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The human glucocorticoid receptor: One gene, multiple proteins and diverse responses

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Abstract

Glucocorticoids are a vital class of endogenous steroid hormones that regulate essential biological processes including growth, development, metabolism, behavior and apoptosis. Most, if not all, of these actions are thought to be mediated through the glucocorticoid receptor. The exact mechanisms of how one hormone, via one receptor, modulates such diverse biological functions are largely unknown. However, recent studies from our lab and others have suggested that a contribution for the diversity results from multiple isoforms of the glucocorticoid receptor that result from alternative RNA splicing and translation initiation of the glucocorticoid receptor mRNA. Additionally, each isoform is subject to several post-translational modifications, including phosphorylation, ubiquitination and sumoylation, which have been shown to modulate the receptor protein stability and/or function. Together these data provide potentially diverse mechanisms to establish cell type specific regulation of gene expression by a single transcription factor. Here, we summarize the recent advances and processes that generate these receptor isoforms and these post-translational modifications. We speculate that the composition and proportion of individual isoforms expressed in particular cellular contexts account for the diverse effects of glucocorticoid hormones.
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Keywords: Steroid; Human glucocorticoid receptor; Isoforms; Alternative RNA splicing; Alternative translation initiation; Post-translational modification

1. Introduction

Glucocorticoids (GCs) are a class of stress-induced, endogenously synthesized steroid hormone molecules. Under control of the hypothalamic–pituitary–adrenal (HPA) axis, these hormones are synthesized in the adrenal cortex and circulated systemically, regulating a variety of cell-, tissue- and organ-specific biological functions including development, growth, metabolism, behavior and apoptosis [1,2]. Clinically, glucocorticoids represent one of the most commonly prescribed drugs worldwide, effectively used for their anti-inflammatory or immune-suppressive effects in asthma, dermatitis, rheumatoid arthritis, prevention of graft rejection, and autoimmune diseases [3–6].

Glucocorticoids are thought to diffuse freely across the cell membrane because of their lipophilicity. Once in the cytoplasm, they interact with the glucocorticoid receptor (GR) which mediates most, if not all, of the hormone-induced actions. Similar to other members of the nuclear receptor superfamily, GR is a modular protein with each domain carrying distinct functions (Fig. 1) [7]. The first 421 amino acids of the protein at the N-terminus encode primarily the constitutive transcriptional activation function 1 (AF1). In contrast, the next 65 amino acids in the protein central region comprise a highly conserved zinc finger DNA-binding domain (DBD). This motif plays a critical role in receptor homodimerization, DNA-binding specificity, and interaction with cofactor proteins. At the C-terminus is a moderately conserved region of approximately 250 amino acids. It encodes primarily the ligand-binding domain (LBD), and contains the motif for ligand-dependent transcriptional activation function 2 (AF2). Functionally, this C-terminal region is also involved in protein–protein interactions with either cytosolic chaperones or co-regulators, depending on the absence or presence of a ligand, respectively. In addition to these three major motifs, nuclear localization signals are identified embedded in both the DBD and LBD regions [8].

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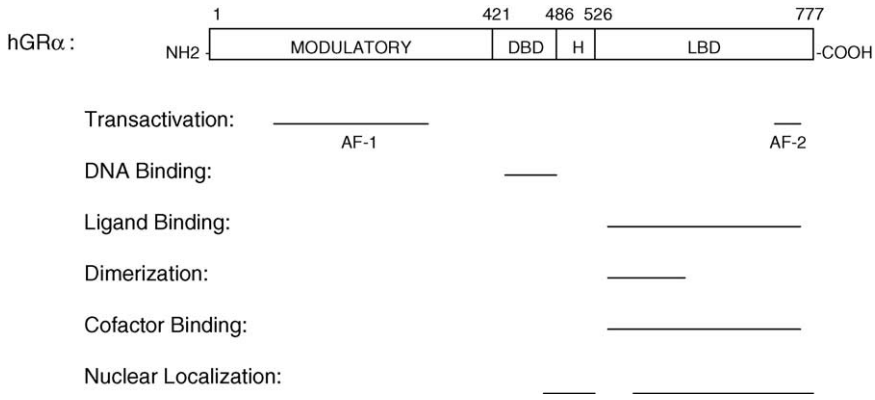

Fig. 1. Functional motifs of the human glucocorticoid receptor (hGR). The N-terminus represents the constitutive transcriptional activation function 1 (AF1), while the C-terminus encodes the ligand-binding domain (LBD) and ligand-dependent activation function 2 (AF2). The highly conserved DNA-binding domain (DBD) is located in the central region of the protein. In addition, the domains involved in nuclear localization, receptor dimerization, and cofactor binding are mainly localized to the C-terminal ligand-binding motif. H, hinge region.

The ligand-free receptor is largely present in the cytoplasm as a multi-protein complex. Upon ligand activation, the receptor translocates into the nucleus where it either enhances or represses transcription of target genes. While this highly simplified signaling cascade has significantly enriched our understanding of the GR's mechanism of action, it does not, however, convey the extreme complexity of gene-, cell- and tissue-specific activity of glucocorticoids. Generally, the nature and magnitude of a cell's response to glucocorticoids are dependent on the hormone levels it is exposed to as well as the concentration of receptor in a cell, in addition to the efficiency of GR-mediated signal transduction [9–13], and the genomic accessibility of glucocorticoid-responsive genes. In particular, multiple GR isoforms are generated as a result of alternative splicing and alternative translation initiation as well as post-translational modifications, and each isoform presents different signal transduction potentials. In this article, we focus on recent advances and processes that produce these diverse receptor isoforms. We speculate that the composition and proportion of individual isoforms expressed in a particular cellular context account for the diverse effects of glucocorticoid hormones differentially.

2. Mechanisms of glucocorticoid action

Prior to ligand-binding, GR primarily resides in the cytoplasm associated with two molecules of heat shock protein 90 and several immunophilins such as FKBP51 (Fig. 2). Following activation by a ligand, the receptor undergoes a series of conformational alterations, leading to its dissociation from the cytoplasmic chaperones and exposure of its nuclear localization signals. These signals are then recognized by a group of nuclear translocation proteins, which actively shuttle the receptor into the nucleus [14,15]. Once inside, readily formed receptor homodimers recognize and interact with specific *cis*-acting sequences called glucocorticoid-responsive elements (GREs) in target gene promoters. The receptor–DNA interaction is further stabilized by the recruitment of coactivators which in turn initiate assembly of the general transcription machinery, leading to the enhancement of gene transcription. Alternatively, ligand-bound GR can bind to a number of poorly-defined negative GREs (nGREs) on promoter region of target genes to suppress transcription. A number of nGRE-mediated genes have been identified including bovine prolactin [16], human immunodeficiency virus type 1 [17], human osteocalcin [18], type 1 vasoactive intestinal polypeptide (VIP) receptor [19], human corticotropin-releasing hormone [20], and neuronal serotonin receptor (5-HT1A) [21]. The exact mechanism of nGRE-mediated repression is largely unclear, but perhaps results from interference with either the assembly of the general transcription machinery [18,22,23] or with transcriptional activation elicited by other positively acting transcription factors [24].

