). Glucocorticoids regulate a greater number of inflammatory genes and elicit a greater anti-inflammatory response in male rats compared to female rats. Sex-specific expression profiles of coregulatory molecules may underlie the sexual dimorphism by modulating the repressive actions of GR on AP1 and NF- $\kappa$ B. The sex-specific differences in the anti-inflammatory actions of glucocorticoids not only provide a mechanistic basis for the predisposition of females to autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus, but also suggest that therapeutic doses of glucocorticoids should be optimized according to the sex of the patient.

### **Non-Classical GR Signaling Pathway**

While the principal effects of glucocorticoids are mediated by transcriptional responses that occur in minutes to hours, a growing body of evidence suggests that GR may also act via non-genomic mechanisms to elicit rapid cellular responses that occur within a few seconds to minutes and do not require changes in gene expression (Figure 3) (44, 45). Multiple mechanisms appear to be involved in these signaling events that ultimately impinge on the activity of various kinases, such as PI3K, AKT, and MAPKs. For example, the glucocorticoid-dependent release of accessory proteins associated with unliganded GR in the cytoplasm, such as the non-receptor tyrosine kinase c-Src, can mediate non-genomic actions

of glucocorticoids. When liberated from the GR complex, c-Src activates multiple kinase cascades that lead to the phosphorylation of annexin 1, inhibition of cytosolic phospholipase A2 activity, and impaired release of arachidonic acid (46, 47). GR has also been reported to localize at the plasma membrane in caveolae via an interaction with caveolin1 (48). Glucocorticoid activation of this membrane associated GR regulates gap junction intercellular communication and neural progenitor cell proliferation by a mechanism that requires c-Src activity and rapid MAPK-dependent phosphorylation of connexin-43 (49). The existence of non-genomic signaling adds greater complexity and diversity to glucocorticoids and their biological actions, and raises the possibility that selective modulators of GR-dependent genomic or non-genomic pathways may be therapeutically advantageous.

### GR Polymorphisms

The capacity of GR to function as a transcriptional activator or repressor is affected by several polymorphisms in the GR gene that alter the amino acid sequence of the encoded receptor. The ER22/23EK GR polymorphism occurs in ~3% of the population and results in an arginine to lysine change at position 23 within the NTD (50, 51). GR with the ER22/23EK polymorphism exhibits reduced transcriptional activity on both glucocorticoid responsive reporters and endogenous genes and has been associated with glucocorticoid insensitivity. Persons with the ER22/23EK polymorphism have a lower tendency to develop impaired glucose tolerance, type-2 diabetes, and cardiovascular disease suggesting the relative resistance of ER22/23EK carriers to endogenous glucocorticoids may result in a more favorable metabolic profile. The N363S polymorphism occurs in ~4% of the population and results in an asparagine to serine substitution in the NTD of GR (50). In contrast to ER22/23EK, the presence of the N363S polymorphism enhances the transcriptional activity of GR and is associated with glucocorticoid hypersensitivity. In addition, a genome-wide microarray analysis has revealed a unique pattern of gene regulation for the N363S polymorphism (52). N363S carriers have been reported to have an increased body mass index and a tendency towards decreased bone mineral density (51). These GR polymorphisms may account at least in part for the variability in the glucocorticoid response observed among individuals treated with these steroids.

### GR SPLICE VARIANTS

The human GR gene is composed of 9 exons. The GR NTD is encoded primarily by exon 2, the DBD is encoded by exons 3 and 4, and the hinge region and LBD are encoded by exons 5-9 (Figure 4). Alternative splicing in exon 9 near the end of the GR primary transcript generates two receptor isoforms, termed GR $\alpha$  and GR $\beta$  (Figure 4) (53, 54). GR $\alpha$  is derived from the end of exon 8 being joined to the beginning of exon 9, whereas GR $\beta$  results from the end of exon 8 being joined to downstream sequences in exon 9. These two proteins are identical through amino acid 727 but then diverge. GR $\alpha$ , the classic GR protein that mediates the actions of glucocorticoids, contains an additional 50 amino acids. The splice variant GR $\beta$  contains an additional, nonhomologous 15 amino acids. The unique GR $\beta$  carboxyl-terminal sequence confers several distinct properties to this receptor isoform. GR $\beta$  does not bind glucocorticoid agonists, resides constitutively in the nucleus of cells, and by



itself is inactive on glucocorticoid-responsive reporter genes (55, 56). However, when co-expressed with GR $\alpha$ , GR $\beta$  functions as a dominant negative inhibitor and antagonizes the activity of GR $\alpha$  on many glucocorticoid-responsive target genes. Competition for GRE binding, competition for transcriptional coregulators, and formation of inactive GR $\alpha$ /GR $\beta$  heterodimers have each been proposed to underlie the antagonism mediated by GR $\beta$ .

