Review

Generating diversity in glucocorticoid receptor signaling: mechanisms, receptor isoforms, and post-translational modifications

Danielle Duma and John A. Cidlowski*
Laboratory of Signal Transduction, Molecular Endocrinology Group, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709, USA

Abstract

Glucocorticoids are necessary for life after birth and regulate numerous homeostatic functions in man, including glucose homeostasis, protein catabolism, skeletal growth, respiratory function, inflammation, development, behavior, and apoptosis. In a clinical setting, they are widely used as anti-inflammatory agents to control both acute and chronic inflammation. Unfortunately, owing to their broad range of physiological actions, patients treated with glucocorticoids for long periods of time experience a variety of serious side effects, including metabolic syndrome, bone loss, and psychiatric disorders including depression, mania, and psychosis. Our understanding of how one hormone or drug regulates all of these diverse processes is limited. Recent studies have shown that multiple glucocorticoid receptor isoforms are produced from one gene via combinations of alternative mRNA splicing and alternative translation initiation. These isoforms possess unique tissue distribution patterns and transcriptional regulatory profiles. Owing to variation in the N-terminal and C-terminal length of glucocorticoid receptor isoforms, different post-translational modifications including ubiquitination, phosphorylation, and sumoylation are predicted, contributing to the complexity of glucocorticoid signaling. Furthermore, increasing evidence suggests that unique glucocorticoid receptor isoform compositions within cells could determine the cell-specific response to glucocorticoids. In this review, we will outline the recent advances made in the characterization of the transcriptional activity and the selective regulation of apoptosis by the various glucocorticoid receptor isoforms.

Keywords: glucocorticoid; isoforms; mechanisms; post-translational modification; receptor.

*Corresponding author: John A. Cidlowski, Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, National Institutes of Health, P.O. Box 12233, 111 T.W. Alexander Drive, Research Triangle Park, NC 27709, USA. Phone: +1-919-541-1564, Fax: +1-919-541-1367, E-mail: cidlowski1@niehs.nih.gov

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Introduction

Glucocorticoids are steroid hormones that are produced and released by the adrenal gland under the control of the hypothalamic-pituitary-adrenal (HPA) axis. In response to a variety of stressors, the hypothalamus releases corticotropin-releasing hormone and vasopressin that stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary gland (1). In the adrenal cells, ACTH stimulates the production and release of glucocorticoids into the bloodstream, affecting all tissues within the body. Their actions are mediated by the ubiquitous cytoplasmic glucocorticoid receptor, which functions as a hormone-activated transcription factor of glucocorticoid target genes. They are important for the maintenance of diverse biological activities such as the regulation of vascular tone, homeostasis of the central nervous system, intermediary metabolism, and water/electrolyte balance. In addition, glucocorticoids exert potent anti-inflammatory and immunomodulatory effects, and are therefore commonly used in the treatment of chronic inflammatory and autoimmune diseases. They are also included in chemotherapeutic regimens for the treatment of leukemia, lymphomas, and myelomas due to their ability to induce lymphocyte apoptosis (2–5).

Synthetic derivatives of glucocorticoids have been applied successfully in a clinical setting for more than half a century (6–8). Although they are considered to be relatively inexpensive drugs, synthetic glucocorticoids are believed to account for a $10 billion (USD) per year market (9). However, their use is limited by serious adverse effects such as diabetes, osteoporosis, myopathy, suppression of the HPA axis, and growth retardation (9). Osteoporosis, for instance, is one of the side effects that can be life-threatening (10). In prolonged glucocorticoid exposure (treatment for 6 months, for example), the incidence of osteoporosis can be as high as 50%, accounting for approximately 25% of cases of osteoporosis. This is a serious problem especially in elderly patients (70–79 years of age) because they frequently receive glucocorticoids prescribed at high-doses (> 7.5 mg/day) for long periods of time (> 2 years) (10, 11).