In addition, transcriptional modulation by GR can be achieved through its cross-talk with other transcription factors such as nuclear factor-κB (NF-κB), activator protein-1 (AP-1), Sma and Mad-related protein (Smad), and signal transduction and activator of transcription (STAT) (Fig. 2). It is generally assumed that GR interaction with NF-κB and/or AP-1, and the subsequent suppression of their target genes, is the major mechanism by which glucocorticoids protect against inflammation [25–27]. Both NF-κB and AP-1 are rapidly activated by proinflammatory cytokines, bacterial and viral infection agents, and proapoptotic stimuli such as UV irradiation. Once activated, they quickly upregulate transcription of immunoregulatory genes, including cytokines, cytokine receptors, chemotactic proteins, and adhesion molecules. Induction of these pro-inflammatory genes is critical for an organism's defense system to identify and eliminate threatening agents. Nevertheless, excessive stimulation of these responses leads to cell or tissue damage and even death. The glucocorticoid receptors are essential to maintain this balance appropriately. Not only does GR attenuate NF-κB and AP-1-mediated production of proinflammatory cytokines by forming an inactive complex with NF-κB

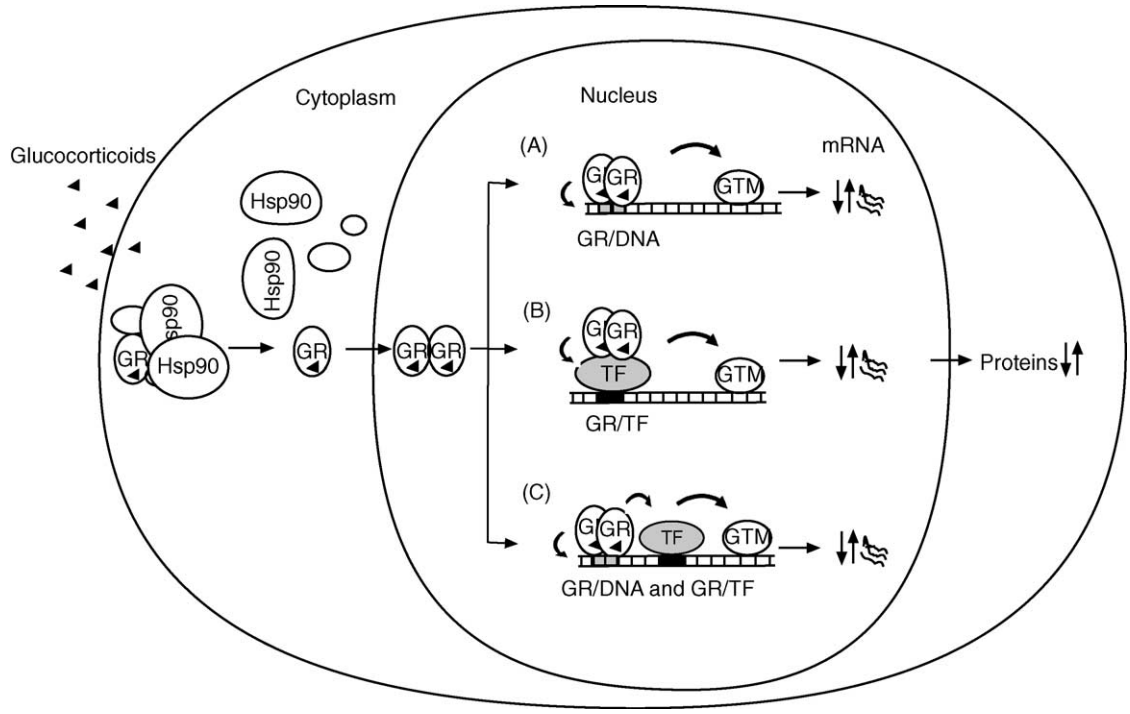

Fig. 2. Signaling pathways of GR-mediated transcriptional regulation. Following binding to glucocorticoids, the cytosolic GR dissociates from chaperone proteins such as Hsp90 and translocates into the nucleus. Once there, GR readily dimerizes and modulates target gene transcription via: (A) direct interaction with *cis*-DNA elements including GREs and nGREs; (B) cross-talk with other DNA-bound transcription factors such as AP-1, NF-κB, Smad, and/or STAT; (C) interaction with both DNA elements and other transcription factors. The resulting modulation of target gene transcripts leads to altered protein expression. GTM, general transcription machinery; TF, other transcription factors.

[28,29] or AP-1 [30,31] through direct protein–protein interactions, but GR also enhances transcription of certain anti-inflammatory cytokines. In contrast to NF-κB and AP-1, the TGF-β-activated Smad family of transcription factors is primarily involved in wound healing and fibrosis. GR has been reported to antagonize TGF-β-induced transcription of genes, such as fibronectin [32], collagen [33,34], and type-1 plasminogen activator inhibitor [35], through its interaction with Smad [35]. Another common class of transcription factor influenced by GR is the STAT family, factors mainly involved in mammary gland development and function. Unlike with the others, physical association of GR with STAT can lead to enhanced transcription of STAT-mediated target genes, as exemplified by β-casein [36,37].

Involvements of GR interactions with both the DNA elements and other transcription factors have also been suggested in several target gene regulations (Fig. 2). Depending on specific promoter contexts and/or transcription factors, these GR dual interactions can lead to either up- or down-regulation of transcription. For example, Hermoso et al. [38] have recently showed that the toll-like receptor 2 (TLR2) are synergistically induced by the cotreatment of A549 cells with TNF-α and dexamethasone. The synergism requires the interaction of NF-κB, STAT, and GR with their cognate enhancer elements in the promoter region of the TLR2 gene, and the potential protein–protein association among these receptors. Whereas in other systems, Stocklin et al. reported that the GR cross-talk with STAT led to a downregulation of GRE-containing MMTV reporter construct [36].

3. Genomic structure of the hGR gene

The hGR cDNA was first cloned in 1985 [39], and later mapped to chromosome 5q31-32 [40,41]. To date there is only one gene for GR. Examination of the receptor genomic structure revealed the presence of 10 exons spanning a 110 kb region [42] (Fig. 3A). The 184 nucleotides of exon 1 represent solely the 5′-untranslated region. Exon 2 (1197 bp) encodes most of the receptor N-terminus, including the constitutive AF1 transactivation domain. The two zinc-finger motifs involved in DNA-binding are separately encoded by exon 3 (167 bp) and exon 4 (117 bp). A total of five exons (exons 5, 6, 7, 8, 9α or 9β) together make up the ligand-binding domain and ligand-dependent AF2 as well as the 3′-untranslated regions [42,43]. Promoter analysis of the GR gene revealed an apparent lack of a TATA box and a CCAAT motif in the 5′-flanking region [42,43]. Instead, multiple GC boxes, AP-1, AP-2, Sp1, cAMP-responsive elements (CRE), Yin Yang1 (YY1), NF-κB and several tissue-specific transcription factor binding sites have been identified [43–47]. This information is consistent with the notion that GR is constitutively expressed in virtually every cell type, but with a tissue-specific pattern.