The ability of GR $\beta$  to inhibit the activity of GR $\alpha$  suggests that high levels of GR $\beta$  may lead to glucocorticoid resistance. GR $\beta$  is prevalent in many cells and tissues but generally is found at lower levels than GR $\alpha$ . However, GR $\beta$  is abundant in certain cell-types such as neutrophils and epithelial cells. Moreover, the expression of GR $\beta$  can be selectively increased by proinflammatory cytokines and other immune activators and lead to reduced glucocorticoid sensitivity (57-59). Elevated levels of GR $\beta$  have been associated with glucocorticoid resistance in a variety of inflammatory diseases including asthma, rheumatoid arthritis, ulcerative colitis, nasal polyposis, systemic lupus erythematosus, sepsis, acute lymphoblastic leukemia, and chronic lymphocytic leukemia (56). Upregulation of GR $\beta$  and diminished GR $\alpha$  signaling is also observed in erythroid cells expanded from polycythemia vera patients (60). The molecular factors that control GR $\beta$  expression are poorly understood, but several studies have implicated the involvement of the splicing factor SRp30c (61-63). Treatment of cells with agents that induce SRp30c expression leads to a selective increase in GR $\beta$  and consequent glucocorticoid insensitivity. A naturally occurring polymorphism (A3669G) in the 3' untranslated region of the GR $\beta$  mRNA also leads to increased expression of GR $\beta$  (64, 65). The polymorphism disrupts an mRNA destabilization motif (AUUUA) resulting in a prolonged half-life of the GR $\beta$  mRNA. A3669G carriers have an elevated risk for pathologies with known inflammatory underpinnings such as autoimmune disease, myocardial infarction, coronary artery disease, and heart failure (66-68). These findings suggest that the rise in GR $\beta$  in persons with the A3669G polymorphism may attenuate the beneficial immunosuppressive and anti-inflammatory actions of GR $\alpha$ .

Recent discoveries suggest an even broader role for GR $\beta$  in cell signaling and physiology. Employing genome wide microarrays on cells engineered to selectively express GR $\beta$ , multiple laboratories have shown that GR $\beta$  directly induces and represses the expression of a large number of genes independent of its dominant negative activity on GR $\alpha$  (69, 70). The ability of GR $\beta$  to recruit histone deacetylases and close local chromatin structure appears to be involved in its repression of certain genes such as interleukin 5 and interleukin 13 (71, 72). GR $\beta$  can also bind the glucocorticoid antagonist mifepristone (RU486) (70). In the presence of RU486, many of the GR $\beta$ -mediated changes in gene expression are abolished. These findings indicate that GR $\beta$  can function as a bonafide transcription factor, and they raise the possibility that GR $\beta$  modulates glucocorticoid responses by genomic actions distinct from its antagonism of GR $\alpha$ . Indeed, GR $\beta$  has recently been shown to directly reduce the expression of histone deacetylase 2 and promote glucocorticoid insensitivity in bronchoalveolar lavage cells from patients with steroid resistant asthma (73). Another interesting finding has been the discovery of GR $\beta$  in zebrafish, mice, and rats (74-76). These isoforms are similar in structure and function to human GR $\beta$  but originate from a mechanism of alternative splicing that differs from that in humans. Knockout of GR $\beta$  in these species promises to shed new light into the physiological and pathological importance of this splice variant.

In addition to GR $\beta$ , alternative splicing of the GR gene gives rise to other receptor isoforms with distinct signaling properties (Figure 4). The use of an alternative splice donor site in the intron separating exons 3 and 4 results in a receptor isoform that contains an insertion of a single arginine residue between the 2 zinc fingers of the GR DBD (77). This receptor variant, termed GR $\gamma$ , is widely expressed and binds glucocorticoids and DNA in a manner similar to GR $\alpha$ . However, GR $\gamma$  is impaired in its ability to regulate glucocorticoid-responsive reporter genes and exhibits a transcriptional profile distinct from GR $\alpha$  on a subset of endogenous genes regulated by glucocorticoids. GR $\gamma$  expression is associated with glucocorticoid resistance in small cell lung carcinoma, corticotroph adenomas, and childhood acute lymphoblastic leukemia (77-79). Two GR splice variants missing large regions of the LBD were initially discovered in glucocorticoid-resistant multiple myeloma cells (80). GR-A is missing middle exons 5-7 which encode the amino-terminal half of the LBD, and GR-P is missing the terminal exons 8-9 which encode the carboxyl-terminal half of the LBD. As expected from these changes in the LBD, GR-A and GR-P do not bind glucocorticoids. Little is currently known about GR-A; however, GR-P has been shown to modulate the transcriptional activity of GR $\alpha$  in a cell-type specific manner (81-83). GR-P is found in many tissues and appears to be the predominant receptor variant expressed in glucocorticoid resistant cancer cells.

## GR TRANSLATIONAL ISOFORMS

Alternative translation initiation from the single GR $\alpha$  mRNA transcript produces an additional cohort of diverse GR proteins (Figure 5). Eight AUG start codons derived from exon 2 of the GR gene give rise to 8 GR $\alpha$  subtypes with progressively shorter NTDs (GR $\alpha$ -A, GR $\alpha$ -B, GR $\alpha$ -C1, GR $\alpha$ -C2, GR $\alpha$ -C3, GR $\alpha$ -D1, GR $\alpha$ -D2, and GR $\alpha$ -D3) (15, 84). All 8 sites are highly conserved across species. GR $\alpha$ -A is the full-length receptor that is generated from the first initiator codon. Production of the truncated isoforms from internal start codons involves both ribosomal leaky scanning and ribosomal shunting mechanisms. The mRNA species encoding the splice variants discussed above (GR $\beta$ , GR $\gamma$ , GR-A, and GR-P) also contain the identical set of start codons and would therefore be expected to give rise to a similar complement of translational isoforms.