Glucocorticoid resistance is another major problem that appears in patients receiving synthetic glucocorticoids for the treatment of inflammatory diseases (12–14). Although glucocorticoids are highly effective in the control of asthma and other chronic inflammatory or immune diseases, a small proportion of patients with asthma fail to respond to treatment (even to high-doses of oral glucocorticoids), and patients with chronic obstructive pulmonary disease are generally unresponsive to glucocorticoids (15). In addition, renal trans-
planted patients receiving steroid therapy also develop glucocorticoid resistance, most likely due to alterations in the expression levels of the glucocorticoid receptor detected in the lymphocytes of these patients (16). Therefore, there is a great medical need for glucocorticoid-like compounds that possess anti-inflammatory and immunomodulatory properties, yet display a reduced risk of side effects and resistance.

Recently, several strategies for development of novel glucocorticoids or glucocorticoid substitutes with an improved therapeutic index have been pursued by different pharmaceutical companies and research groups (8). For example, chemical optimization of compounds has been done to improve activity, to generate prodrugs that have enhanced bioavailability, to release the compounds in target tissues, or to generate novel glucocorticoid ligands, such as selective glucocorticoid receptor agonists (SEGRAs). SEGRAs preferentially induce transcription of glucocorticoid regulated genes (responsible for the anti-inflammatory effects of glucocorticoids) with little or no transactivating activity (suggested to be involved with the development of side effects) (8, 17). Although, the selective agonist receptors are currently in Phase II clinical trials, as an alternative topical treatment for atopic dermatitis and inflammation following cataract surgery (18, 19), it remains to be seen whether selective receptor agonists cause significantly less side effects than classical corticoids in clinical applications, such as oral administration. Furthermore, some of the glucocorticoid-like compounds identified have undesired effects after prolonged treatment in vivo (20–22), highlighting the need for further research to better understand the mechanism of action of glucocorticoids.

To understand the molecular basis of glucocorticoid-induced side effects and resistance, it is necessary to obtain an understanding of their actions in homeostatic signaling processes. Multiple factors in the glucocorticoid signaling system such as ligand availability, ultradian hormone pulsing, alternative splicing of the glucocorticoid receptor, promoter association, attraction of cofactors, and clearance of receptors from target genes can influence the ultimate transcriptional activity of the glucocorticoid receptor (23, 24). Recent data from our laboratory suggest that multiple glucocorticoid receptor isoforms are generated from the primary glucocorticoid receptor transcript through alternative initiation of translation. Furthermore, the glucocorticoid receptor translational isoforms are expressed in a tissue specific manner and regulate distinct genes (25, 26). This could represent a general mechanism that confers target-tissue sensitivity to glucocorticoid hormones. This discovery could help to explain why patients taking glucocorticoids frequently develop serious adverse effects and resistance, and increase the possibility of new targets for the development of selective glucocorticoid receptor isoforms agonists.

The glucocorticoid receptor: modular organization, mechanism of action, and transcriptional regulation

The glucocorticoid receptor is a member of the nuclear receptor superfamily of transcription factors and is ubiquitously expressed at a density varying from 2000 to 30,000 receptors per cell according to the cell type and tissue analyzed (27–29). The glucocorticoid receptor gene is conserved from *Xenopus* to human (30) and encodes a modular protein organized into three major functional domains: the N-terminal domain (NTD), the central DNA-binding domain (DBD), and the C-terminal ligand-binding domain (LBD) (Figure 1). When compared to other steroid hormone receptors, the NTD is poorly conserved in terms of size and sequence homology, with the exception of a region rich in negatively charged acidic amino acids known as AF-1 (activation function 1). The AF-1 region has the ability to activate target genes in a hormone-independent manner (31, 32) and has been shown to interact directly with the basal transcriptional machinery as well as with cofactors that participate in transcriptional regulation. For example, the AF-1 domain recruits the multimeric chromatin-remodeling ATPase brahma-related gene 1, a homolog of yeast SWI/SNF, that in turn assembles the histone acetylases P/CAF and CBP/p300 and facilitates the initiation of transcription (33, 34). Adjacent to the AF-1 region is the highly conserved DBD. The DBD is organized into two zinc fingers that contain four cysteines per loop (34). Specific amino acid sequences within the first zinc finger mediate the interaction of the glucocorticoid receptor with specific DNA elements termed glucocorticoid response elements (GREs). The second zinc finger region stabilizes the glucocorticoid receptor/GRE interaction (34). Finally, the C-terminal LBD is highly conserved among ster-

