# Tissue-specific glucocorticoid action: a family affair

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## Abstract

Glucocorticoids exert a wide variety of physiological and pathological responses, most of which are mediated by the ubiquitously expressed glucocorticoid receptor (GR). The glucocorticoid response varies among individuals, as well as within tissues from the same individual, and this phenomenon can be partially explained through understanding the process of generating bioavailable ligand and the molecular heterogeneity of GR. This review focuses on the recent advances in our understanding of prereceptor ligand metabolism, GR subtypes and GR polymorphisms. Furthermore, we evaluate the impact of tissue- and individual-specific diversity in the glucocorticoid pathway on human health and disease.

## Introduction

Glucocorticoids were named for their ability to promote the conversion of proteins and lipids into glucose during the stress-induced activation of the hypothalamic–pituitary–adrenal (HPA) axis [1]. In addition to this role in intermediary metabolism, glucocorticoids regulate numerous tissue-specific activities including immune function, the inflammatory response, embryogenesis, behavior, and cell proliferation and survival. The pleiotropic effects of glucocorticoids provide an abundance of opportunities for pharmacological exploitation. Indeed, synthetic glucocorticoid agonists are one of the most widely prescribed classes of drugs worldwide and are indispensable in the treatment of autoimmune diseases, inflammatory disorders and cancer [2,3]. However, the physiological and supraphysiological response to glucocorticoids is not uniform, differing not only among individuals but also within tissues of the same individual.

The individual- and tissue-specific nature of the glucocorticoid response can be illustrated by examining the effect of glucocorticoids in the treatment of acute lymphoblastic leukemia (ALL). Glucocorticoids are routinely included in chemotherapeutic regimens for ALL because of their ability to induce lymphocyte apoptosis, thereby inhibiting the expansion of cells that have escaped the normal constraints of the cell cycle [4]. Unfortunately, subpopulations of individuals undergoing glucocorticoid-based therapy harbor cells that are resistant to glucocorticoid-induced apoptosis and fail to respond to treatment [4,5]. Thus, glucocorticoids do not offer a therapeutic advantage in all cases of ALL. Although glucocorticoids promote lymphocyte apoptosis, they protect against apoptosis in the endometrium, ovarian follicle, hepatocyte, fibroblast and mammary epithelium [6]. Due to this tissue specificity, glucocorticoids are not useful in treating all malignancies [7]. The therapeutic response to glucocorticoid therapy is further complicated by the fact that individuals undergoing chronic treatment commonly develop dose-limiting side-effects, such as an increased risk of hypertension, truncal obesity, bone fracture and osteoporosis [8,9]. These adverse

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pharmacological effects likely represent an exaggeration of the physiological response. For example, the aberrant induction of apoptosis in osteocytes and osteoblasts plays a part in glucocorticoid-induced osteoporosis [8]. The variability in the glucocorticoid response with respect to resistance, tissue-specificity and side-effects presents a challenge for researchers and clinicians and has prompted the design of selective, safer synthetic glucocorticoids that evade resistance [9].

The physiological and pharmacological actions of glucocorticoids are predominantly mediated through the ubiquitously expressed glucocorticoid receptor (GR). To date, only one GR gene has been identified in all species examined. How can one gene mediate such diverse responses? This review focuses on recent advances in our understanding of prereceptor ligand metabolism and glucocorticoid signaling through the heterogeneous GR. These molecular mechanisms have the potential to explain the etiology of disorders such as obesity and the variability in the glucocorticoid response.

## Ligands, receptors and genes

### Prereceptor ligand metabolism

Cortisol (the most abundant, endogenous glucocorticoid in man) is synthesized by the adrenal gland and transported in the blood predominantly bound (~80%) to corticosteroid-binding globulin (CBG) [10]. In addition to facilitating cortisol distribution, CBG has a role in tissue-specific cortisol release [10]. Several lines of evidence indicate that cortisol delivery to precise microenvironments is accomplished through human-leukocyte-elastase-mediated CBG cleavage or membrane-bound CBG receptors, both of which stimulate the release of cortisol from CBG [10]. CBG-free cortisol readily diffuses across cellular membranes to exert its effect.