The GR $\alpha$  translational isoforms display a similar affinity for glucocorticoids and a similar capacity to interact with GREs following ligand activation; however, they show marked differences in other properties (8, 15). The GR $\alpha$ -A, GR $\alpha$ -B, and GR $\alpha$ -C isoforms are localized in the cytoplasm of cells in the absence of hormone and translocate to the nucleus upon glucocorticoid binding. In contrast, the GR $\alpha$ -D isoforms reside constitutively in the nucleus of cells. In addition, nuclear localized GR $\alpha$ -D associates with GRE-containing promoters of specific target genes independent of glucocorticoid treatment. Most striking, however, is that each GR $\alpha$  subtype possesses a distinct gene regulatory profile. When the individual isoforms are expressed at similar levels in U2OS osteosarcoma or Jurkat T lymphoblastic leukemia cells, they each regulate a unique set of genes (8, 85). Less than 10% of the genes were commonly regulated by all the subtypes, indicating that the vast majority of genes were selectively regulated by different GR $\alpha$  isoforms. These isoform-unique gene regulatory profiles lead to functional differences in glucocorticoid-induced apoptosis (8, 85, 86). Cells expressing GR $\alpha$ -C3 were the most sensitive to the cell-killing



effects of glucocorticoids whereas cells expressing GR $\alpha$ -D3 were the most resistant. The heightened activity of the GR $\alpha$ -C3 isoform has also been observed on glucocorticoid responsive reporters and has been linked to an N-terminal motif (residues 98-115) that is sterically hindered in the larger receptor isoforms (15, 87). When unobstructed in GR $\alpha$ -C3, this motif increases the activity of the AF1 domain presumably through enhanced recruitment of various coregulators. The complete absence of the AF-1 domain likely underlies the reduced transcriptional activity of the GR $\alpha$ -D isoforms. GR $\alpha$ -D3 does not efficiently repress the expression of multiple anti-apoptotic genes due to deficits in its ability to interact with the p65 subunit of NF- $\kappa$ B and to be recruited to the promoters of these glucocorticoid-responsive genes (86).

The discovery that each GR $\alpha$  translational isoform regulates a unique transcriptome suggests that the cellular response to glucocorticoids will be governed by the expressed complement of receptor subtypes. The GR $\alpha$ -A and GR $\alpha$ -B isoforms are the most abundant GR proteins in many cell-types. However, trabecular meshwork cells from the human eye preferentially express the GR $\alpha$ -C and GR $\alpha$ -D isoforms (88). In addition, immature dendritic cells predominantly express the GR $\alpha$ -D isoforms whereas mature dendritic cells predominantly express the GR $\alpha$ -A subtype (89). In rodents, GR $\alpha$ -C isoforms are highest in pancreas and colon and the GR $\alpha$ -D subtypes are highest in spleen and lung (15). Recent studies have also demonstrated that the composition of GR $\alpha$  subtypes in a given cell or tissue can change in response to different conditions. Treatment of differentiated murine skeletal muscle cells with a selective estrogen-related receptor  $\beta/\gamma$  agonist induced a preferential increase in the GR $\alpha$ -D isoforms (90), and the GR $\alpha$ -C isoforms are selectively upregulated following mitogen activation of human primary T cells (85). In the human brain, alterations in the expression of the GR subtypes were observed during development and during the ageing process (91). Moreover, a selective increase in GR $\alpha$ -D was measured in specific regions of the brain in patients with schizophrenia and bipolar disorder (92, 93). Since preferential increases in the expression of the GR $\alpha$ -C or GR $\alpha$ -D isoforms may lead to glucocorticoid hypersensitivity or hyposensitivity, respectively, an important goal of future studies will be to define the operative cellular mechanisms that control the expressed complement of translational isoforms. Currently, the efficiency with which a particular start codon is used has been reported to be influenced by the ER22/23EK polymorphism in the GR gene and heterogeneity in the 5' untranslated region of the GR $\alpha$  mRNA (94, 95). Post translational modifications that differentially affect the half-life of the various receptor subtypes may also contribute to alterations in the expression profile of the GR $\alpha$  isoforms.

## POST-TRANSLATIONAL MODIFICATION OF GR ISOFORMS

Each individual GR isoform originating from alternative processing of the GR gene is subject to a variety of post-translational modifications that further modulate its function and expand the repertoire of receptor subtypes available for glucocorticoid signaling. The first identified and most extensively studied covalent modification of GR is phosphorylation (96-98). Human GR $\alpha$  is phosphorylated on at least 7 serine residues (Ser-113, Ser-134, Ser-141, Ser-203, Ser-211, Ser-226, and Ser-404), all of which are located in the NTD of the receptor (Figure 2). The major kinases that phosphorylate GR $\alpha$  include MAPKs, cyclin dependent kinases, casein kinase II, and GSK-3 $\beta$ . Many of the sites exhibit a low level of

basal phosphorylation and become hyperphosphorylated following the binding of glucocorticoids (99). The structure of the bound glucocorticoid can influence both the pattern and extent of GR $\alpha$  phosphorylation (100). Ser-134 is unique in that it is phosphorylated in a glucocorticoid-independent manner by stress-activating stimuli (101).