![Figure 1](image_url) Linear schematic of the human glucocorticoid receptor (hGR) protein and generation of multiple GR isoforms from a single gene. The three major domains, the N-terminal domain (NTD), DNA-binding domain (DBD), and the ligand binding-domain (LBD) of hGR are shown. As discussed and referenced in the text, approximate locations are indicated of some defined regions and functions concerning the AF-1 (activation function 1), the AF-2 (activation function 2) helix, and cofactor/chaperone binding.
Glucocorticoid receptor splice variants and N-terminal isoforms

The human glucocorticoid receptor (hGR) is the product of one gene located on chromosome 5 and consists of nine exons. Exon 1 and the first part of exon 2 contain the 5’UTR, exons 2–9 contain the protein-coding sequences, and the 3’UTR is found within the latter part of exon 9 (30). Through alternative promoter usage and splicing of pre-mRNA one gene gives rise to several isoforms that have been designated hGRα, hGRβ, hGRA, hGRP, hGRγ, and hGR-low sensitiv-
Mechanisms of glucocorticoid receptor signaling within cells. 1) Passive diffusion of glucocorticoids into the cell; 2) glucocorticoids are subject to pre-receptor metabolism by 11β-hydroxysteroid dehydrogenase-2 (11β-HSD2); 3) binding of glucocorticoids to the cytosolic glucocorticoid receptor; 4) dissociation of the glucocorticoid receptor from chaperones; and 5) translocation of the glucocorticoid receptor to the nucleus and regulation of gene transcription in four different ways: (A) interaction with glucocorticoid response elements (GREs), (B) interaction with negative GRE (nGRE), (C) interaction with GRE and transcription factors (TFs) at the composite response elements, and (D) direct interaction with TFs independent of chromatin interaction. TF-RE, transcription factor responsive element; nGRE, negative glucocorticoid response element; GR-Iso, glucocorticoid receptor isoform; HPS, heat shock protein.

Figure 2

Figure 2  Mechanisms of glucocorticoid receptor signaling within cells. 1) Passive diffusion of glucocorticoids into the cell; 2) glucocorticoids are subject to pre-receptor metabolism by 11β-hydroxysteroid dehydrogenase-2 (11β-HSD2); 3) binding of glucocorticoids to the cytosolic glucocorticoid receptor; 4) dissociation of the glucocorticoid receptor from chaperones; and 5) translocation of the glucocorticoid receptor to the nucleus and regulation of gene transcription in four different ways: (A) interaction with glucocorticoid response elements (GREs), (B) interaction with negative GRE (nGRE), (C) interaction with GRE and transcription factors (TFs) at the composite response elements, and (D) direct interaction with TFs independent of chromatin interaction. TF-RE, transcription factor responsive element; nGRE, negative glucocorticoid response element; GR-Iso, glucocorticoid receptor isoform; HPS, heat shock protein.

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Figure 3  Alternative splicing of exon 9 generates GRα and GRβ. GRα mRNA produces additional isoforms as a result of alternative translation initiation. Translation can be initiated at each of the AUG codons corresponding to methionines at positions 1, 27, 86, 90, 98, 316, 331, and 336 of the GR N-terminal domain resulting in a set of hGR isoforms with different lengths, sizes, and functional domains. Each GR protein is subjected to post-translational modifications by phosphorylation (P) at residues S113, S141, S203, S211, S226, and S404, SUMOylation (S) at residues K277, K293, and K703, and ubiquitination (u) at residue K417.

induced inflammatory lesions in glucocorticoid-insensitive asthmatics (5, 14, 57). Interestingly, a recent study from our group suggests a novel physiological role for hGRβ. In unstimulated U2 OS osteosarcoma cells stably expressing hGRβ, GRβ was found to regulate the expression of over 5000 genes. Thus, hGRβ can act not only as a dominant negative to hGRα but also as an endogenous manipulator of gene expression under basal conditions. Moreover, an additional 997 genes were regulated by hGRβ in the presence of the glucocorticoid receptor antagonist RU-486, suggesting that RU-486 acts as an exogenous ligand for hGRβ (58). This observation highlights the potential for development of GRβ-selective ligands.