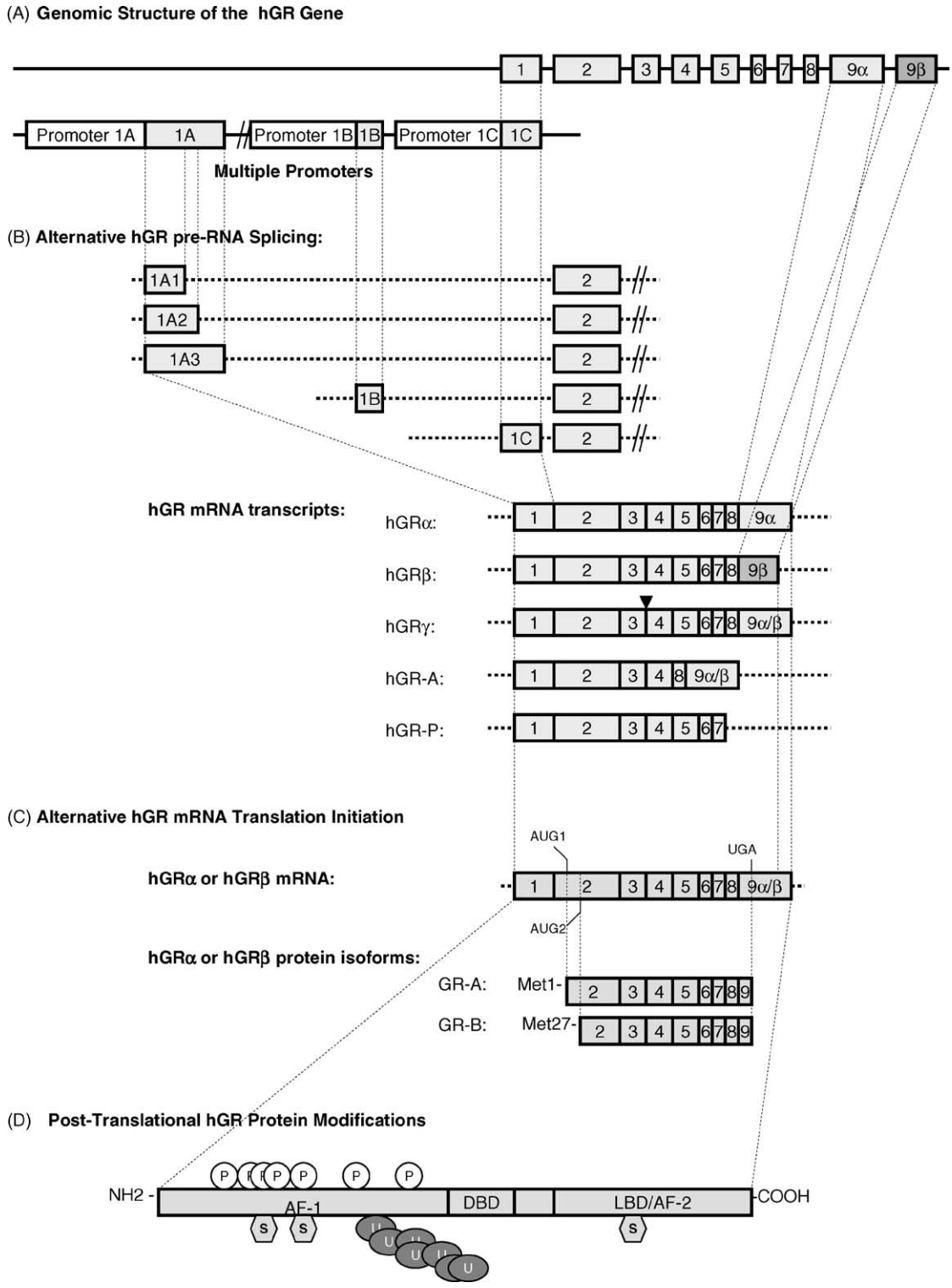

Fig. 3. Generation of multiple hGR isoforms from a single gene. (A) Genomic structure of the hGR gene. The gene contains a total of 10 exons, spanning a 110 kb genomic region. Exon 1 represents solely the 5′-untranslated sequence, and contains three isoforms (1A, 1B and 1C), each controlled by a diverse upstream region (promoters 1A, 1B and 1C). (B) Generation of multiple hGR transcripts as a result of alternative RNA splicing. Five exon 1 variants (1A1, 1A2, 1A3, 1B and 1C) can join to the same acceptor site on exon 2, yielding transcripts containing various 5′-untranslated sequences. Alternative splicing of exon 9 gives rise to two mRNAs coding for hGRα or hGRβ. Alternative splicing of other exons has been described, including GR-γ containing a three-nucleotide insertion between exons 3 and 4; GR-A missing exons 5–7; and GR-P lacking exons 8 and 9. (C) Generation of multiple hGR protein isoforms as a result of alternative translation initiation. Translation can be initiated at the first AUG (Met1) to give rise to GR-A (hGRα or hGRβ) or internal AUG (Met27) to produce GR-B. Alternative translation initiation has been demonstrated for both hGRα and hGRβ transcripts. The amino acid labels refer to the full-length hGRα receptor and are from references listed in the text. (D) Post-translational modifications of the protein. P, phosphorylation; S, sumoylation; U, ubiquitination.

4. Alternative splicing and promoter usage

A more detailed analysis of the hGR promoter sequence revealed at least three distinct regulatory regions present with each controlling a unique exon 1 isoform (1A, 1B and 1C) [45] (Fig. 3A). Exon 1C (184 bp) is identical to the exon 1 originally characterized in [39], which is under the control of promoter 1C. Immediately upstream of the promoter 1C is exon 1B (77 bp), driven by promoter 1B of approximately 1 kb in size. Much further upstream (27 kb) of the transcription start site for exon 1C is exon 1A (981 bp), which is regulated by promoter 1A (1075 bp). Three alternative splice donor sites are separately contained on exon 1A, giving rise to three 1A transcripts including 1A1 (212 bp), 1A2 (383 bp) and 1A3 (981 bp). All of these five exon 1 isoforms (1A1, 1A2, 1A3, 1B and 1C) are spliced onto the same acceptor site on exon 2 (Fig. 3B).

Despite the fact that none of the exon 1 isoforms encodes protein information, they, together with their respective promoters, play an important role in regulating cell-type specific hGR gene expression. For example, exon 1A3-containing transcripts were detected at higher levels in cancer cells of hematopoietic origin such as IM-9, CEM-C7, and Jurkat than other cancer cells such as Hela and WI-38 [45], whereas no apparent differences were observed for exon 1B and 1C transcripts among the same cells lines. Similarly expression pattern of exon 1A1- and 1A2-containing transcripts was observed, but this pattern was drastically different from that of 1A3. Furthermore, when CEM-C7 and IM-9 cells were transiently transfected with the same construct containing the promoter 1A and subsequently treated with dexamethasone, an enhanced transcript expression in CEM-C7 cells, but a repression in IM-9 cells, was detected. Together these data suggest that glucocorticoid-responsive elements are contained in the promoter 1A, and that these elements are utilized in a cell-type specific manner. Nevertheless, it remains unknown whether the diversity of exon 1-containing transcripts would necessarily lead to distinct expression profiles of the hGR protein.

In addition to splicing events at the 5′-end of hGR cDNA generating a heterogeneous population of untranslated exon 1, alternative splicing has also been observed for exon 9, giving rise to two mRNAs encoding hGRα and hGRβ, respectively (Fig. 3B) [39]. The hGRα and hGRβ are identical up to exon 8 (encoding amino acids 1–727) after which they diverge. hGRα contains exon 9α of 2475 nucleotides in size, of which the first 150 bp encode for 50 amino acid residues. Separated by a 158 bp short intron sequence downstream from exon 9α, the exon 9β continues for another 1478 nucleotides with the first 45 coding for 15 distinct residues of the hGRβ C-terminus.

The existence of two alternatively spliced transcripts, hGRα and hGRβ, was established ever since the cloning of hGR cDNA [39]. Similar transcripts are found in rat [48], but not in mouse which contains only GRα [49]. However, functional analyses indicate apparent distinctions between these two receptor isoforms. hGRα binds to the classical receptor agonist corticosteroids or the antagonist RU486, translocates into the nucleus upon ligand activation, and modulates transcription of target genes in a hormone-dependent manner [39]. This information is consistent with the expectations of the glucocorticoid receptor acting as a ligand-dependent transcription factor, and therefore, extensive attention has been focused on this isoform for quite a few years. In contrast, hGRβ does not interact with glucocorticoids and exhibits transcriptional inertness on glucocorticoid-responsive genes [7,39,50]. Furthermore, when hGRβ was transiently transfected together with hGRα, a dominant negative effect of hGRβ was observed on the functional potentials of hGRα [50,51] and mineralcorticoid receptor [52], leading to the hypothesis that the cellular ratio of hGRα to hGRβ may have a profound influence on a cell's sensitivity to glucocorticoids.