One of the major effects of GR $\alpha$  phosphorylation is that it changes the transcriptional activity of the receptor. Early studies demonstrated that phosphorylation-deficient GR $\alpha$  mutants were impaired in their ability to activate some glucocorticoid-responsive promoters but not others (102). Subsequent reports showed that phosphorylation at Ser-211 correlated with increased transcriptional activity of GR $\alpha$  whereas phosphorylation at Ser-226 decreased the signaling capacity of the receptor (99, 103, 104). A deficiency in Ser-211 phosphorylation may contribute to the resistance to glucocorticoid-induced apoptosis that develops in malignant lymphoid cells (105, 106). Conversely, increased phosphorylation at Ser-226 may account for the impaired glucocorticoid signaling in the pathophysiology of depression (107). Phosphorylation of Ser-404 also has major consequences on glucocorticoid responses, as the ability of GR $\alpha$  to both activate and repress target genes is diminished by phosphorylation at this site (108). In cells expressing a GR $\alpha$  mutant incapable of Ser-404 phosphorylation, the global transcriptional response to glucocorticoids is redirected to favor the activation of distinct signaling pathways. Consistent with many of the phosphorylation sites residing within the AF1 domain, differences in cofactor recruitment appear to be responsible for the transcriptional alterations that accompany GR $\alpha$  phosphorylation. GR $\alpha$  recruitment of the coactivator MED14 is enhanced by glucocorticoid-dependent phosphorylation at Ser-211 (104), whereas the interaction of GR $\alpha$  with the coactivator p300/CBP and the p65 subunit of NF- $\kappa$ B are both diminished by phosphorylation at Ser-404 (108).

Phosphorylation alters other properties of GR that impact the profile of glucocorticoid signaling. Degradation of the GR $\alpha$  protein is enhanced by glucocorticoid-dependent phosphorylation of the receptor, as phosphorylation deficient mutants are stabilized in the presence of glucocorticoids (102). In addition, the ligand-free GR $\alpha$  is protected from degradation by its association with the tumor suppressor gene TSG101 which preferentially interacts with the non-phosphorylated receptor (109). The cellular distribution of GR $\alpha$  is also altered by receptor phosphorylation. GR $\alpha$  phosphorylated on Ser-203, Ser-226, or Ser-404 spends less time in the nuclear compartment due to greater cytoplasmic retention and/or enhanced nucleocytoplasmic transport. As a consequence, GR $\alpha$  phosphorylated on either of these residues exhibits reduced transcriptional activity on glucocorticoid-responsive target genes (99, 103, 108, 110).

Additional post-translational modifications of GR have been described that regulate the function of the receptor. Ubiquitin is a 76 amino acid protein that when attached to specific lysine residues marks proteins for proteasomal degradation. Ubiquitination of GR $\alpha$  at a conserved lysine residue (Lys-419) has been shown to target the receptor for turnover by the proteasome (Figure 2) (111, 112). Mutant receptors that cannot be ubiquitinated at this residue are resistant to ligand-dependent downregulation and exhibit potentiated transcriptional activity on glucocorticoid-responsive reporter genes. Moreover, changes in the expression of an E3 ubiquitin ligase for GR $\alpha$  leads to alterations in both receptor levels

and cellular sensitivity to glucocorticoids (113). GR $\alpha$  is also post-translationally modified by sumoylation, a process in which SUMO (small ubiquitin-related modifier) peptides are covalently attached to specific lysine residues (Lys-277, Lys-293, Lys-703) within the receptor (Figure 2). The addition of SUMO peptides to the receptor occurs in the absence of ligand and is increased by the binding of glucocorticoids. Depending on the site of sumoylation, the transcriptional activity of GR $\alpha$  can be enhanced or repressed via alterations in the recruitment and/or activity of specific coregulators (114-119). Finally, it has been suggested that GR $\alpha$  becomes acetylated on lysine residues (Lys-494 and Lys-495) located within the hinge region in response to glucocorticoid binding (Figure 2) (120). Deacetylation of GR by histone deacetylase 2 was reported to be required for the receptor to efficiently repress the transcriptional activity of NF- $\kappa$ B, suggesting that acetylation of GR limits the inhibitory actions of glucocorticoids on NF- $\kappa$ B signaling. More recently, studies have shown that the clock transcription factor acetylates GR $\alpha$  and attenuates its ability to both activate and repress glucocorticoid responsive genes, conferring glucocorticoid insensitivity to target tissues (121). Clearly, post translational modifications can regulate multiple aspects of GR $\alpha$  function, providing cells with additional receptor heterogeneity for controlling glucocorticoid responses. An important goal of future research will be to determine to what extent the various splicing and translational isoforms of GR are subject to and regulated by these post translational modifications.

