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Author manuscript

Endocr Dev. Author manuscript; available in PMC 2016 February 29.

Published in final edited form as:

Endocr Dev. 2013 ; 24: 41–56. doi:10.1159/000342502.

Exploring the Molecular Mechanisms of Glucocorticoid Receptor Action from Sensitivity to Resistance

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Abstract

Glucocorticoids regulate a variety of physiological processes, and are commonly used to treat disorders of inflammation, autoimmune diseases, and cancer. Glucocorticoid action is predominantly mediated through the classic glucocorticoid receptor (GR), but sensitivity to glucocorticoids varies among individuals, and even within different tissues from the same individual. The molecular basis of this phenomenon can be partially explained through understanding the process of generating bioavailable ligand and the molecular heterogeneity of the GR. The molecular mechanisms that regulate glucocorticoid action highlight the dynamic nature of hormone signaling and provide novel insights into genomic glucocorticoid actions and glucocorticoid sensitivity. Although glucocorticoids are highly effective for therapeutic purposes, long-term and/or high-dose glucocorticoid administration often leads to reduced glucocorticoid sensitivity or resistance. Here, we summarize our current understanding of the mechanisms that modulate glucocorticoid sensitivity and resistance with a focus on GR-mediated signaling.

Glucocorticoids are a class of stress-induced steroid hormones synthesized by the adrenal cortex under control of the hypothalamic-pituitary-adrenal axis [1]. Endogenous glucocorticoid levels in the serum display a classic circadian pattern, peaking at the beginning of the period of highest activity. Additionally, glucocorticoid levels are strongly elevated in response to a variety of physical and psychological stresses [1]. Cortisol in humans and corticosterone in rodents act to regulate diverse cellular functions including development, homeostasis, metabolism, cognition and inflammation. Glucocorticoids play a significant role in maintaining the immune system, acting to prevent excessive and harmful responses to injury or infection. The anti-inflammatory and immunomodulatory properties underlie the use of glucocorticoids in the clinic where they are used to treat inflammatory diseases and oncological disorders [1].

A plethora of synthetic glucocorticoids have been developed for therapeutic use, and the worldwide market in oral and topical glucocorticoids is estimated to be worth more than USD 10 billion per year [2]. Glucocorticoid agonists are frequently used to treat many inflammatory conditions, from inflammatory arthritis, ulcerative colitis to asthma and skin

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diseases. Proapoptotic properties of glucocorticoids make them a major component of chemotherapeutic regimens for the treatment of cancers of hematological origins including Hodgkin's lymphoma, acute lymphoblastic leukemia (ALL) and multiple myelomas. Glucocorticoid usage continues to grow every year, driven by increased prevalence of chronic diseases in an ageing population and by increased duration of treatment in certain patients. Although glucocorticoids are highly effective for therapeutic purposes, long-term and/or high-dose glucocorticoid administration is commonly associated with adverse side effects, like hyperglycemia, weight gain, hypertension, osteoporosis, depression and decreased immunological function. Furthermore, patients on glucocorticoids can develop reduced glucocorticoid sensitivity and even resistance. Current research is focused on developing synthetic glucocorticoids with increased tissue selectivity to minimize the side effects by dissociating the desired anti-inflammatory effects from undesirable side effects [3]. Here, we summarize the recent advances and molecular processes involved in glucocorticoid sensitivity and function and discuss in detail the mechanisms that contribute to glucocorticoid resistance. The potential role of glucocorticoid receptor (GR) gene in determining cellular responsiveness to glucocorticoids is emphasized.

The Glucocorticoid Receptor

Organization of the hGR Gene, mRNA and Protein

Glucocorticoids mediate their effect through intracellular GR, which belongs to a large family of transcription factors known as the nuclear hormone receptors. The human GR (NR3C1) is the product of one gene that is located in chromosome 5q31–32. The hGR promoter lacks a consensus TATA box and CCAAT motif, however contains binding sites for transcription factors like AP1, SP1, AP2 nuclear factor-κB (NF-κB) and CREB. The hGR gene consists of 9 exons; exon 1 forms the 5′-untranslated region, while exon 2–9 code for the GR protein. Recent studies have identified 9 alternative first exons (1A, 1B, 1C, 1D, 1E, 1F, 1H, 1I, and 1J) that are generated from unique promoter usage, and likely account for tissue-specific expression of GR. Exon 2 forms the N-terminal domain of GR, exon 3–4 form the central DNA-binding domain (DBD), while exons 5–9 code for the ligand-binding domain (LBD; fig. 1a).

Alternative splicing of the hGR gene in exon 9 generates two highly homologous mRNA transcripts that results in the production of two GR isoforms termed GRα and GRβ. Both the isoforms contain exons 1–8 with different version of exon 9, exon 9α and -β, respectively. The two isoforms are identical up to amino acid 727, but vary beyond this position. The predominant form of GR (GRα) is a protein composed of 777 amino acids. In GRβ, the carboxy terminal 50 amino acids of GRα are replaced by 15 non-homologous amino acids [4].