Interest in the physiological significance of hGRβ did not emerge until the development of hGRβ-specific antibodies and the demonstration of its natural existence in a variety of human cell lines and tissues [53,54]. Since then, elevated levels of hGRβ have been positively correlated with glucocorticoid resistance in several disease states such as asthma, rheumatoid arthritis, and ulcerative colitis [55–62]. Recently, Hauk et al. [63] demonstrated that overexpression of the hGRβ isoform in mouse hybridoma cells generated a GC-resistant phenotype, in strong accordance with the notion that increased hGRβ expression contributes to glucocorticoid insensitivity. However, given the low levels of endogenous hGRβ detected in numerous cell lines and tissues [53,54,64,65], several researchers have argued that the amount of hGRβ may not be sufficient to underlie its dominant negative activity [66,67]. In support of this, no correlation has been found between the levels of hGRβ and cytokine-induced glucocorticoid insensitivity [68] or glucocorticoid-resistant childhood leukemia [69]. While further studies are certainly necessary to reconcile this contradiction, it is possible that the contribution of hGRβ to glucocorticoid-inertness is restricted to certain cell lines or diseases. In addition, the relative expression of hGRβ to hGRα that are detected at a single stage or time point in cells or tissues may not reflect the actual progression of glucocorticoid-resistance or the corresponding alteration in protein levels of these two isoforms. For example, studies from our lab have shown that even within the same tissues, the levels of hGRβ may vary considerably among different cell types [53]. Additionally, exposure of cells to agents such as proinflammatory cytokines such as TNF-α and IL-1 [47], microbial superantigens [59], and cortisol [70] can lead to induction of hGRβ, but suppression of hGRα, and thus a preferential proportion of hGRβ. Consequently, the enhanced ratio of hGRβ to hGRα may lead to diminished glucocorticoid responsiveness.

It is clearly of importance to identify factors that regulate the alternative splicing from the same pre-mRNA precursor. A recent study by Xu et al. [71] suggested that serine–arginine-rich protein p30 (SRp30c) was responsible for the specific generation of hGRβ transcripts over its coun-

terpart in neutrophils. In addition, the RNA splicing may also be influenced by other factors such as transcription factors interacting with their cognate elements in target gene promoters [72], or hormones [73]. Thus, preferential production of a single transcript, hGRα or hGRβ, is possible depending on particular cellular contexts and the agents the cells are exposed to. The molecular mechanisms underlying the selective generation of hGRβ by SRp30c are currently unclear. Nevertheless, further studies should provide insights about how pathologic conditions may modulate the level of this specific enzyme, thereby affecting the alternative hGR splicing.

Studies from our lab and others have indicated that hGRβ can bind to the GRE, heterodimerize with hGRα, and interact with Hsp90 [51,74]. The molecular basis for the dominant negative effect of hGRβ has been localized to two critical residues, L733 and N734, within the unique 15 amino acids of hGRβ [75]. Although the exact mechanism by which hGRβ plays its inhibitory roles via these two residues is still unclear, the current hypothesis attributes to the formation of a transcriptionally inactive hGRβ and hGRα heterodimer.

In addition to hGRα and hGRβ, Rivers et al. [76] have detected another splicing variant hGRγ, which is expressed at a level between 3.8 and 8.7% of the total GR transcripts (Fig. 3B). hGRγ differs from the others by a 3-base, thus one amino acid (arginine), insertion between exons 3 and 4 encoding the DNA-binding domain. Previous studies have suggested that this single residue insertion decreases the receptor transactivation potential by nearly half [77]. Consistent with this, a recent report suggests a positive correlation of hGRγ expression levels with childhood acute lymphoblastic leukemia [78]. Additionally, two other splice variants have been identified in human multiple myeloma cells by Moalli et al. [79] (Fig. 3B). hGR-P misses exons 8 and 9, and hGR-A lacks exons 5, 6 and 7. Both variants have been associated with glucocorticoid-resistant phenotypes [79–81]. Interestingly, although the hGR-P truncation mutant by itself exhibited lower transactivation activity than hGRα, cotransfection of both constructs suggested an enhanced effect of hGR-P on the ligand-induced hGRα activity [81]. However, the mechanism of this enhancement is currently unclear. Taken together, several transcripts have been described from the common hGR precursor RNA, including hGRα, hGRβ, hGRγ, hGR-P and hGR-A. In comparison to hGRα, most of them have decreased transactivation potentials. As of yet none have been evaluated for their potential to induce rapid glucocorticoid or non-genomic actions. Thus, it is reasonable to predict that the relative levels of these variants play a role, at least partly, in differential glucocorticoid-induced responsiveness, particularly as it relates to human disease.

5. Alternative translation initiation of the GR

Since the initial cloning of hGR in 1985 by Hollenberg et al., it had been thought that each of the two alternative transcripts, hGRα and hGRβ, gave rise to only a single protein. Studies from our lab by Yudt et al. have clearly demonstrated that this is not the case [75] (Fig. 3C). When a single hGRα cDNA was transiently transfected into receptor-less COS-1 cells or synthesized using transcription and translation reaction in vitro, a doublet band was clearly detected by Western Blot analysis. A similar result with hGRβ was observed as well (Jewell and Cidlowski, unpublished data). The receptors were named hGR-A (94 kDa) and hGR-B (91 kDa) based on their molecular weight. Further studies demonstrated that the shorter GR-B resulted from alternative translation initiation at the internal ATG codon corresponding to methionine 27 (M27). Site-directed mutagenesis of the ATG encoding methionine 1 or 27 to ACG specifically eliminated the corresponding GR-A or GR-B proteins, indicating that leaky ribosomal scanning was responsible for the generation of these two isoforms. Functional analyses indicate that both GR-A and GR-B isoforms exhibit similar sub-cellular distribution and ligand-induced nuclear translocation. However, the transactivation, but not transrepression, activity of the two isoforms differed, with GR-B being nearly twice as effective as the longer form GR-A. Interestingly, the shorter isoform GR-B was also observed endogenously in several cell lines such as HeLa, HEK293, CEM-C7, suggesting that the alternative hGR translation initiation is a naturally occurring phenomenon.

6. Post-translational modification of the hGR

In addition to the remarkable complexity of multiple receptor isoforms generated by alternative splicing and alternative translation initiation, each isoform is subject to a variety of post-translational modifications including phosphorylation, ubiquitination and sumoylation (Fig. 3D). The post-translational modifications of the GR have been a subject of research for the past two decades. As with other proteins, studies indicate that these modifications play important roles in the receptor's subcellular distribution, protein turnover, and transcriptional activities.

6.1. Phosphorylation

Among the nuclear receptor superfamily, the GR has been one of the earliest proteins evaluated for potential phosphorylation. Early evidence that the receptor was an endogenous phosphoprotein came from studies using ligand-bound affinity columns, [^{32}P]-ATP incubation, and Western Blot analysis [82–85]. The receptor is constitutively phosphorylated under physiological conditions, but also undergoes an agonist-induced and cell cycle-dependent hyperphosphorylation [86–89]. Eight phosphorylation sites on the mouse GR have been identified, most of which are serine residues located on its N-terminus at positions 122, 150, 212, 220, 234, 315 and 412, and one threonine at 159 [90]. Sequence comparisons of the mouse receptor with human and rat suggest that most of these residues are conserved among species, and

they are indeed the major sites of phosphorylation as demonstrated later [91]. The major kinases postulated to be responsible for the receptor phosphorylation include mitogen-activated protein kinases (MAPK), cyclin-dependent kinases (CDK) [92], glycogen synthase kinase-3 (GSK-3) [93] and c-Jun N-terminal kinases (JNK) [93,94], with each having distinct specificities for potential phosphorylation residues.

To understand how GR phosphorylation status affects receptor function, Mason and Housley [95] initially tested the effect of a series of single or multiple phosphorylation site mutations on mouse GR activation of a MMTV-driven reporter construct. Surprisingly, even when all seven residues were simultaneously substituted with alanine, only a slight reduction in GR's transcriptional activity was observed. Consistent with this, Webster et al. [96] showed that a combination of receptor mutants devoid of various phosphorylation sites had little effect on receptor expression, distribution, ligand-induced nuclear translocation, and transcriptional activation of the similar MMTV reporter. However, significant decreases of transcription by the mutant receptors were observed on another reporter construct under the control of minimal GREs, suggesting that the effect of phosphorylation on GR's activity is probably promoter-specific. Therefore, it is likely that the phosphorylation status of GR contributes to endogenous gene-specific regulation, perhaps in a cell type specific manner where the use of kinases might differ.