Recently, our laboratory has revealed cell-specific expression of multiple translational hGRα isoforms (25). The GRα variant mRNA is translated into multiple GRα isoforms (GRα-A, GRα-B, GRα-C, and GRα-D) from at least eight initiation sites through mechanisms involving ribosomal leaky scanning (25). The expression pattern of endogenous translational GRα isoforms was determined using multiple glucocorticoid receptor selective antibodies. The GRα isoforms were found to be expressed in different cell lines and tissues of rats, mice, and in human primary cultures of trabecular meshwork cells (25, 59, 60). However, the relative expression levels vary considerably among tissues. For example, in spleen of CD1 mouse, the amount of GRα-D isoform was similar to GRα-A, -B, and -C isoforms, whereas in bone and in C2C12 myotube cells, the amount of GRα-D isoform is lower when compared to the other isoforms. The GRα (A–D) isoforms bind to [3H] dexamethasone and have similar apparent $K_d$ values (26) (Table 1). In the absence of glucocorticoids, the GRα isoforms, except the D isoform, localize to the cytoplasm, and as expected, after hormone treatment, the GRα translational forms A–C translocate to the nucleus (25).

Another interesting difference among the GRα isoforms lies in their transcriptional activity. The GRα isoforms display distinct transactivation or transrepression patterns on global gene expression as examined by cDNA microarray analyses using cells lines that stably express the different translational forms. Almost 500 genes in U2 OS cells are commonly regulated by the GRα isoforms and each of the isoforms regulates an additional unique set of genes (Table 1) (26). To determine the DNA binding ability of each isoform,
Table 1 Glucocorticoid receptor alpha (GRα) isoforms.