## SUMMARY AND FUTURE PERSPECTIVES

Glucocorticoids act through GR to regulate numerous physiological processes, and synthetic derivatives of these hormones are widely prescribed for treating inflammatory diseases, autoimmune disorders, and various cancers. A challenge to clinicians and patients alike is that cell, tissue, and individual responses to glucocorticoids are markedly different and can change over time and in response to various extracellular cues. The discovery that multiple GR isoforms arise from a single gene has advanced our understanding of the molecular basis for the diversity in glucocorticoid signaling. GR isoforms with unique expression, gene regulatory, and functional profiles are generated by alternative splicing of the primary transcript, alternative translation initiation of the mature mRNA, and post-translational modifications of the encoded protein. The potential for these GR subtypes to undergo various post-translational modifications and to function as monomers, homodimers, and heterodimers provides cells with a wealth of possibilities for generating diverse glucocorticoid responses with fine-tuned precision. Alterations in the relative levels of the GR subtypes may underlie pathologies characterized by hyposensitivity or hypersensitivity to glucocorticoids.

The cellular response to glucocorticoids will depend not only on the GR isoform composition but also on the glucocorticoid that binds and activates the receptor as well as the concentration of the administered steroid. Not all glucocorticoids are created equal, as structurally different but similarly potent steroids used in the clinic regulate both common and unique sets of genes (88, 122). The distinct transcriptional signature of these glucocorticoids suggests that their binding confers unique conformations on GR that lead to differences in DNA binding, chromatin remodeling, and/or coregulator recruitment. Recent discoveries also suggest that the concentration of glucocorticoids needed to achieve the

desired transcriptional response can vary in a context-specific manner. For example, GR repression of pro-inflammatory genes via tethering to NF- $\kappa$ B occurs at much lower glucocorticoid concentrations than the induction of gene expression (123). Global GR recruitment assays have revealed that different GREs and their associated target genes also exhibit profound differences in their sensitivity to glucocorticoids (29). In addition, dose-response studies in the livers of male and female rats have discovered several genes in females that are 10- to 100-fold less sensitive to glucocorticoid regulation than they are in males (43). Remarkably, even the polarity of the transcriptional response of certain GR-regulated genes can vary depending on the dose of glucocorticoid (124). Collectively, these findings suggest that as the specific genes underlying various pathologies become identified, careful consideration of the structure and dose of the employed glucocorticoid may help fine-tune the glucocorticoid response and lead to improved benefit/risk ratios for patients on steroid therapy.

Glucocorticoid resistance remains a major barrier to the effective treatment of a variety of immune and inflammatory diseases (125, 126). Advances in our understanding of glucocorticoid signaling have uncovered a variety of mechanisms that contribute to reduced glucocorticoid responsiveness, including increased expression of the GR $\beta$  and GR $\alpha$ -D isoforms, changes in GR phosphorylation, and homologous down-regulation of the receptor. Understanding the molecular mechanisms of resistance permits not only the prediction of patient responsiveness to glucocorticoids but also the design of novel therapeutic strategies for combatting the insensitivity. For instance, altered patterns of GR phosphorylation thought to contribute to glucocorticoid resistance in patients with severe asthma can be reversed by co-administration of long acting  $\beta$ 2AR agonists or p38 MAPK inhibitors (125). Development of glucocorticoid analogues deficient in their ability to down regulate the expression of GR may also prove to be an effective strategy for overcoming steroid resistance.

The harmful side effects of medicinal glucocorticoids also remain a frustration for clinicians and their patients. Therefore, intense efforts have been made over the last decade to develop novel GR ligands, termed dissociated or selective GR agonists (SEGRA), that retain the negative regulation of gene expression thought to be important for many of the anti-inflammatory actions of glucocorticoids but lose the positive regulation of gene expression thought to underlie many of their adverse effects. A number of SEGRAs have been developed but few have made it to clinical trials, and several recent studies have challenged both the premise and utility of these analogues as therapeutic agents (127, 128). One potential short-coming of SEGRAs is that mounting evidence indicates that glucocorticoid-dependent activation of many genes also plays a critical role in the anti-inflammatory mechanisms of glucocorticoids. Clearly, more research is needed to unravel the molecular details by which glucocorticoids repress the inflammatory response and induce undesirable side effects. A greater understanding of the heterogeneity in GR signaling responses in both healthy and diseased tissues will aid in the development of safer and more effective glucocorticoid therapies.