Our laboratory has identified N-terminal isoforms of GR, which are generated by alternative translation initiation. In 2001, Yudt and Cidlowski [5] demonstrated that COS-1 cells transiently transfected with cDNA encoding hGRα, yield two GR proteins of 94 and 91 kDa termed hGR-A and hGR-B, respectively. Subsequent analysis revealed that hGRα mRNA is translated from at least eight alternative initiation sites producing multiple GRα isoforms termed GR-A, B, C1, C2, C3, D1, D2 and D3. The 94-kDa protein is the classic GRα which

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is now termed GRα-A, the 91-kDa protein is GRα-B, the 82-to 84-kDa proteins form GRα-C1, C2 and C3, and the 53-to 56-kDa proteins produce D1, D2 and D3 (fig. 1a). The internal AUG codons for all the isoforms are conserved among the human, rat and mouse gene. The mechanism for generating the translational isoforms involves leaky 5′ ribosomal scanning and/or ribosomal shunting, both of which are regulated by mRNA-specific elements. Since all the alternative start codons are located in the amino terminal half of the receptor, hGR-α isoforms have identical DBD and LBD, but differ at their N-terminal region [6].

Similar to other steroid hormone receptors, GR is a modular protein organized into three major functional domains: the N-terminal domain, the central DBD and the C-terminal LBD. The N-terminal domain covering amino acids 1–420 is poorly conserved and is the most variable domain between the steroid hormone receptors. The N-terminal domain contains the transactivation domain AF-1 (activation function-1), which activates target genes in a ligand-independent fashion. The central domain is highly conserved and harbors the DBD, which contains two zinc finger motifs. The carboxy-terminal of GR contains the LBD (aa 527–777), which recognizes and binds ligand. A second activation function domain AF-2 is located within this carboxy-terminal region. The C-terminus also contains sequences important for interaction with heat shock proteins and coregulators (fig. 1b).

The classic mode of action of steroids occurs by direct regulation of gene transcription. In the absence of ligand, GRα is mainly found in the cytoplasm as a heterocomplex by coordinated associations with molecular chaperones, such as heat shock proteins 40 (hsp40), hsp70 and hsp90, and cochaperones including hsp70-interacting protein (hip) and hsp70/hsp90-organizing protein (hop). These chaperones maintain the receptor in the proper confirmation to bind the ligand in a hydrophobic pocket in the C-terminus. When lipophilic glucocorticoids diffuse across the plasma membrane and bind GR, this induces rearrangement of the GR heterocomplex leading to GR homodimerization and nuclear translocation. Once inside the nucleus, GR can regulate transcription positively or negatively [7].

Transcriptional Activation by the Glucocorticoid Receptor

Once inside the nucleus, GR binds directly to DNA elements called glucocorticoid response elements (GREs) to stimulate target gene expression. Binding to GRE induces conformational changes in GR leading to coordinated recruitment of coactivators and chromatin-remodeling complexes that influence the activity of RNA polymerase II and activate gene transcription (fig. 2). The classic view that GR induces gene expression by binding to GREs located only in the promoter proximal region of the target gene has been questioned by recent discoveries using ChIP-chip and next-generation sequencing (ChIP-seq) [8]. In these studies, GR-binding sites are isolated by chromatin immunoprecipitation and then identified by ChIP-seq or by hybridization to tile microarray. The comprehensive genomic map of GR:DNA binding derived from these studies revealed that many GR-binding sites identified are located far from the promoter proximal region of target genes and showed an unexpected difference between the activation and repressive functions of the GR. A significant proportion of the GR-binding sites lacked a consensus GRE element, which suggests that binding of GR to the chromatin may in many cases occur by tethering to

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other transcription factors [8]. Additionally, GR can physically interact with the members of the signal transducer and activator of transcription (STAT) family, either in conjunction with binding a GRE or apart, to enhance transcription of certain target genes. What remains to be established, however, is the functionality of these distant GR-binding sites in relation to the transcription of genes or other undiscovered functions encoded in the GR protein.

In contrast to the classic model where binding of transcription factors to DNA is characterized by stable complexes, the binding of GR to chromatin and the hormone-dependent remodeling of chromatin are highly dynamic and differentially affected by ligand type. Using fluorescently tagged receptor coupled with photo-bleaching experiments, GR was found to rapidly cycle on and off the chromatin in living cells within seconds to minutes. Even though various important findings have emerged from these large-scale GR-chromatin interaction studies, several questions still remain to be addressed. GR-chromatin binding data alone do not prove that the binding of GR at a specific site is important in the regulation of a particular target gene. Therefore, a combination of location analysis and expression profiling is required to validate if GR binding sites are functional.

Glucocorticoid-induced gene expression is frequently cell type-specific and only a small proportion of genes are commonly activated between different tissues. Tissue-specific target gene activation by glucocorticoids has been shown to be dependent on accessibility of the GR-binding site which in turn is determined by DNA methylation and higher order chromatin structures like long-range chromatin loops. Thus, tissue-specific target gene activation may be determined by the tissue-specific chromatin landscape, which influences binding of GR to the cognate DNA elements.