<table>
<thead>
<tr>
<th></th>
<th>GRα-A</th>
<th>GRα-B</th>
<th>GRα-C3</th>
<th>GRα-D3</th>
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<tbody>
<tr>
<td>AUG codon (25)</td>
<td>1</td>
<td>27</td>
<td>98</td>
<td>336</td>
</tr>
<tr>
<td>Translational mechanism (25)</td>
<td>Ribosomal scanning (first start codon)</td>
<td>Ribosomal leaky scanning</td>
<td>Ribosomal leaky scanning</td>
<td>Ribosomal shunting</td>
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<tr>
<td>Amino acid peptide size (25)</td>
<td>777</td>
<td>751</td>
<td>680</td>
<td>442</td>
</tr>
<tr>
<td>Molecular weight (25)</td>
<td>94 kDa</td>
<td>91 kDa</td>
<td>82–84 kDa</td>
<td>53–56 kDa</td>
</tr>
<tr>
<td>Subcellular localization (25)</td>
<td>AL = cytoplasm</td>
<td>AL = cytoplasm</td>
<td>AL = cytoplasm</td>
<td>AL = nucleus</td>
</tr>
<tr>
<td>Binding to [3H] dexamethasone Kd (nM ± SEM)</td>
<td>13.33 ± 7.6</td>
<td>11.28 ± 4.51</td>
<td>8.7 ± 1.83</td>
<td>9.06 ± 1.73</td>
</tr>
<tr>
<td>Binding to GRE in the promoter region of GC regulated genes (25)</td>
<td>ATP4 = PL</td>
<td>ATP4 = PL</td>
<td>ATP4 = PL</td>
<td>ATP4 = PL</td>
</tr>
<tr>
<td>Ligand-dependent recruitment of cofactors to the promoter region of the GZMA gene (26)</td>
<td>(–) CBP</td>
<td>(–) CBP</td>
<td>(↑) CBP</td>
<td>(↓) CBP</td>
</tr>
<tr>
<td>Transactivation activity (GRE2) (25)</td>
<td>0.81 ± 0.24</td>
<td>0.82 ± 0.14</td>
<td>0.90 ± 0.18</td>
<td>0.47 ± 0.27</td>
</tr>
<tr>
<td>Gene regulation profile (no. of genes from Agilent whole human arrays) (26)</td>
<td>3451</td>
<td>2945</td>
<td>3252</td>
<td>1761</td>
</tr>
<tr>
<td>Half-life of GR isoforms (25)</td>
<td>AL = 12–15 h</td>
<td>AL = 12–15 h</td>
<td>AL = 12–15 h</td>
<td>AL = 12–15 h</td>
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<tr>
<td>PL = 6 h</td>
<td>PL = 6 h</td>
<td>PL = 6 h</td>
<td>PL = 6 h</td>
<td></td>
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<tr>
<td>Cell expression (human) (25, 58)</td>
<td>HeLa, A549, CEM cells and primary trabecular meshwork cells</td>
<td>HeLa, A549, CEM cells and primary trabecular meshwork cells</td>
<td>HeLa, A549, CEM cells and primary trabecular meshwork cells</td>
<td>HeLa, A549, CEM cells and primary trabecular meshwork cells</td>
</tr>
<tr>
<td>Induction of apoptosis (dexamethasone 100 nM–48 h) (26)</td>
<td>(–) Propidium iodide positive</td>
<td>(–) Propidium iodide positive</td>
<td>(↑) Propidium iodide positive</td>
<td>(↓) Propidium iodide positive</td>
</tr>
<tr>
<td>(–) Annexin positive and PI negative</td>
<td>(–) Annexin positive and PI negative</td>
<td>(↑) Annexin positive and PI negative</td>
<td>(↓) Annexin positive and PI negative</td>
<td>(↓) Annexin positive and PI negative</td>
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<tr>
<td>(–) Activated PARP</td>
<td>(–) Activated PARP</td>
<td>(↑) Activated PARP</td>
<td>(↓) Activated PARP</td>
<td>(↓) Activated PARP</td>
</tr>
<tr>
<td>(–) DNA degradation</td>
<td>(–) DNA degradation</td>
<td>(↑) DNA degradation</td>
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<tr>
<td>(–) Caspase activation</td>
<td>(–) Caspase activation</td>
<td>(↑) Caspase activation</td>
<td>(↓) Caspase activation</td>
<td>(↓) Caspase activation</td>
</tr>
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</table>

AL, absence of ligand; PL, presence of ligand; –, no change related to wild type; ≠, significant increase related to wild type; x, significant decrease related to wild type; ATP4, adenosine 5'-triphosphate 4; VDR, vitamin D receptor; I-κBa, nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, alpha; CASP6, caspase 6; GZMA, granzyme A; CBP, cAMP-responsive element (CRE) binding protein (CREB)-binding protein; ₪H4, acetylated histone H4; ₪Pol II, RNA polymerase II; PI, propidium iodide; PARP, poly (ADP-ribose) polymerase.

chromatin immunoprecipitation (ChIP) assays using GRα-selective antibodies were performed. Results indicate that upon ligand binding the GRα (A–D) isoforms have similar binding affinities for the GREs found in the ATPase, H+/K+ exchanging, alpha polypeptide (ATP4A), vitamin D receptor (VDR), nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (I-κBa), caspase-6 (CASP6), and granzyme A (GZMA) genes. However, the
GRα-D isoform was found to bind to certain promoters (e.g., VDR and IκBα) even in the absence of ligand. In addition, another subset of ChIP assays revealed that the individual GRα isoforms differ in their ability to recruit cofactors, modify chromatin, and recruit active RNA polymerase, and these differences seem to be gene specific. For example, significantly higher amounts of CBP and p300 were recruited to the GZMA promoter region by the GRα-C isoform (26). Thus, these experiments provide important evidence suggesting that the GRα isoforms are inherently different proteins that differentially transduce glucocorticoid hormone signals.