The functional significance of individual phosphorylation residues was further explored in a recent study using phospho-specific antibodies recognizing either hGR S203 or S211 [97]. Wang et al. showed that distinct kinetics was observed for ligand-induced hyperphosphorylation of these two residues. S211 displayed a more robust and sustained phosphorylation in comparison to S203. Intriguingly, following ligand activation, the S211-phosphorylated hGR receptor was detected solely in the nucleus by both Western Blot analysis and immunofluorescence. In marked contrast, the S-203 phosphorylated isoform was located primarily in the cytoplasm. This diverse pattern of localization was not only observed in U2-OS cells with stably transfected hGR, but also with the endogenous receptor in A549 cells. Consistent with the subcellular distribution, when a panel of receptor agonists/antagonists was examined for their effects on ligand-induced phosphorylation at these two residues and on receptor's transactivation potential, the extent of receptor containing the phosphorylated S211 residue showed a strong correlation with the receptor's activity. Together, these data suggest that the phosphorylation status of individual residues may have profound effects on the receptor subcellular localization, and more importantly, on its modulation of target gene transcription. However, caution needs to be exercised when interpreting these data, since previous reports have shown that the receptor mutants lacking all identified phosphorylation sites behave similarly to the wild type receptor with regard to ligand-induced nuclear translocation of the receptor [95,96]. In addition, the cytoplasmic retention of S203-phosphorylated receptor is somewhat inconsistent with published data [96,98] which indicate that most, if not all, of the glucocorticoid receptor translocates into the nucleus following treatment with dexamethasone.

It has been suggested that GR undergoes a dynamic process of phosphorylation and dephosphorylation in response to the presence of receptor ligands, variances in cell-cycle, and/or physiological state. By analogy to kinases, phosphatases are also critical for proper function of the receptor. For example, protein phosphatase type 1 (PP1), type 2a (PP2a) [99] and type 5 (PP5) [100] have been shown to regulate the nucleocytoplasmic shuttling of the receptor. In addition, the treatment of cells with okadaic acid, a phosphatase-specific inhibitor, resulted in enhancement of GR's transcriptional activity [101]. Likewise, suppression of PP5 expression induced both constitutive and ligand-activated GR transcriptional potential [102]. Nevertheless, the exact mechanisms by which these phosphatases affect the receptor's localization and activity are largely unclear. For example, it is unknown if the effect is directly resulted from the dephosphorylation of GR by these phosphatases, or indirectly from the dephosphorylation of receptor-associated chaperones such as Hsp90. Also, it is currently unclear which residues are most affected by these enzymes.

6.2. Ubiquitination

Ubiquitination is another important post-translational modification process that cells use to target specific proteins, via the attachment of multiple ubiquitin molecules, to the proteasome for degradation. Ubiquitin is a highly conserved 76 amino acid molecule universally distributed among eukaryotes. The molecule is first activated by E-1 activating enzymes, then transferred to E-2 conjugating enzymes, and subsequently passed on to E-3 ligases. E-3 ligases recognize a wide range of target substrates by their conserved ubiquitination motifs and attach ubiquitin to the appropriate residues on the target proteins. Once tagged, the proteins are degraded by proteasome complexes in a series of programmed steps.

In vitro studies by Wallace and Cidlowski have indicated that GR is a potential substrate for ubiquitination [103]. Treatment of COS-1 cells expressing mouse GR with the proteasome inhibitor MG132 leads to a block of ligand-induced GR degradation and an enhancement of GR transcriptional activity, suggesting that the proteasome is involved in receptor turnover. Direct ubiquitination of GR was demonstrated using co-immunoprecipitation assays in COS-1 cells transiently cotransfected with constructs encoding GR and ubiquitins. Consistent with this, computer analysis of primary GR sequences from human, mouse, and rat identified a conserved PEST motif, which is important for substrate recognition by E2/E3 enzymes [104]. When residue K426 of this GR PEST motif was mutated to alanine, the receptor became resistant to ligand-induced degradation and possessed an enhanced transcriptional activity, similar to the results obtained with MG132 treatment. These data suggest that this residue is

critical for GR protein degradation, possibly as an ubiquitin acceptor site. However, it is not clear if this K426 residue has any direct effect on receptor ubiquitination, and if that is the only site involved. Interestingly, when Hela cells were treated with MG132, increased transcriptional activity of GR was also observed, suggesting that ubiquitination-induced proteasome degradation might occur for the endogenous receptors as well. However, further studies are necessary in order to fully establish the involvement of ubiquitination in regulating receptor degradation endogenously.

6.3. Sumoylation

Small ubiquitin-related modifier (SUMO) is an 11 kDa protein moiety that can be covalently ligated to lysine residues in a variety of target proteins. The protein is similar to ubiquitin in both size and three-dimensional structure, yet the functional consequences of sumoylation are distinct. While ubiquitination largely leads to the proteasome-mediated target protein degradation, modifications by SUMO regulate more diverse biological effects including protein–protein interactions, subcellular localization, protein stability, and transcriptional capacity [105–108]. Recent work from in vitro studies indicate that hGR is a sumoylation target protein with three lysine residues (K277, K293 and K703) identified as the potential acceptor sides for SUMO attachment [109–111]. However, interpretation of the data has been controversial. With mutation of all three lysine residues to arginine, diminished GR sumoylation and enhanced transcriptional activity of the receptor was reported by Tian et al. [110], suggesting that sumoylation might inhibit the receptor's transactivation potential. In marked contrast, Le Drean et al. published data indicating that overexpression of SUMO-1 together with GR increased the receptor's transactivation potential by up to eight-fold [111]. Despite the apparent controversy regarding the functional consequences of sumoylation on GR activity, both articles demonstrate that GR is a potential sumoylation target protein, and that the covalent modification by SUMO affects the receptor's function in a promoter- and cell-specific manner. Nevertheless, as both studies utilized transient transfections, it is unclear whether endogenous GR is sumoylated, and thus its transcriptional activity modulated.

7. Glucocorticoid receptor mutations and polymorphisms

Studies of GR mutations and polymorphisms present a clear example of how small changes in the gene may affect its protein expression, structure, function and thus have diverse clinical manifestations. So far, a total of fifteen missense, three nonsense, three frameshift, one splice site, and seventeen polymorphisms have been identified from either patients or in vitro cell lines derived from leukemias or myelomas (for a recent review, see [112]). Most of these have been associated with glucocorticoid resistance. However, not all of the mutations necessarily cause a glucocorticoid-insensitive phenotype. For example, the N363S mutant leads to glucocorticoid hypersensitivity and may contribute to male obesity although controversy exists on the issue [113].

8. Conclusion

It is clear that multiple isoforms of the GR protein are generated endogenously as a result of alternative RNA splicing and alternative translation initiation. In addition, each isoform is subject to a variety of post-translational modifications including phosphorylation, ubiquitination and sumoylation. Consequently, the potential existence of an enormous number of receptor variants, each having differential characteristics in expression, localization, transcriptional activity, and/or degradation, comprises a tissue- or cell-specific hGR population contributing substantially to unique biological responses.