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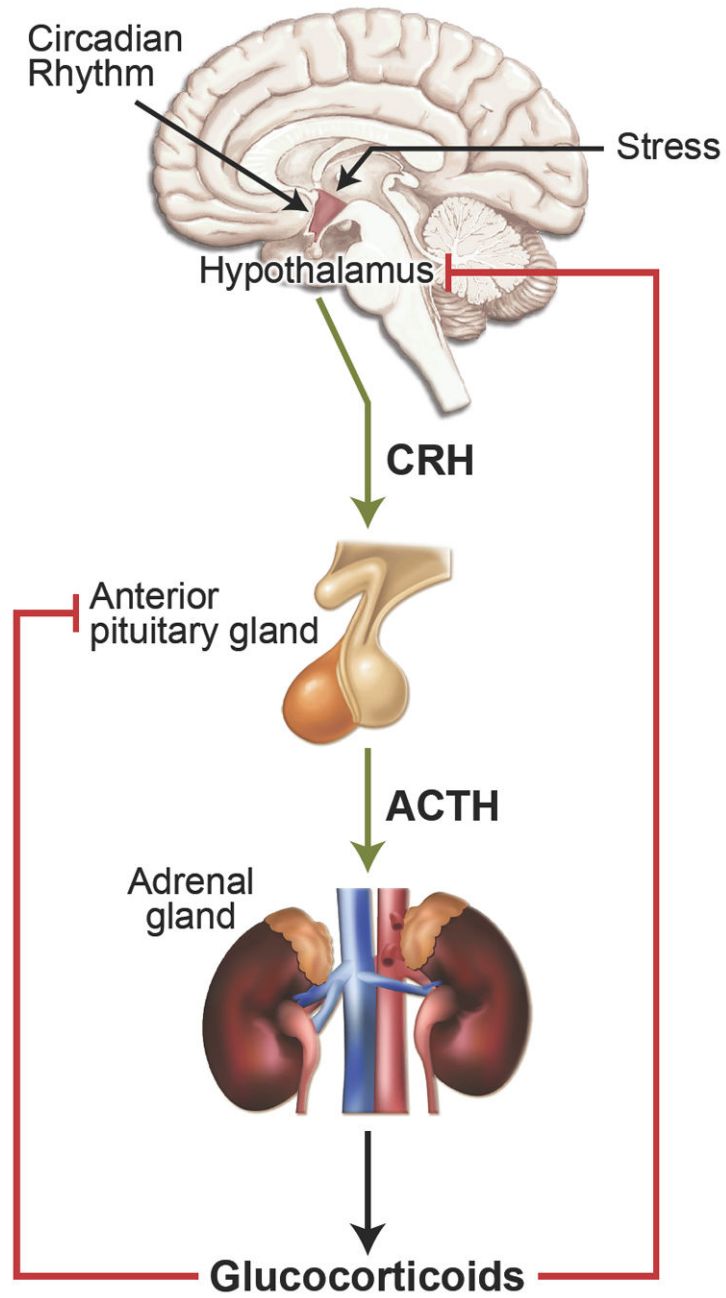
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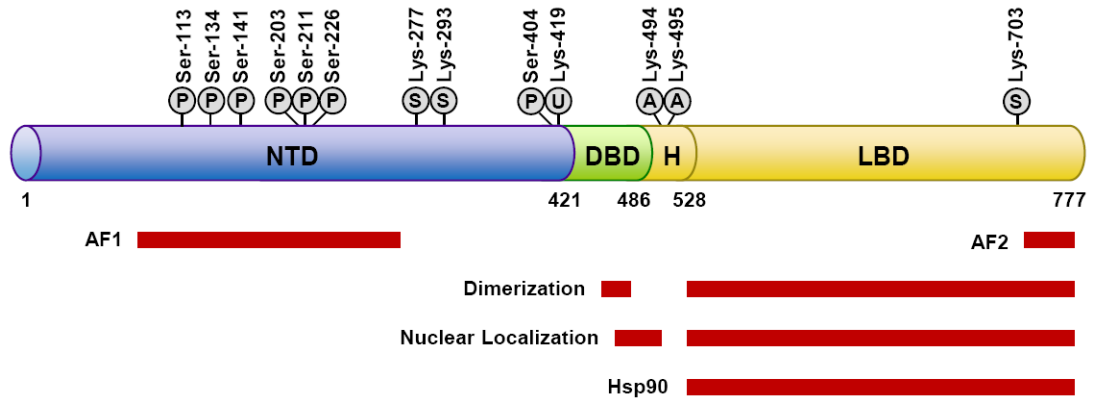
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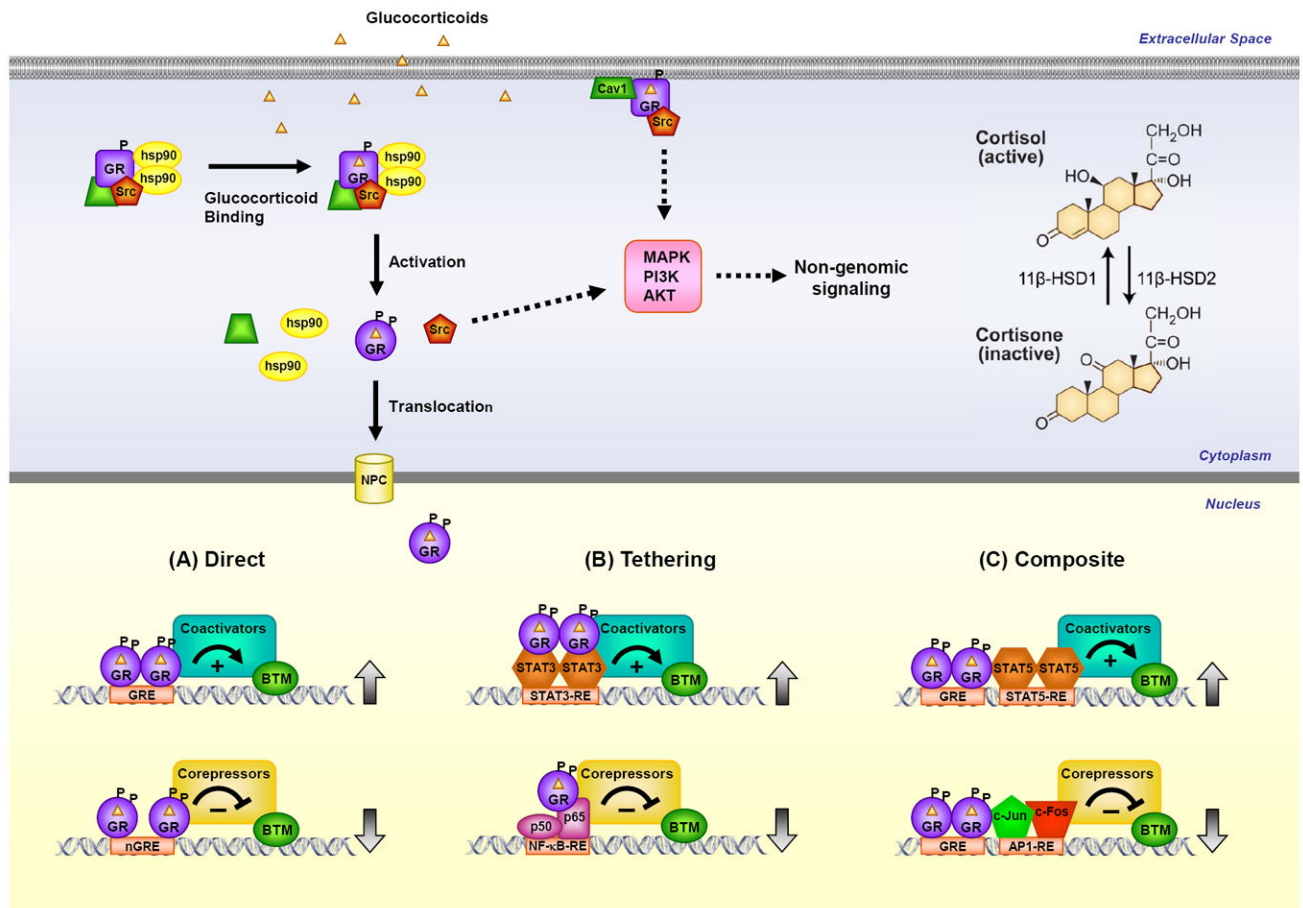