**Selective regulation of apoptosis by GR isoforms**

The physiological consequence of differential expression of GRα isoforms was evaluated in a model of dexamethasone-induced apoptosis in U2 OS cells stably transfected with the individual translational GRα isoforms. Cell death and markers of apoptosis such as the externalization of phosphatidyl serine, PARP cleavage, DNA degradation, and caspase activity, were induced by hormone in a GRα isoform-selective manner (Table 1). Cells expressing the isoform C3 have elevated levels of apoptotic markers and are more sensitive to dexamethasone-induced apoptosis when compared to the other isoforms A, B, and D3. The D3 isoform expressing cells are relatively resistant to glucocorticoid-induced apoptosis. Consistent with this observation, the GRα isoforms regulate cell death-related genes in a selective manner. Furthermore, many of the genes involved in apoptosis, such as caspase-6 and -7, tumor necrosis factor (TNF) receptor superfamily members, and caspase recruitment domain apoptotic genes are regulated by at least one of the translational GRα isoforms (26). These novel findings suggest the GRα isoforms display distinct apoptotic-inducing activities by selectively regulating genes involved in apoptosis and strengthen the idea of tissue-specific expression of GRα isoforms as a general mechanism defining target-tissue sensitivity to glucocorticoids.

**Post-translational modifications**

Most proteins undergo post-translational modifications, which are known to play a role in normal physiological processes and the progression of many diseases. Although glucocorticoid receptors are hormone activated transcription factors, their expression and many of their activities can also be highly regulated by post-translational modifications, including phosphorylation, ubiquitination, and sumoylation. The generation of multiple receptor isoforms by alternative splicing and alternative translation accompanied by post-translational modification exponentially increases the complexity in glucocorticoid signaling. Taken together, multiple processes probably regulate the structure/biological function of the glucocorticoid receptor.

Protein phosphorylation is a widespread mechanism for controlling the activity, structure, and cellular localization of many proteins. One-third of the 100,000 or so proteins in a typical mammalian cell are thought to be phosphorylated at any given time (61). Phosphorylation of the glucocorticoid receptor is an important modification, potentially affecting the glucocorticoid receptor protein structure, stability, nuclear localization, and transcriptional activity (62–65). There are at least six serine residues located in or near the AF-1 transactivation domain at the N-terminus of the hGR that can be phosphorylated (S113, S141, S203, S211, S226, and S404) and eight phosphorylation sites were identified at serines 122, 150, 212, 220, 234, 315, and 412 and at threonine 159 in the mouse glucocorticoid receptor (66, 67). The glucocorticoid receptor is partially phosphorylated in the absence of glucocorticoid; however, phosphorylation increases upon ligand binding. Several protein kinases such as mitogen-activated protein kinase (MAPK), cyclin-dependent kinase (CDK), glycogen synthase kinase-3 (GSK-3), and c-Jun N-terminal kinase (JNK) (66, 68, 69) are responsible for the phosphorylation of glucocorticoid receptor and each kinase has distinct preferences for potential residues.

The phosphorylation status of the glucocorticoid receptor alters its transcriptional activity at some genes but not others. For example, mutation of five serine residues did not alter the ability of glucocorticoid receptor to transactivate a GRE-TK promoter in COS-7 cells but decreased transcriptional activity of the glucocorticoid receptor at the GRE-TATA promoter. These data suggest that the role of glucocorticoid receptor phosphorylation is dependent on the promoter context of the target gene (70). Furthermore, the phosphorylation status of the glucocorticoid receptor can impact transcriptional activity due to alterations in the recruitment of coactivators and corepressors. Utilizing a modified yeast two-hybrid approach, Hittelman and colleagues (71) identified the tumor susceptibility gene 101 (TSG101) and the vitamin D receptor-interacting protein 150 (DRIP150) as proteins that interact specifically with a functional glucocorticoid receptor AF-1 surface. In yeast and mammalian cells, TSG101 represses whereas DRIP150 enhances glucocorticoid receptor AF-1-mediated transactivation. Thus, the phosphorylation status of residues within the AF-1 transactivation domain can significantly impact cofactor recruitment. As the D isoform is missing all of the phosphorylation sites described so far (25), we speculate that phosphorylation and recruitment of coregulators could contribute to the functional differences observed between the GRα translational isoforms.