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References

[1] Barnes PJ. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. Clin Sci (Lond) 1998;94:557–72.
[2] Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev 2000;21:55–89.
[3] Coghlan MJ, Elmore SW, Kym PR, Kort ME. The pursuit of differentiated ligands for the glucocorticoid receptor. Curr Top Med Chem 2003;3:1617–35.
[4] Barnes PJ, Greening AP, Crompton GK. Glucocorticoid resistance in asthma. Am J Respir Crit Care Med 1995;152:125–40.
[5] Langhoff E, Ladefoged J, Jakobsen BK, Platz P, Ryder LP, Svejgaard A, et al. Recipient lymphocyte sensitivity to methylprednisolone affects cadaver kidney graft survival. Lancet 1986;1:1296–7.
[6] Kirkham BW, Corkill MM, Davison SC, Panayi GS. Response to glucocorticoid treatment in rheumatoid arthritis: in vitro cell mediated immune assay predicts in vivo responses. J Rheumatol 1991;18:821–5.
[7] Giguere V, Hollenberg SM, Rosenfeld MG, Evans RM. Functional domains of the human glucocorticoid receptor. Cell 1986;46:645–52.
[8] Tang Y, Getzenberg RH, Vietmeier BN, Stallcup MR, Eggert M, Renkawitz R, et al. The DNA-binding and tau2 transactivation domains of the rat glucocorticoid receptor constitute a nuclear matrix-targeting signal. Mol Endocrinol 1998;12:1420–31.
[9] Vanderbilt JN, Miesfeld R, Maler BA, Yamamoto KR. Intracellular receptor concentration limits glucocorticoid-dependent enhancer activity. Mol Endocrinol 1987;1:68–74.
[10] Danielsen M, Stallcup MR. Down-regulation of glucocorticoid receptors in mouse lymphoma cell variants. Mol Cell Biol 1984;4:449–53.

[11] Cidlowski JA, Cidlowski NB. Regulation of glucocorticoid receptors by glucocorticoids in cultured hela s3 cells. Endocrinology 1981;109:1975–82.
[12] Hoeck W, Rusconi S, Groner B. Down-regulation and phosphorylation of glucocorticoid receptors in cultured cells. Investigations with a monospecific antiserum against a bacterially expressed receptor fragment. J Biol Chem 1989;264:14396–402.
[13] Silva CM, Powell-Oliver FE, Jewell CM, Sar M, Allgood VE, Cidlowski JA. Regulation of the human glucocorticoid receptor by long-term and chronic treatment with glucocorticoid. Steroids 1994;59:436–42.
[14] Isohashi F, Okamoto K. Atp-stimulated translocation promoter that enhances the nuclear binding of activated glucocorticoid receptor complex. Biochemical properties and its function. Receptor 1993;3:113–24 [mini-review].
[15] Okamoto K, Hirano H, Isohashi F. Molecular cloning of rat liver glucocorticoid-receptor translocation promoter. Biochem Biophys Res Commun 1993;193:848–54.
[16] Sakai DD, Helms S, Carlstedt-Duke J, Gustafsson JA, Rottman FM, Yamamoto KR. Hormone-mediated repression: a negative glucocorticoid response element from the bovine prolactin gene. Genes Dev 1988;2:1144–54.
[17] Soudeyns H, Geleziunas R, Shyamala G, Hiscott J, Wainberg MA. Identification of a novel glucocorticoid response element within the genome of the human immunodeficiency virus type 1. Virology 1993;194:758–68.
[18] Morrison N, Eisman J. Role of the negative glucocorticoid regulatory element in glucocorticoid repression of the human osteocalcin promoter. J Bone Miner Res 1993;8:969–75.
[19] Pei L. Identification of a negative glucocorticoid response element in the rat type 1 vasoactive intestinal polypeptide receptor gene. J Biol Chem 1996;271:20879–84.
[20] Malkoski SP, Dorin RI. Composite glucocorticoid regulation at a functionally defined negative glucocorticoid response element of the human corticotropin-releasing hormone gene. Mol Endocrinol 1999;13:1629–44.
[21] Ou X-M, Storring JM, Kushwaha N, Albert PR. Heterodimerization of mineralocorticoid and glucocorticoid receptors at a novel negative response element of the 5-ht1a receptor gene. J Biol Chem 2001;276:14299–307.
[22] Meyer T, Carlstedt-Duke J, Starr DB. A weak tata box is a prerequisite for glucocorticoid-dependent repression of the osteocalcin gene. J Biol Chem 1997;272:30709–14.
[23] Stromstedt PE, Poellinger L, Gustafsson JA, Carlstedt-Duke J. The glucocorticoid receptor binds to a sequence overlapping the tata box of the human osteocalcin promoter: a potential mechanism for negative regulation. Mol Cell Biol 1991;11:3379–83.
[24] Subramaniam N, Cairns W, Okret S. Studies on the mechanism of glucocorticoid-mediated repression from a negative glucocorticoid response element from the bovine prolactin gene. DNA Cell Biol 1997;16:153–63.
[25] Hermoso MA, Cidlowski JA. Putting the brake on inflammatory responses: the role of glucocorticoids. IUBMB Life 2003;55:497–504.
[26] De Bosscher K, Vanden Berghe W, Haegeman G. The interplay between the glucocorticoid receptor and nuclear factor-kappab or activator protein-1: molecular mechanisms for gene repression. Endocr Rev 2003;24:488–522.
[27] De Bosscher K, Vanden Berghe W, Haegeman G. Mechanisms of anti-inflammatory action and of immunosuppression by glucocorticoids: negative interference of activated glucocorticoid receptor with transcription factors. J Neuroimmunol 2000;109:16–22.
[28] Bamberger CM, Schulte HM, Chrousos GP. Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. Endocr Rev 1996;17:245–61.
[29] Scheinman R, Gualberto A, Jewell C, Cidlowski J, Baldwin Jr A. Characterization of mechanisms involved in transrepression of nf-kappa b by activated glucocorticoid receptors. Mol Cell Biol 1995;15:943–53.
[30] Lucibello FC, Slater EP, Jooss KU, Beato M, Muller R. Mutual transrepression of fos and the glucocorticoid receptor: involvement of a functional domain in fos which is absent in fosb. EMBO J 1990;9:2827–34.
[31] Schule R, Rangarajan P, Kliewer S, Ransone LJ, Bolado J, Yang N, et al. Functional antagonism between oncoprotein c-jun and the glucocorticoid receptor. Cell 1990;62:1217–26.
[32] Guller S, Wozniak R, Kong L, Lockwood CJ. Opposing actions of transforming growth factor-beta and glucocorticoids in the regulation of fibronectin expression in the human placenta. J Clin Endocrinol Metab 1995;80:3273–8.
[33] Slavin J, Unemori E, Hunt TK, Amento E. Transforming growth factor beta (tgf-beta) and dexamethasone have direct opposing effects on collagen metabolism in low passage human dermal fibroblasts in vitro. Growth Factors 1994;11:205–13.
[34] Meisler N, Keefer KA, Ehrlich HP, Yager DR, Myers-Parrelli J, Cutroneo KR. Dexamethasone abrogates the fibrogenic effect of transforming growth factor-beta in rat granuloma and granulation tissue fibroblasts. J Invest Dermatol 1997;108:285–9.
[35] Song C-Z, Tian X, Gelehrter TD. Glucocorticoid receptor inhibits transforming growth factor-beta signaling by directly targeting the transcriptional activation function of smad3. PNAS 1999;96:11776–81.
[36] Stocklin E, Wissler M, Gouilleux F, Groner B. Functional interactions between stat5 and the glucocorticoid receptor. Nature 1996;383:726–8.
[37] Groner B. Transcription factor regulation in mammary epithelial cells. Domest Anim Endocrinol 2002;23:25–32.
[38] Hermoso MA, Matsuguchi T, Smoak K, Cidlowski JA. Glucocorticoids and tumor necrosis factor alpha cooperatively regulate toll-like receptor 2 gene expression. Mol Cell Biol 2004;24:4743–56.
[39] Hollenberg SM, Weinberger C, Ong ES, Cerelli G, Oro A, Lebo R, et al. Primary structure and expression of a functional human glucocorticoid receptor cdna. Nature 1985;318:635–41.
[40] Francke U, Foellmer BE. The glucocorticoid receptor gene is in 5q31-q32. Genomics 1989;4:610–2 [corrected].
[41] Theriault A, Boyd E, Harrap SB, Hollenberg SM, Connor JM. Regional chromosomal assignment of the human glucocorticoid receptor gene to 5q31. Hum Genet 1989;83:289–91.
[42] Encio IJ, Detera-Wadleigh SD. The genomic structure of the human glucocorticoid receptor. J Biol Chem 1991;266:7182–8.
[43] Zong J, Ashraf J, Thompson EB. The promoter and first, untranslated exon of the human glucocorticoid receptor gene are gc rich but lack consensus glucocorticoid receptor element sites. Mol Cell Biol 1990;10:5580–5.
[44] Nobukuni Y, Smith CL, Hager GL, Detera-Wadleigh SD. Characterization of the human glucocorticoid receptor promoter. Biochemistry 1995;34:8207–14.
[45] Breslin MB, Geng CD, Vedeckis WV. Multiple promoters exist in the human gr gene, one of which is activated by glucocorticoids. Mol Endocrinol 2001;15:1381–95.
[46] Breslin MB, Vedeckis WV. The human glucocorticoid receptor promoter upstream sequences contain binding sites for the ubiquitous transcription factor, Yin Yang 1. J Steroid Biochem Mol Biol 1998;67:369–81.
[47] Webster JC, Oakley RH, Jewell CM, Cidlowski JA. Proinflammatory cytokines regulate human glucocorticoid receptor gene expression and lead to the accumulation of the dominant negative beta isoform: a mechanism for the generation of glucocorticoid resistance. Proc Natl Acad Sci USA 2001;98:6865–70.
[48] Korn SH, Koerts-de Lang E, Engel GE, Arends JW, Wouters EF, Thunnissen FB. Alpha and beta glucocorticoid receptor mrna expression in skeletal muscle. J Muscle Res Cell Motil 1998;19:757–65.