Transcriptional regulation of GR is also modulated by recruitment of coactivators, which mediate posttranslation modifications of histones (acetylation and methylation). This property aids in altering the chromatin structure and recruiting other cofactors, thus making the chromatin more accessible for the assembly of general transcription factors and the RNA polymerase complex at the target gene promoter. The identity of coregulators that contribute to GR transactivation has grown in the recent years to numbers in the hundreds. Some of the well-studied GR coactivators are the SRC family proteins, mediator complex and SWI/SNF complexes [7]. It has been shown that binding of liganded GR to the GRE results in a conformational change that facilitates the binding of SRC to GR and cooperates in the assembly of the transcription initiation complex at the promoter of the target gene.

Posttranslational modifications of GR further modulate the transcriptional landscape of the receptor. Phosphorylation is the most studied covalent modification of GR. At least seven serine residues (Ser-113, Ser-134, Ser-141, Ser-203, Ser-211, Ser-226 and Ser-404) are phosphorylated in GR, and all these sites are also conserved in mouse and rat (fig. 1b). Other sites of phosphorylation include Ser-45 and 267 [9]. The receptor displays a basal level of phosphorylation and becomes hyperphosphorylated upon binding glucocorticoids. Phosphorylation of GRα changes its transcriptional activity, often in a gene-specific manner. Ligand-dependent phosphorylation of Ser-404 has been shown to impact transcriptional activity of GR by impairing both activation and repression of target genes. Differences in cofactor recruitment have been implicated in these impaired transcriptional effects due to

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phosphorylation at Ser-404. Recently, our laboratory has identified a new hormone independent phosphorylation site Ser134. Serine 134 is hyperphosphorylated under an array of stressful conditions, including glucose starvation, oxidative stress, UV irradiation and osmotic shock. Phosphorylation of Ser-134 significantly increased the association of the GR with the ζ-isoform of the 14-3-3 class of signaling proteins (14-3-3ζ) on promoter proximal regions, resulting in a blunted hormone-dependent transcriptional response of specific genes. This study shows the level of molecular stress, as measured by Ser134-GR phosphorylation, has a global impact on the function of the signaling property of GR within animal cells [10].

GR is also subject to a variety of other posttranslational modifications. GR is ubiquitinated at a conserved lysine residue located in a PEST degradation motif, and this modification targets the receptor for degradation by the 26S proteasome. Mutation of this conserved Lys residue enhances the glucocorticoid-induced transcriptional activity of GR 4-fold and blocks ligand-dependent degradation of GR [11]. Another important posttranslational modification of GR is the covalent addition of a small ubiquitin-related modifier-1 (SUMO-1) termed sumoylation. GR is sumoylated at residues Lys-277, Lys-293 and Lys-703 [4]. Sumoylation of GRα has been shown to promote its degradation and inhibits the transcriptional activity of GR in a promoter-specific manner by recruiting corepressors.

Transcriptional Repression by the Glucocorticoid Receptor

Ligand-bound GR can repress target gene transcription by binding directly to DNA or by binding to other transcription factors. The former mechanism involves the binding of GR to less well-defined glucocorticoid-responsive elements called nGREs. It has been suggested that the GR functions passively at these nGRE by hampering the assembly of an activating transcription complex or the RNA polymerase. Recently, Surjit et al. [12] have shown that GR can actively repress target gene transcription by recruiting corepressors. They have identified yet another class of DNA response elements which contain inverted repeats of the sequence CTCC separated by either zero, one or two nucleotides. These novel nGREs have been shown to recruit NCoR1 and SMRT, which in turn engage HDACs to repress target gene transcription. This active repression mechanism has been linked to glucocorticoid effects on metabolism and bone [12]. However, few inflammatory genes that are repressed by glucocorticoids have been reported to utilize these nGREs, and further research will likely be necessary to define their role in this process.

Most of the anti-inflammatory effects of glucocorticoids appear to result from an important negative regulatory mechanism called transrepression, in which ligand-bound GR is recruited to chromatin by protein-protein interactions with DNA-bound transcription factors, particularly NF-κB and activator protein-1 (AP-1). It has been shown that transrepression requires the DBD of GR, but it does not depend on direct DNA binding. Repression is also accomplished on some genes by binding both a GRE and a transcription factor bound adjacent to the GRE in a composite manner [13]. The mechanism of transcriptional regulation by GR is complex (fig. 2). Operating through these diverse mechanisms, GR has been shown by microarray analysis to regulate up to 10–20% of the human genome in different cell types. Thus, when considering the transcriptional responses mediated by GR, one should take into account the absolute and relative abundance of the GR isoforms in

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different tissues, the location, accessibility and architecture of GREs as a function of chromatin landscape, the level of expression and activation of transcription factors with which GR associates, and the expression and availability of coregulators. Moreover, positive or negative regulatory activity appears likely to be dictated not simply by GR-binding site sequence but by chromatin context and by other transcription factors that bind in the vicinity of GR.