**Figure 1.** Regulation of glucocorticoid hormone secretion by the hypothalamic-pituitary-adrenal (HPA) axis.



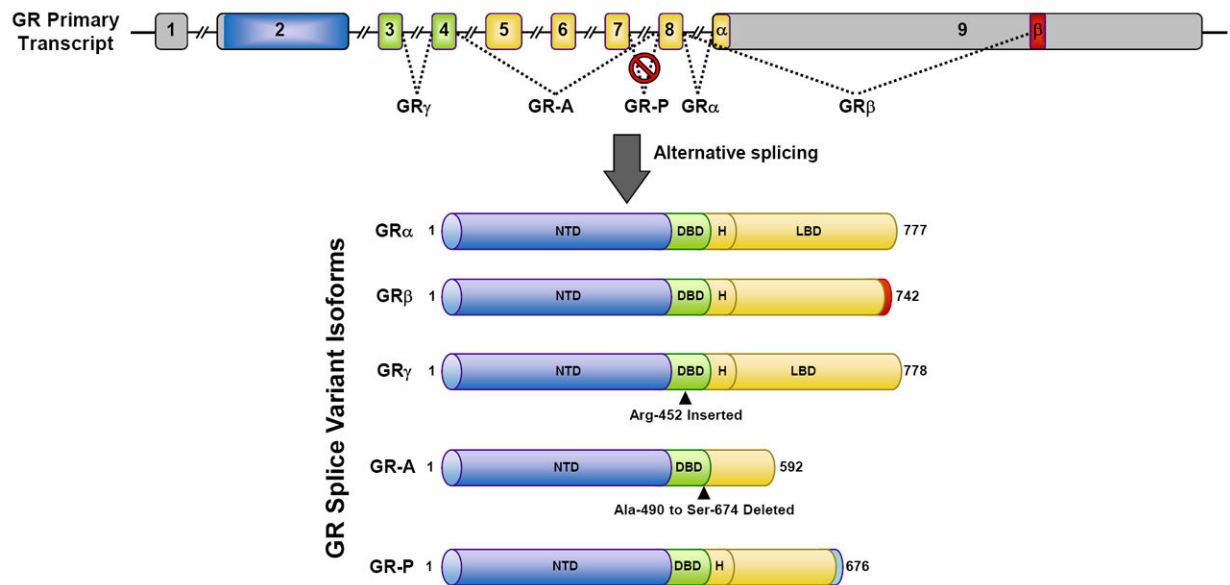
**Figure 2.**

GR domain structure and sites of post-translational modification. Shown are the domains of GR and regions of the receptor involved in transactivation (AF1 and AF2), dimerization, nuclear localization, and hsp90 binding. Also depicted are the amino acid residues modified by phosphorylation (P), sumoylation (S), ubiquitination (U), and acetylation (A). Numbers are for human GR.