[49] Otto C, Reichardt HM, Schutz G. Absence of glucocorticoid receptor-beta in mice. J Biol Chem 1997;272:26665–8.
[50] Oakley RH, Sar M, Cidlowski JA. The human glucocorticoid receptor beta isoform. Expression, biochemical properties, and putative function. J Biol Chem 1996;271:9550–9.
[51] Bamberger CM, Bamberger AM, de Castro M, Chrousos GP. Glucocorticoid receptor beta, a potential endogenous inhibitor of glucocorticoid action in humans. J Clin Invest 1995;95:2435–41.
[52] Bamberger CM, Bamberger AM, Wald M, Chrousos GP, Schulte HM. Inhibition of mineralocorticoid activity by the beta-isoform of the human glucocorticoid receptor. J Steroid Biochem Mol Biol 1997;60:43–50.
[53] Oakley RH, Webster JC, Sar M, Parker Jr CR, Cidlowski JA. Expression and subcellular distribution of the beta-isoform of the human glucocorticoid receptor. Endocrinology 1997;138: 5028–38.
[54] de Castro M, Elliot S, Kino T, Bamberger C, Karl M, Webster E, et al. The non-ligand binding beta-isoform of the human glucocorticoid receptor (hgr beta): tissue levels, mechanism of action, and potential physiologic role. Mol Med 1996;2:597–607.
[55] Chikanza IC. Mechanisms of corticosteroid resistance in rheumatoid arthritis: a putative role for the corticosteroid receptor beta isoform. Ann NY Acad Sci 2002;966:39–48.
[56] Hamid QA, Wenzel SE, Hauk PJ, Tsicopoulos A, Wallaert B, Lafitte JJ, et al. Increased glucocorticoid receptor beta in airway cells of glucocorticoid-insensitive asthma. Am J Respir Crit Care Med 1999;159:1600–4.
[57] Sousa AR, Lane SJ, Cidlowski JA, Staynov DZ, Lee TH. Glucocorticoid resistance in asthma is associated with elevated in vivo expression of the glucocorticoid receptor beta-isoform. J Allergy Clin Immunol 2000;105:943–50.
[58] Honda M, Orii F, Ayabe T, Imai S, Ashida T, Obara T, et al. Expression of glucocorticoid receptor beta in lymphocytes of patients with glucocorticoid-resistant ulcerative colitis. Gastroenterology 2000;118:859–66.
[59] Hauk PJ, Hamid QA, Chrousos GP, Leung DY. Induction of corticosteroid insensitivity in human pbmcs by microbial superantigens. J Allergy Clin Immunol 2000;105:782–7.
[60] Fakhri S, Tulic M, Christodoulopoulos P, Fukakusa M, Frenkiel S, Leung DY, et al. Microbial superantigens induce glucocorticoid receptor beta and steroid resistance in a nasal explant model. Laryngoscope 2004;114:887–92.
[61] Leung DY, de Castro M, Szefler SJ, Chrousos GP. Mechanisms of glucocorticoid-resistant asthma. Ann NY Acad Sci 1998;840:735–46.
[62] Leung DY, Hamid Q, Vottero A, Szefler SJ, Surs W, Minshall E, et al. Association of glucocorticoid insensitivity with increased expression of glucocorticoid receptor beta. J Exp Med 1997;186:1567–74.
[63] Hauk PJ, Goleva E, Strickland I, Vottero A, Chrousos GP, Kisich KO, et al. Increased glucocorticoid receptor beta expression converts mouse hybridoma cells to a corticosteroid-insensitive phenotype. Am J Respir Cell Mol Biol 2002;27:361–7.
[64] Pujols L, Mullol J, Roca-Ferrer J, Torrego A, Xaubet A, Cidlowski JA, et al. Expression of glucocorticoid receptor alpha- and beta-isoforms in human cells and tissues. Am J Physiol Cell Physiol 2002;283:1324–31.
[65] Pujols L, Mullol J, Benitez P, Torrego A, Xaubet A, de Haro J, et al. Expression of the glucocorticoid receptor alpha and beta isoforms in human nasal mucosa and polyp epithelial cells. Respir Med 2003;97:90–6.
[66] Vottero A, Chrousos GP. Glucocorticoid receptor beta: view i. Trends Endocrinol Metab 1999;10:333–8.
[67] Carlstedt-Duke J. Glucocorticoid receptor beta: view ii. Trends Endocrinol Metab 1999;10:339–42.
[68] Torrego A, Pujols L, Roca-Ferrer J, Mullol J, Xaubet A, Picado C. Glucocorticoid receptor isoforms {alpha} and {beta} in in vitro cytokine-induced glucocorticoid insensitivity. Am J Respir Crit Care Med 2004;170:420–5.
[69] Haarman EG, Kaspers GJ, Pieters R, Rottier MM, Veerman AJ. Glucocorticoid receptor alpha, beta and gamma expression vs in vitro glucocorticod resistance in childhood leukemia. Leukemia 2004;18:530–7.
[70] Whorwood CB, Donovan SJ, Wood PJ, Phillips DI. Regulation of glucocorticoid receptor alpha and beta isoforms and type i 11beta-hydroxysteroid dehydrogenase expression in human skeletal muscle cells: a key role in the pathogenesis of insulin resistance. J Clin Endocrinol Metab 2001;86:2296–308.
[71] Xu Q, Leung DY, Kisich KO. Serine–arginine-rich protein p30 directs alternative splicing of glucocorticoid receptor pre-mrna to glucocorticoid receptor beta in neutrophils. J Biol Chem 2003;278:27112–8.
[72] Auboeuf D, Honig A, Berget SM, O'Malley BW. Coordinate regulation of transcription and splicing by steroid receptor coregulators. Science 2002;298:416–9.
[73] Lai GJ, McCobb DP. Opposing actions of adrenal androgens and glucocorticoids on alternative splicing of slo potassium channels in bovine chromaffin cells. Proc Natl Acad Sci USA 2002;99: 7722–7.
[74] Oakley RH, Jewell CM, Yudt MR, Bofetiado DM, Cidlowski JA. The dominant negative activity of the human glucocorticoid receptor beta isoform Specificity and mechanisms of action. J Biol Chem 1999;274:27857–66.
[75] Yudt MR, Jewell CM, Bienstock RJ, Cidlowski JA. Molecular origins for the dominant negative function of human glucocorticoid receptor beta. Mol Cell Biol 2003;23:4319–30.
[76] Rivers C, Levy A, Hancock J, Lightman S, Norman M. Insertion of an amino acid in the DNA-binding domain of the glucocorticoid receptor as a result of alternative splicing. J Clin Endocrinol Metab 1999;84:4283–6.
[77] Ray DW, Davis JR, White A, Clark AJ. Glucocorticoid receptor structure and function in glucocorticoid-resistant small cell lung carcinoma cells. Cancer Res 1996;56:3276–80.
[78] Beger C, Gerdes K, Lauten M, Tissing WJ, Fernandez-Munoz I, Schrappe M, et al. Expression and structural analysis of glucocorticoid receptor isoform gamma in human leukaemia cells using an isoform-specific real-time polymerase chain reaction approach. Br J Haematol 2003;122:245–52.
[79] Moalli PA, Pillay S, Krett NL, Rosen ST. Alternatively spliced glucocorticoid receptor messenger rnas in glucocorticoid-resistant human multiple myeloma cells. Cancer Res 1993;53:3877–9.
[80] Krett NL, Pillay S, Moalli PA, Greipp PR, Rosen ST. A variant glucocorticoid receptor messenger rna is expressed in multiple myeloma patients. Cancer Res 1995;55:2727–9.
[81] de Lange P, Segeren CM, Koper JW, Wiemer E, Sonneveld P, Brinkmann AO, et al. Expression in hematological malignancies of a glucocorticoid receptor splice variant that augments glucocorticoid receptor-mediated effects in transfected cells. Cancer Res 2001;61:3937–41.
[82] Grandics P, Miller A, Schmidt TJ, Litwack G. Phosphorylation in vivo of rat hepatic glucocorticoid receptor. Biochem Biophys Res Commun 1984;120:59–65.
[83] Housley PR, Pratt WB. Direct demonstration of glucocorticoid receptor phosphorylation by intact l-cells. J Biol Chem 1983;258:4630–5.
[84] Kurl RN, Jacob ST. Phosphorylation of purified glucocorticoid receptor from rat liver by an endogenous protein kinase. Biochem Biophys Res Commun 1984;119:700–5.
[85] Singh VB, Moudgil VK. Phosphorylation of rat liver glucocorticoid receptor. J Biol Chem 1985;260:3684–90.
[86] Orti E, Mendel DB, Smith LI, Munck A. Agonist-dependent phosphorylation and nuclear dephosphorylation of glucocorticoid receptors in intact cells. J Biol Chem 1989;264:9728–31.