Mechanisms Contributing to Glucocorticoid Resistance

The anti-inflammatory and immunosuppressive effects of glucocorticoids are exploited extensively for the treatment of many inflammatory conditions. Due to the chronic nature of the inflammatory conditions, the treatment paradigms involve long-term glucocorticoid administration, which results in tolerance and induces the development of glucocorticoid resistance. Resistance to the therapeutic effects of glucocorticoids presents a considerable problem in managing these inflammatory diseases. In general, glucocorticoid resistance is defined as the inability of the cells to respond to all or a restricted number of glucocorticoid responses. At the molecular level, glucocorticoid resistance can be induced by several mechanisms and likely differs among patients. Glucocorticoid resistance can be attributed to reduced expression of GR, altered affinity of GR for the ligand, reduced ability of GR to bind DNA, or increased expression of inflammatory transcription factors like AP-1 that compete for DNA binding. Since resistance to glucocorticoids limits the therapeutic benefit of glucocorticoids, it is of clinical importance to elucidate molecular mechanisms of glucocorticoid resistance.

Polymorphisms and Somatic Mutations in the Glucocorticoid Receptor Gene

GR protein is the product of single gene located in chromosome 5 in humans. Somatic mutations in the gene are associated with specific types of disease, while numerous loss-of-function mutations in the GR gene have been observed in glucocorticoid-resistant human ALL cell lines. The combination of glucocorticoid and chemotherapy, with its mutagenic potential, might indeed favor the development of and subsequent selection for GR mutations. Hillman and colleagues showed that the glucocorticoid-resistant CCRF-CEM cell line contains one GR allele with the L753F mutation [14]. The LBD mutation (Δ702) of GR observed in glucocorticoid-resistant ALL patients emerged as the dominant population at relapse. Since the conventional assays for detection of aberrant GR mutations are inadequate in a heterogeneous cell population, GR mutations in patients are underrepresented as a mechanism of glucocorticoid resistance.

A polymorphism is defined as an inheritable genetic germ line variant of a single locus (most frequently a single nucleotide variation) that is present in at least 1% of the population. Inactivating single nucleotide polymorphisms within the LBD or the DBD of the receptor, and a 4-bp deletion at the 3′ boundary of exon 6 of the gene, have been described in glucocorticoid-resistant patients. Most of these mutations were heterozygous, indicating that complete loss-of-function of the receptor is incompatible with life. Severe impairment of the transactivation function of GR was observed in the cases of R477H, I559N, V571A,

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and D641V mutations. Furthermore, the mutant receptors hGRα I559N, F737L, I747M and L773P exerted a dominant negative effect upon the wild-type receptor [15]. Dexamethasone binding studies showed a variable reduction in the affinity of the mutant receptors for the ligand, with the most severe reduction observed in the cases of I559N. The ER22/23EK polymorphism that occurs in ~3% of the population results in an arginine (R) to lysine (K) change at position 23 (R23K) within the N terminus (fig. 1c). ER22/23EK is associated with decreased GR transcriptional activity in reporter assays and decreased expression of endogenous glucocorticoid responsive genes when compared to wild-type GR. Russcher and colleagues have shown an association between the ER22/23EK polymorphism and increases in the ratio of GRα-A to GRα-B. Adult carriers of the ER22/23EK polymorphism were shown to have a lower tendency to develop impaired glucose tolerance, type 2 diabetes and cardiovascular disease [16].

The N363S polymorphism, located within exon 2, occurs in ~4% of individuals, results in modest increases in GR transcriptional activity, and is associated with generalized increases in glucocorticoid sensitivity. Interestingly, microarray analysis revealed a unique polymorphism-specific pattern of gene regulation for N363S when compared to wild-type GRα. Furthermore, some studies associate N363S with an increased body mass index, coronary artery disease and decreased bone mineral density [17].

The A3669G polymorphism that is located within the 3′ untranslated region of GRβ results in increased stability of GRβ mRNA and the enhanced expression of GRβ protein (fig. 1c) [18]. Interestingly, A3669G is less capable of transrepressing the NFκB-regulated gene IL-2 than wild-type GRβ. Moreover, A3669G is associated with reduced immunosuppression. Individuals harboring A3669G have a higher incidence of rheumatoid arthritis and cardiovascular disease. Homozygous carriers of A3669G are associated with a proinflammatory phenotype that included an increased risk of myocardial infarction and coronary heart disease [16]. However, the spectrum of clinical manifestations in patients with GR mutations is quite broad, as a large number of subjects are asymptomatic and show only biochemical changes. Although the impact of GR polymorphisms and altered chaperone or cochaperone expression on glucocorticoid responsiveness in hematological malignancies is not well established, GR polymorphisms are emerging as an important biomarker for diseases of metabolic origin.