Cortisol is crucial for maintaining physiological homeostasis, as exemplified by phenotypes associated with cortisol dysregulation. For example, tumors of the adrenal gland commonly produce excess cortisol, causing a pathological condition known as Cushing's syndrome, which is characterized by visceral obesity and metabolic disease (type-2 diabetes, insulin resistance, dyslipidemia and hypertension) [11]. The detrimental effect of cortisol excess is attributed to the induction of multiple metabolic and hemodynamic pathways in a variety of tissues, such as liver, kidney and adipocytes [11,12]. Although similar features exist between Cushing's syndrome and the more common metabolic syndrome, metabolic syndrome seems to occur in the absence of systemic cortisol excess [11,12]. This observation gave rise to the suggestion that intracellular cortisol excess, which is not detected by conventional methods, might contribute to the development of metabolic syndrome [11,12].

Upon diffusion into the cytoplasm, the intracellular bioavailability of cortisol is controlled by a mechanism that has been termed 'prereceptor ligand metabolism'. 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) catalyzes the conversion of cortisol to cortisone, the inactive glucocorticoid metabolite, whereas 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts cortisone to cortisol [13] (Figure 1a). Thus, the relative levels of 11β-HSD enzymes are important factors in determining the intracellular concentration of cortisol. The major role of 11β-HSD2 is to prevent cortisol from gaining access to high-affinity mineralocorticoid receptors and, therefore, is predominantly expressed in the mineralocorticoid-responsive cells of the kidney [12,14]. By contrast, 11β-HSD1 ensures intracellular cortisol bioavailability in the glucocorticoid-responsive metabolic tissues of the liver, fat, the lung and the central nervous system [12,15]. This cell-type-specific expression pattern reflects selective activation of the 11β-HSD promoters and is important for fine-tuning glucocorticoid responses [16].

Because cortisol has a crucial role in maintaining homeostasis, inappropriate tissue-specific alterations in 11β-HSD enzymes might have potentially detrimental effects on physiological processes. Indeed, recent studies support initial data that indicated a role for 11β-HSD and intracellular cortisol excess in obesity and the development of metabolic disease. For example, 11β-HSD1-knockout mice are protected from high-fat-diet-induced preadipocyte differentiation and obesity [17], and parameters of cortisone-induced diabetes are improved in 11β-HSD1-knockdown mice [18]. By contrast, transgenic mice that overexpress 11β-HSD1 specifically in the liver exhibit signs of metabolic disease, such as fatty liver, insulin resistance and hypertension, even in the absence of obesity [19]. Furthermore, mice that overexpress 11β-HSD2 specifically in adipocytes are protected from high-fat-diet- induced obesity [20]. Clinical studies in humans have found elevated levels of *11β-HSD1* mRNA [21–23], decreased levels of *11β-HSD2* mRNA [22] and increased cortisol levels [24] in adipose tissue from obese subjects when compared to lean subjects. Moreover, Engeli *et al.* [22] observed an association between high levels of *11β-HSD1* mRNA and metabolic abnormalities in obese women. Together, these findings indicate that prereceptor ligand metabolism regulates intracellular cortisol bioavailability and controls tissue-specific, endogenous glucocorticoid action. Because of the implications for the development of obesity and metabolic disease, these discoveries have prompted the design of 11β-HSD1 inhibitors for the treatment of metabolic syndrome [25].