[87] Bodwell JE, Hu JM, Orti E, Munck A. Hormone-induced hyperphosphorylation of specific phosphorylated sites in the mouse glucocorticoid receptor. J Steroid Biochem Mol Biol 1995;52: 135–40.
[88] Hu JM, Bodwell JE, Munck A. Cell cycle-dependent glucocorticoid receptor phosphorylation and activity. Mol Endocrinol 1994;8:1709–13.
[89] Hu JM, Bodwell JE, Munck A. Control by basal phosphorylation of cell cycle-dependent, hormone-induced glucocorticoid receptor hyperphosphorylation. Mol Endocrinol 1997;11:305–11.
[90] Bodwell JE, Orti E, Coull JM, Pappin DJ, Smith LI, Swift F. Identification of phosphorylated sites in the mouse glucocorticoid receptor. J Biol Chem 1991;266:7549–55.
[91] Almlof T, Wright AP, Gustafsson JA. Role of acidic and phosphorylated residues in gene activation by the glucocorticoid receptor. J Biol Chem 1995;270:17535–40.
[92] Krstic MD, Rogatsky I, Yamamoto KR, Garabedian MJ. Mitogen-activated and cyclin-dependent protein kinases selectively and differentially modulate transcriptional enhancement by the glucocorticoid receptor. Mol Cell Biol 1997;17:3947–54.
[93] Rogatsky I, Waase CL, Garabedian MJ. Phosphorylation and inhibition of rat glucocorticoid receptor transcriptional activation by glycogen synthase kinase-3 (gsk-3). Species-specific differences between human and rat glucocorticoid receptor signaling as revealed through gsk-3 phosphorylation. J Biol Chem 1998;273: 14315–21.
[94] Itoh M, Adachi M, Yasui H, Takekawa M, Tanaka H, Imai K. Nuclear export of glucocorticoid receptor is enhanced by c-jun n-terminal kinase-mediated phosphorylation. Mol Endocrinol 2002;16:2382–92.
[95] Mason SA, Housley PR. Site-directed mutagenesis of the phosphorylation sites in the mouse glucocorticoid receptor. J Biol Chem 1993;268:21501–4.
[96] Webster JC, Jewell CM, Bodwell JE, Munck A, Sar M, Cidlowski JA. Mouse glucocorticoid receptor phosphorylation status influences multiple functions of the receptor protein. J Biol Chem 1997;272:9287–93.
[97] Wang Z, Frederick J, Garabedian MJ. Deciphering the phosphorylation "code" of the glucocorticoid receptor in vivo. J Biol Chem 2002;277:26573–80.
[98] Yudt MR, Cidlowski JA. Molecular identification and characterization of a and b forms of the glucocorticoid receptor. Mol Endocrinol 2001;15:1093–103.
[99] DeFranco DB. Functional implications of glucocorticoid receptor trafficking. Ernst Schering Res Found Workshop 2002:91–109.
[100] Dean DA, Urban G, Aragon IV, Swingle M, Miller B, Rusconi S, et al. Serine/threonine protein phosphatase 5 (pp5) participates in the regulation of glucocorticoid receptor nucleocytoplasmic shuttling. BMC Cell Biol 2001;2:6.
[101] Somers JP, DeFranco DB. Effects of okadaic acid, a protein phosphatase inhibitor, on glucocorticoid receptor-mediated enhancement. Mol Endocrinol 1992;6:26–34.
[102] Zuo Z, Urban G, Scammell JG, Dean NM, McLean TK, Aragon I, et al. Ser/thr protein phosphatase type 5 (pp5) is a negative regulator of glucocorticoid receptor-mediated growth arrest. Biochemistry 1999;38:8849–57.
[103] Wallace AD, Cidlowski JA. Proteasome-mediated glucocorticoid receptor degradation restricts transcriptional signaling by glucocorticoids. J Biol Chem 2001;276:42714–21.
[104] Rogers S, Wells R, Rechsteiner M. Amino acid sequences common to rapidly degraded proteins: the pest hypothesis. Science 1986;234:364–8.
[105] Muller S, Hoege C, Pyrowolakis G, Jentsch S. Sumo, ubiquitin's mysterious cousin. Nat Rev Mol Cell Biol 2001;2:202–10.
[106] Wilson VG, Rangasamy D. Intracellular targeting of proteins by sumoylation. Exp Cell Res 2001;271:57–65.
[107] Freiman RN, Tjian R. Regulating the regulators: lysine modifications make their mark. Cell 2003;112:11–7.
[108] Verger A, Perdomo J, Crossley M. Modification with sumo. A role in transcriptional regulation. EMBO Rep 2003;4:137–42.
[109] Holmstrom S, Van Antwerp ME, Iniguez-Lluhi JA. Direct and distinguishable inhibitory roles for sumo isoforms in the control of transcriptional synergy. Proc Natl Acad Sci USA 2003.
[110] Tian S, Poukka H, Palvimo JJ, Janne OA. Small ubiquitin-related modifier-1 (sumo-1) modification of the glucocorticoid receptor. Biochem J 2002;367:907–11.
[111] Le Drean Y, Mincheneau N, Le Goff P, Michel D. Potentiation of glucocorticoid receptor transcriptional activity by sumoylation. Endocrinology 2002;143:3482–9.
[112] Bray PJ, Cotton RG. Variations of the human glucocorticoid receptor gene (nr3c1): pathological and in vitro mutations and polymorphisms. Hum Mutat 2003;21:557–68.
[113] Koper JW, Stolk RP, de Lange P, Huizenga NA, Molijn GJ, Pols HA, et al. Lack of association between five polymorphisms in the human glucocorticoid receptor gene and glucocorticoid resistance. Hum Genet 1997;99:663–8.