Glucocorticoid Receptor Expression Level

It is well documented that the level of GR protein determines the magnitude of glucocorticoid response. Several studies have shown that decreased GR expression in primary ALL cells is associated with initial resistance to glucocorticoid therapy, relapse and poor prognosis. GR levels in cells are dynamic, and are regulated in a cell type-specific manner by the surrounding concentration of ligand. In different cell lines and tissues, ligand induces downregulation of both GR mRNA and protein. Glucocorticoid-induced downregulation of GR mRNA has been attributed to reduce transcription of the GR gene as well as decreased stability of the GR mRNA [18, 19]. Previous studies from our laboratory have shown that the ligand-mediated downregulation of GR mRNA is mediated through the exonic region on the GR gene [20]. Additionally, proteosome-mediated degradation

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contributes to increased turnover of the GR protein in a ligand-dependent manner [11, 21]. Since GR can be ubiquitinated and tagged for proteasomal degradation, proteasome inhibitors might increase glucocorticoid responsiveness. However, this has not yet been shown in glucocorticoid-resistant disease. While hormone-induced downregulation of GR represents a mechanism for maintaining glucocorticoid homeostasis in normal cells, it has the potential to limit therapeutic responses to glucocorticoids in malignant cells. On the contrary, ligand induced upregulation of GR in lymphocytes is associated with glucocorticoid sensitivity. Thus, T cell lines that fail to auto-induce GR are resistant to glucocorticoid-induced apoptosis. Taken together, these findings suggest that GR expression level may be an important determinant of the glucocorticoid response [16]. The mechanisms underlying these processes are poorly understood, and further research is required to dissect their contribution to glucocorticoid resistance.

Glucocorticoid Receptor Heterogeneity

Glucocorticoid Receptor Isoforms Generated by Alternative Splicing

The α-isoform is the functional receptor and is encoded for by exons 2–9α. It is located in the cytoplasm in the absence of ligand, but translocates to the nucleus upon glucocorticoid binding. Alternative splicing of the GR primary transcript has been shown to generate a variant termed GRβ, a shorter protein with 742 residues. GRβ does not bind glucocorticoids, resides constitutively in the nucleus of cells, and does not directly regulate glucocorticoid-responsive reporter genes. However, when coexpressed with GRα, the splice variant functions as a dominant-negative inhibitor of GRα on genes both positively and negatively regulated by glucocorticoids. Various mechanisms, including competition for GRE binding, competition for transcriptional coregulators and formation of inactive GRα/GRβ heterodimers, have been proposed to underlie the antagonism [22]. Recent data show that GRβ, when introduced into cells in the absence GRα, does bind the synthetic GRα antagonist RU-486. In many cells and tissues examined, GRβ is expressed at low levels when compared to GRα, and in vitro studies have indicated that reductions in the cellular GRα:GRβ ratio contribute to glucocorticoid resistance. Resistance to glucocorticoid therapy in patients with leukemia and other diseases has been associated with high cellular levels of GRβ when compared to GRα, but this relationship has not been observed in other studies [23]. However, an association has been shown between reduced GRα:GRβ ratio and mood disorders such as schizophrenia, bipolar and major depressive disorder. As mentioned earlier, polymorphisms in GRβ have been linked to increased risk of myocardial infarction and coronary heart disease [24]. Another important finding has been the discovery of GRβ in mouse (mGRβ). This arises from a distinct mechanism that employs alternative splice donor sites in the intron separating exons 8 and 9. The resulting GRβ isoform is similar in structure and functionality to human GRβ. In addition, mGRβ exhibits ubiquitous expression, nuclear localization, inability to bind glucocorticoid agonists and antagonism of GRα [25]. Although GRβ does not bind to glucocorticoid, it is transcriptionally active, and the GR antagonist mifepristone can bind to it [24]. The endogenous ligand for GRβ is currently unknown.

The γ-isoform of GR (GRγ) is a splice variant in which exon 4 is alternatively spliced to exon 3, thereby including 3 bp of the intron region resulting in an additional arginine

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residue. This isoform is expressed at 3.8–8.7% of total GR mRNA in different human tissues. Ray and colleagues reported that the biological activity of the γ-isoform is reduced to 50% of the wild-type receptor. Gerdes and colleagues reported preliminary results showing a possible role for the γ-isoform in poor prednisone response in childhood ALL [16]. GRγ expression is also associated with glucocorticoid resistance in small cell lung carcinoma cells and corticotroph adenomas. The GR-A variant has an excision of exons 5, 6 and 7, resulting in an in-frame juxtaposition of exon 8 to 4. Little information is known about the expression levels and function of this variant. GR-P is missing exons 8 and 9, which encode the C-terminal half of the LBD due to a failure to splice at the exon 7/8 boundary. The GR-P transcripts account for up to 10–20% of total GR mRNA [4]. This GR variant has been reported to be upregulated in a small group of hematological malignancies (ALL, non-Hodgkin's lymphoma and multiple myeloma).