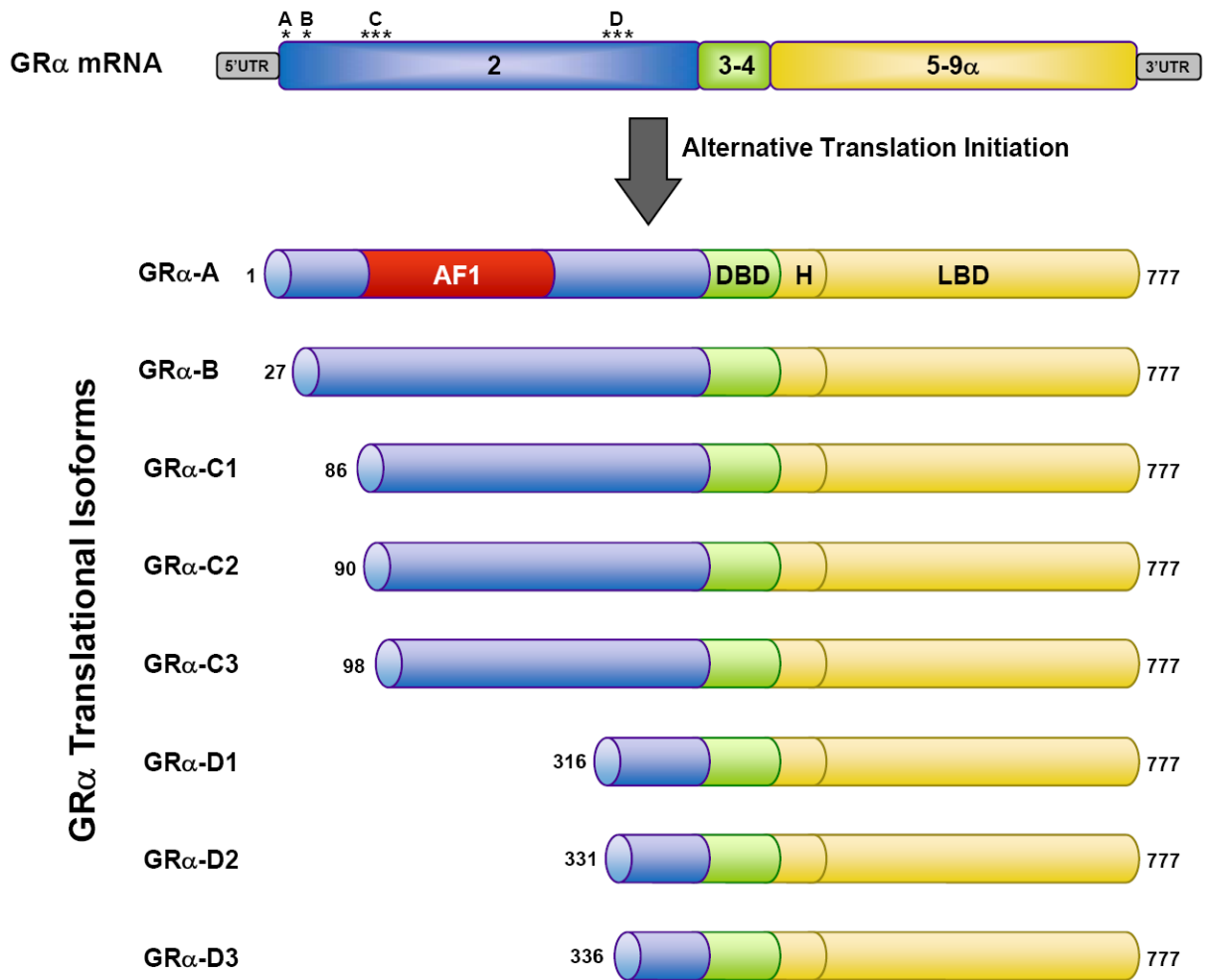


**Figure 3.**

GR signaling pathways. Glucocorticoid-activated GR regulates gene expression in 3 primary ways: binding directly to DNA (A), tethering itself to other DNA-bound transcription factors (B), or binding directly to DNA and interacting with neighboring DNA-bound transcription factors (C). GR can also signal in a non-genomic manner through alterations in the activity of various kinases.

**Figure 4.**

GR splice variants. The GR primary transcript is composed of 9 exons. Exon 2 encodes the NTD, exons 3-4 encode the DBD, and exons 5-9 encode the hinge region (H) and LBD. GR $\alpha$  results from splicing exon 8 to the beginning of exon 9. GR $\beta$ , GR $\gamma$ , GR-A, and GR-P are generated by the depicted alternative splicing events.



**Figure 5.**

GR translational isoforms. Translation initiation from 8 different AUG start codons in the single GR $\alpha$  mRNA (location of the AUG start codons indicated by an asterisk) produces 8 receptor isoforms with progressively shorter amino NTDs. The numbers for the initiator methionines and AF1 region (amino acids 77-262) are for the human GR $\alpha$  protein.