## The glucocorticoid signaling pathway

Glucocorticoids signal through genomic and non-genomic pathways. The classic, genomic actions of glucocorticoids are mediated through cytosolic GR. GR is a member of the nuclear receptor superfamily of ligand-dependent transcription factors [26], and the α-isoform is the prototypic, most well-studied protein product of the GR gene. GRα is organized into modules that consist of a unique, immunogenic N terminus, a highly conserved central DNA-binding domain (DBD) and a C-terminal ligand-binding domain (LBD) [27]. A ligand-independent transactivation domain (AF-1) is embedded within the N terminus, whereas a ligand-dependent transactivation domain (AF-2), nuclear localization signals and protein–protein interaction domains are mapped toward the C-terminal region [27] (Figure 1b).

The mature GRα that is capable of ligand binding resides in the cytoplasm, where it exists as a multi-protein heterocomplex that contains chaperones and co-chaperones [28,29]. In the classic model of glucocorticoid signaling, ligand-activated GRα undergoes an initial conformational change that results in the exposure of its nuclear localization signal followed by importin-mediated nuclear entry [30] (Figure 1). Ligand-activated GRα influences gene transcription in various ways. For example, *cis* regulation is accomplished through a direct interaction between GRα and DNA (Figure 1c), whereas interactions between GRα and other transcription factors facilitate *trans* regulation [31] (Figure 1d). In the *cis* model of transcriptional activation, GRα homodimers bind to conserved glucocorticoid response elements (GREs) located within promoters of responsive genes and, after the recruitment of basal transcriptional machinery and cofactors, induce gene transcription. This simplistic model of transcriptional activation might not be applicable to all genes, however, because recent studies implied that GRα-DNA binding activity is not required for its synergism with the Nurr1 [32] and Stat5 [33] transcription factors (Figure 1d). The mechanism of GRα-mediated transcriptional repression seems to be more promiscuous and involves negative GREs (nGREs), composite binding sites and/or *trans* repression [31]. nGREs are low-affinity GRα-binding sites that do not exhibit a highly conserved consensus sequence, whereas composite-binding sites contain a GRE or nGRE that overlaps the binding site of another transcription factor [31]. The mechanism of GRα *cis* repression involves either a nGRE-mediated conformational change in GRα that results in GRα inactivation or interference with another transcription factor through a nGRE composite-binding site [31] (Figure 1c). It is generally

accepted that the anti-inflammatory properties of glucocorticoids involve *trans* repression and are caused by interference with AP-1- and NFκB-mediated gene transcription [2,34]. For example, GRα prevents the p65 subunit of NFκB from binding to endogenous promoters [35] through steric occlusion [36] and/or direct protein–protein interaction (Figure 1d) [37]. Recently, however, Cuzzocrea *et al.* [38] suggested a role for the peroxisome proliferator-activated receptor-α (PPAR-α), another member of the nuclear receptor superfamily, in mediating the anti-inflammatory actions of glucocorticoids. They found that the anti-inflammatory properties of the synthetic glucocorticoid dexamethasone were weakened in PPARα-knockout mice when compared to wild-type mice [38]. Furthermore, the anti-inflammatory response was increased when wild-type mice were treated with dexamethasone in combination with a PPARα agonist [38]. Therefore, the anti-inflammatory effects of glucocorticoids seem to involve crosstalk with other signaling pathways and receptors.

The development of large-scale gene-expression profiling techniques has prompted several studies examining GRα-directed gene expression. Although these studies differ with respect to cell type, ligand, microchip and *p* value used for analysis, it is evident that GRα is actively involved in the transcription, whether upregulation or downregulation, of many genes. For example, Lu *et al.* [39] observed that ~3000 out of 41 000 genes were regulated by ligand-activated GRα in U-2 OS osteosarcoma cells. Genes involved in glucose metabolism, cell adhesion, cell proliferation, cell survival, growth and development are among those identified and reflect the diverse, tissue-specific nature of glucocorticoid action.