Glucocorticoid Receptor Isoforms Generated by Alternative Translation Initiation

Our laboratory has identified N-terminal GR isoforms that are generated by alternative translation initiation from a single GR mRNA species. Internal conserved AUG codons corresponding to methionines 27, 86, 90, 98, 316, 331 and 336 were identified as bona fide translation start sites, generating proteins that have been termed GRα-B, C1, C2, C3, D1, D2 and D3, respectively. All GRα translational isoforms are expressed in various tissues in rat and mouse, but differences in expression levels between tissues are noted. Since the translational isoforms differ only by the length of their N-terminus, all 8 isoforms exhibit a similar affinity for ligand and most undergo hormone-induced nuclear localization. The GRα-D3 variant is the exception in that it localizes to the nucleus and can bind certain GREs even in the absence of hormone. The GRα-C3 variant is the most transcriptionally active, whereas the GRα-D proteins were the least active in reporter assays. These data suggest that the glucocorticoid-induced transcriptional response reflects the composite actions of GRα isoforms and that the specific intracellular pool of GRα subtypes may determine cellular sensitivity to glucocorticoids [26].

Microarray analysis and functional study of the various GRα isoforms revealed that the expression of the more active GRα-C3 correlated with increased sensitivity to glucocorticoid-induced apoptosis, and expression of the relatively inactive GRα-D3 was associated with resistance to glucocorticoid-induced apoptosis in U2-OS osteosarcoma cells stably expressing the individual translational isoforms. A recent study from our laboratory has shown that GRα-D, unlike the other receptor isoforms, does not inhibit the activity of an NF-κB-responsive reporter gene and does not efficiently repress either the transcription or translation of the antiapoptotic genes Bcl-xL, cellular inhibitor of apoptosis protein 1 and survivin. The inability of GRα-D to downregulate the expression of these genes appears to be associated with a diminished interaction between GRα-D and NF-κB. Thus, the D-isoform fails to interact with NF-κB in cells and promote apoptosis in response to glucocorticoids [27]. These data suggest that the N-terminal translational isoforms of GRα selectively regulate antiapoptotic genes and that the GRα-D isoform may contribute to the resistance of certain cancer cells to glucocorticoid-induced apoptosis. Genetic manipulation of the GR translational isoforms in animal models may shed new light on the biological importance of these intriguing GR variants, and it will be important to determine the

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contributions of a single GR isoform in whole animal. Furthermore, it is critical to verify if glucocorticoid-resistant cells exhibit an altered pattern of GR isoform expression.

Other Mechanisms of Glucocorticoid Resistance

Insufficient intracellular level of biologically active glucocorticoid is also responsible for glucocorticoid resistance. Insufficient levels of glucocorticoids may result from impaired uptake (regulated by P glycoprotein, multidrug resistance-associated protein, lung-resistance protein), increased steroid-binding proteins in the circulation or reduced converting enzyme activity (11β-hydroxysteroid dehydrogenase type 2) [16]. Nuclear translocation of GR is an important determinant of glucocorticoid sensitivity. Phosphorylation modulates the cellular trafficking of the receptor as GRα phosphorylated on Ser-203 is preferentially retained in the cytoplasm. A large proportion of patients with glucocorticoid-resistant asthma showed reduced nuclear translocation of GR and reduced GRE binding in PBMCs after glucocorticoid exposure, and this might be explained by GR phosphorylation [9, 23]. Since the mature GR heterocomplex is required for optimal ligand binding and subsequent activation of the transcriptional response, abnormalities in the chaperones and cochaperones that make up the heterocomplex may contribute to decreased glucocorticoid responsiveness. Kojika's group showed that alterations in hsp90 and hsp70 were associated with decreased cellular sensitivity [16]. Excessive activation of the transcription factors that GR interacts with has been identified as a mechanism of glucocorticoid resistance. Increased activation of AP1 has been recognized as a mechanism of glucocorticoid resistance in asthma, since this protein binds to GR and thus prevents its interaction with GRE and other transcription factors [23]. Furthermore, mutual antagonism between NF-κB and GR mediated by physical interaction (which is enhanced by CREB-binding protein) may be involved in decreased glucocorticoid sensitivity [28].

Conclusion

Glucocorticoids regulate numerous physiological processes, and are vital in the treatment of inflammation, autoimmune disease and cancer. The chronic nature of many of the inflammatory conditions and treatment paradigms frequently results in glucocorticoid resistance. Technological progress in molecular biology has advanced our understanding of the molecular mechanisms involved in glucocorticoid resistance, which includes reduced GR expression, GR downregulation, and acquired GR mutations. It is vital that our current understanding of these molecular mechanisms is translated into the clinic to aid in the development of safer and more effective glucocorticoid therapies.

Acknowledgments

We thank members of our laboratory for their critical reading of the manuscript. We thank NIEHS Arts and Photography for their help with the figures. This research was supported by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences.