The ubiquitin-proteasome pathway regulates the glucocorticoid signaling system by controlling degradation of the glucocorticoid receptor as well as other molecules in the cascade. This was first demonstrated in COS-1 cells expressing mouse glucocorticoid receptor. Pretreatment of cells with the proteasome inhibitor MG132 blocked ligand-induced glucocorticoid receptor degradation and enhanced glucocorticoid receptor transcriptional activity. Mutagenesis of lysine 426, which lies within a candidate PEST degradation motif, abrogated ligand-dependent glucocorticoid receptor down-
regulation and enhanced hormone responsiveness (72). GRα translational isoforms contain the ubiquitination site (K426), and as predicted there is no difference in the half-lives among the glucocorticoid receptor isoforms. The half-life of the GRα isoforms dropped from 12 to 15 h in the absence of ligand to 6 h in the presence of glucocorticoids (26).

Post-translational modification of the glucocorticoid receptor can also be accomplished by addition of a small ubiquitin-related modifier-1 (SUMO-1). Three consensus sumoylation sites have been identified within the glucocorticoid receptor peptide sequence (K277, K293, and K703). The first two reside in the N-terminal transactivation region, whereas the third lies within the ligand-binding domain of the glucocorticoid receptor. The two N-terminal sites are the major acceptor sites for SUMO-1 attachment. Mutation of these sites enhances transcriptional activity of the glucocorticoid receptor on minimal promoters, but has no clear effect on the more complex mouse mammary tumor virus promoter (73). Once again, the GR-D isoform (shortest form of the glucocorticoid receptor) does not contain two of the three sumoylation sites, suggesting that these sites could also contribute to differences observed among the glucocorticoid receptor isoforms in cell signaling and biological effects.

**Concluding remarks**

The therapeutic effects of glucocorticoids have been known and exploited for more than 60 years. They represent standard therapy for reducing inflammation and immune activation in many pathological conditions such as asthma, rheumatoid arthritis, collagen, vascular, dermatological, and inflammatory bowel diseases. However, long-term treatment with glucocorticoids is limited because they induce several severe side effects. Furthermore, endogenous glucocorticoids are also implicated in the etiology of other common diseases, such as hypertension, obesity, type II diabetes, and depression. With rapid advances in our knowledge of the human genome, the development of new techniques and the increase in our understanding of cell signaling, considerable progress has been achieved in the past decade elucidating the molecular mechanisms that mediate glucocorticoid effects. It appears that there are multiple mechanisms at each step of the glucocorticoid receptor signaling cascade through which target cells alter sensitivity and specificity of the response to glucocorticoids. A recent discovery by our laboratory suggests that the N-terminal domain of the glucocorticoid receptor substantially contributes to this regulatory diversity. The GRα isoforms were found to have the ability to regulate a unique set of genes in response to glucocorticoids and to selectively regulate apoptosis. In addition, the relative levels of these isoforms were shown to differ among tissues, suggesting that cellular expression levels and availability of coactivators and corepressors also influence the transcriptional activity of the glucocorticoid receptor by directly affecting formation of the transcription initiation complexes accumulated on target genes promoters. Thus, we propose another level of regulation in glucocorticoid receptor signaling that involves the expression of a rich repertoire of unique glucocorticoid receptor isoforms. Owing to the complex pattern of tissue-specific isoform expression, mapping protein interactions and transactions (such as post-translational modifications and degradation) within a cell or organism is essential to developing a molecular understanding of physiology and to the discovery of new potential therapeutic targets.

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