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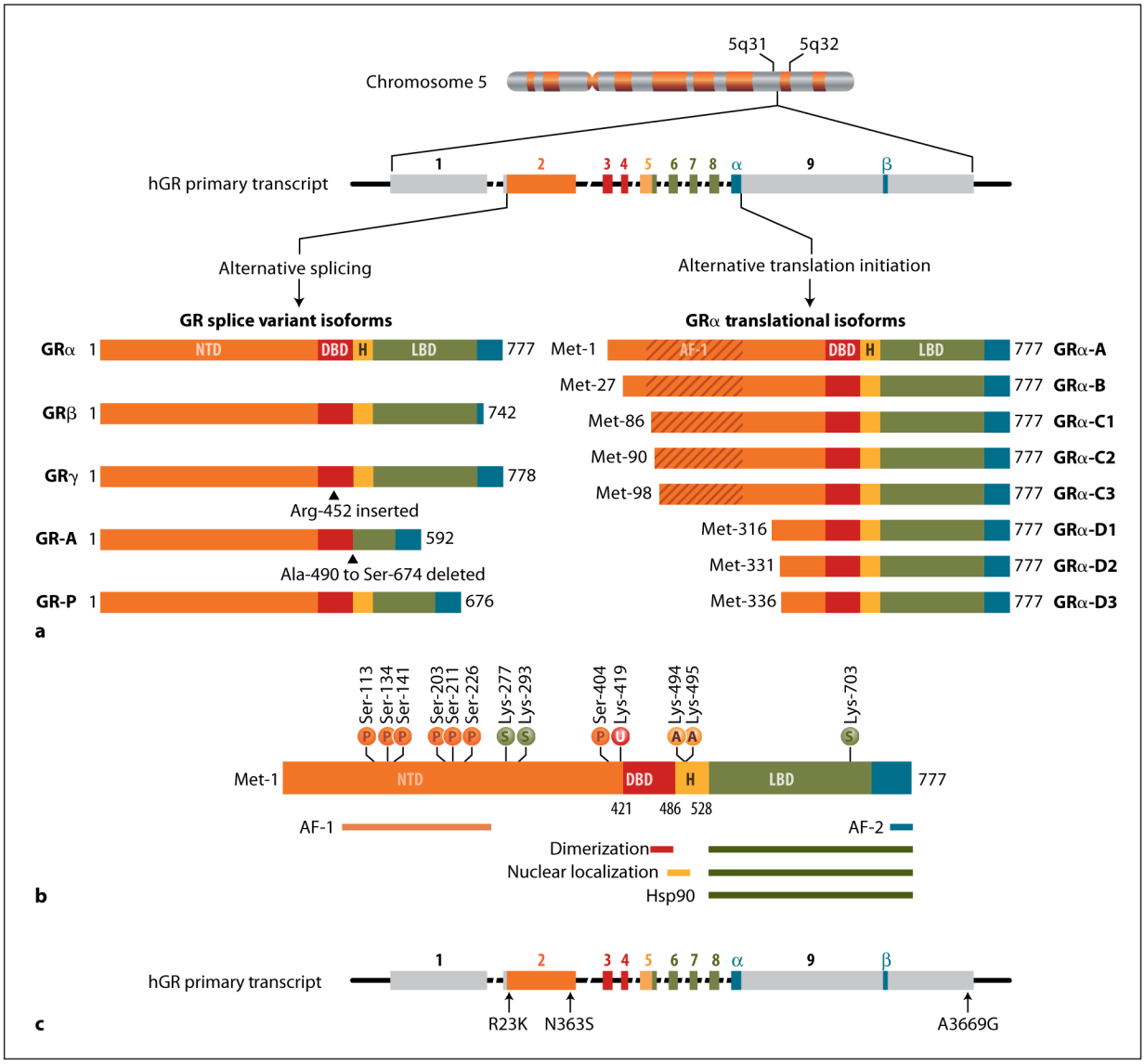

Fig. 1.

Genomic location and organization of the human GR. **a** Alternative splicing and translation initiation of hGR primary transcript. The hGR gene (NR3C1) is one locus on chromosome 5q31–32. The hGR primary transcript is composed of 9 exons, with exon 2 encoding most of the N-terminal domain (NTD), exons 3 and 4 encoding the DBD, and exons 5–9 encoding the hinge region (H) and LBD. GR splice variant isoform: The classic GRα protein results from splicing of exon 8 to the beginning of exon 9. GRβ is produced from an alternative splice acceptor site that links the end of exon 8 to downstream sequences in exon 9, encoding a variant with a unique 15 amino acid at C terminus (positions 728–742). GRγ is generated by an alternative splice donor site in the intronic sequence separating exons 3 and 4, resulting in a protein with an arginine insertion (Arg-452) between the two zinc fingers of the DBD. GR-A is produced from alternative splicing that joins exon 4 to exon 8, deleting the proximal 185 amino acids of the LBD (Ala-490-Ser-674) encoded by exons 5–7. GR-P

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is formed by a failure to splice exon 7 to exon 8. The retained intronic sequence introduces a stop codon, resulting in a truncated receptor mutant missing the distal half of the LBD. GRα translational isoforms: Domain organization of the GRα translational isoforms. Initiation of translation from eight different AUG start codons in a single GR-mRNA generates receptor isoforms with progressively shorter N-terminal domains. This generates the GRα translational isoforms GRα-A, B, C1, C2, C3, D1, D2 and D3. **b** Domain structure and posttranslational modifications of hGR-α. GR contains three major functional regions, the N-terminal transactivation domain (NTD), the central DBD and the C-terminal LBD. The region located between the DBD and LBD is known as the hinge region (H). Regions involved in transcriptional activation (AF1 and AF2), dimerization, nuclear localization and chaperone hsp90 binding are indicated. Sites of posttranslational modifications like phosphorylation (P), sumoylation (S), ubiquitination (U) and acetylation (A) are indicated. **c** hGR polymorphisms. Arrows indicate polymorphisms that result in amino acid changes and A3669G which leads to GR stability.

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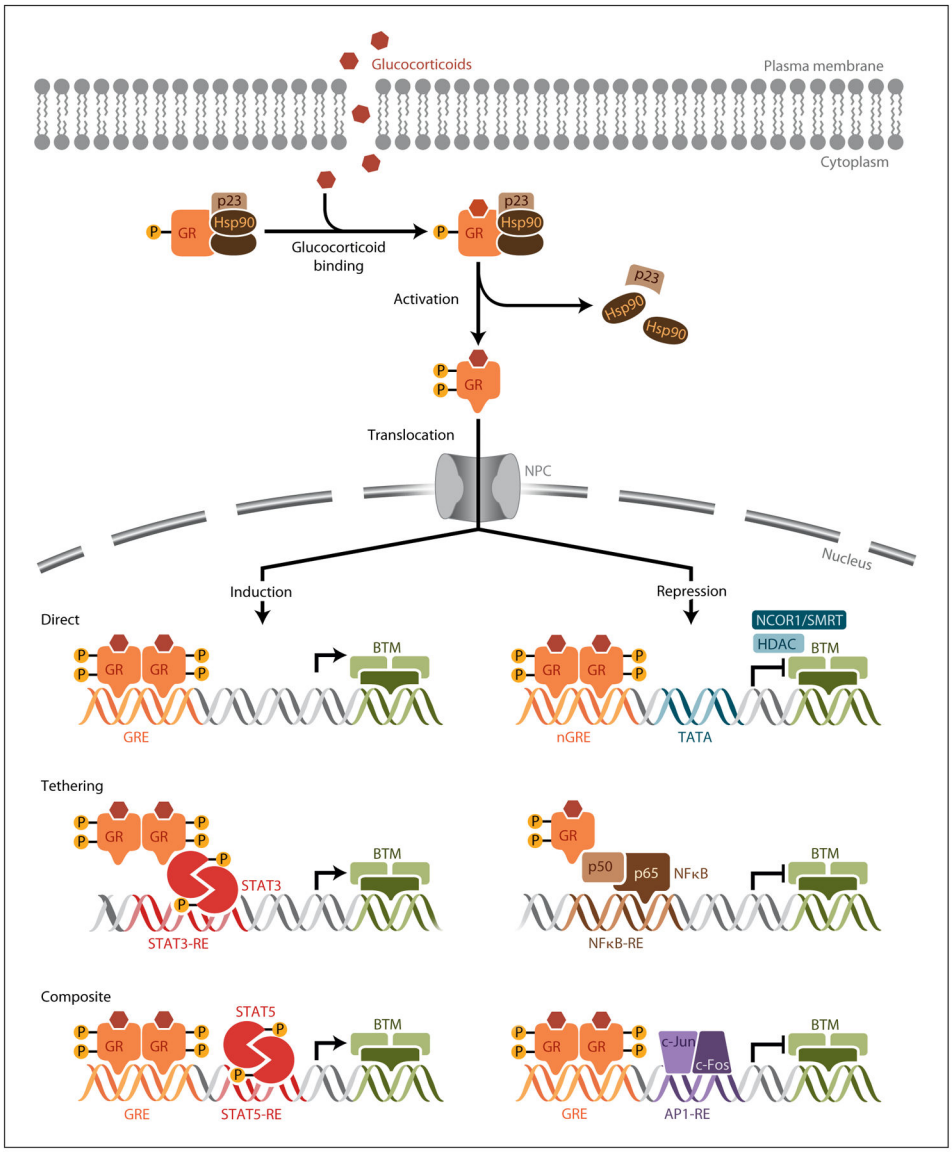

Fig. 2.

Genomic action of GR. Upon binding glucocorticoids, cytoplasmic GR undergoes a conformation change (activation), becomes hyper-phosphorylated (P), dissociates from heterocomplex, and translocates into the nucleus, where it regulates gene expression. GR activates or represses transcription of target genes by direct GRE binding, by tethering itself to other transcription factors apart from DNA binding, or in a composite manner by both direct GRE binding and interactions with transcription factors bound to neighboring sites. NPC = Nuclear pore complex; BTM = basal transcription machinery; TBP = TATA-binding protein; nGRE = negative GRE; RE = response element.