Rapid, non-genomic glucocorticoid actions are thought to be mediated through physiochemical interactions with cellular membranes, the cytosolic GR or membrane-bound GR [40,41]. These mechanisms are just beginning to be understood and are briefly described here. The intercalation of glucocorticoids in plasma and mitochondrial membranes rapidly alters cellular function by impairing cation transport across the plasma membrane and by increasing the mitochondrial proton leak [40]. Because immune-cell function crucially depends on optimal intracellular ion balance and coupling of oxidative phosphorylation, it has been suggested that this non-genomic pathway has a role in glucocorticoid-induced immunosuppression [40]. The cytosolic GR that mediates genomic glucocorticoid actions (described previously) might also play a part in the induction of non-genomic glucocorticoid actions. Croxtall *et al.* showed that glucocorticoids stimulate the rapid release of Src kinase from cytoplasmic GR heterocomplexes, resulting in lipocortin 1 activation and the inhibition of arachidonic acid release (reviewed in Ref. [40]). Thus, glucocorticoids might exert some of their anti-inflammatory and anti-proliferative effects through the non-genomic, cytoplasmic GR-mediated inhibition of arachidonic acid release (reviewed in Ref. [40]). Many rapid glucocorticoid actions are thought to occur through membrane-bound GR, but the underlying mechanisms remain largely undefined. Recently, membrane-bound GR was identified as a component of the T-cell receptor (TCR) multi-protein complex. Glucocorticoids rapidly induced dissociation of this complex and disrupted TCR signaling [41,42]. These data provide additional insight into the non-genomic mechanism of glucocorticoid-induced immunosuppression, specifically in T cells [41,42]. Clearly, the non-genomic mechanisms of glucocorticoid action warrant further investigation because they might emerge as important targets for pharmacological manipulation.

## Glucocorticoid receptor heterogeneity

### GRβ splice variant

Alternative splicing of the GR primary transcript has been shown to generate the classic GRα prototype (described previously) and a variant termed 'GRβ' [5] (Figure 2a). These two isoforms share identical N-termini encoded by exons 2–8 and are distinguished only by their unique C-terminal LBD [5]. GRα is composed of 777 amino acids, including 50 C-terminal

residues derived from exon 9α, whereas GRβ is a shorter protein with 742 residues, the final 15 of which are unique and encoded for by exon 9β [43]. GRβ has been reported not to bind endogenous or synthetic agonists, and reporter assays indicate that GRβ regulates gene expression only by antagonizing GRα-mediated gene transcription, thereby acting as a dominant-negative GRα inhibitor [5,43–45]. However, recent data show that GRβ, when introduced into cells in the absence GRα, does in fact bind the synthetic GRα antagonist RU-486 [46]. GRβ also regulates gene expression independent of GRα, and RU-486 diminishes this capacity [46]. Moreover, in a manner similar to GRα, GRβ represses basal activity of the interleukin 5 (IL-5) and IL-13 cytokine promoters through the recruitment of histone deacetylase 1 [47]. Taken together, these data indicate that the presence of a unique C terminus exerts a profound influence on GRβ ligand selectivity and transcriptional capability [43].

In many cells and tissues examined, GRβ is expressed at low levels when compared to GRα [46]. In support of a dominant-negative function for GRβ, *in vitro* studies have indicated that reductions in the cellular GRα:GRβ ratio contribute to glucocorticoid resistance. For example, glucocorticoid insensitivity in cytokine-treated, cultured human airway smooth muscle cells is associated with GRβ upregulation [48], and increases in the GRα:GRβ ratio in human peripheral blood mononuclear cells (PBMC) are associated with methotrexate-induced glucocorticoid sensitization [49]. Although these studies indicate that perturbations in the cellular GRα:GRβ ratio impact glucocorticoid responsiveness, Torrego *et al.* showed that elevated GRβ levels were not involved in cytokine-induced glucocorticoid insensitivity in PBMCs [50]. Clinical studies have proven to be just as contradictory. Resistance to glucocorticoid therapy in patients with leukemia [51] and other diseases [52] has been associated with high cellular levels of GRβ when compared to GRα, but this relationship has not been observed in other studies [53–56]. Thus, the significance of the GRα:GRβ ratio in predicting glucocorticoid sensitivity in individuals undergoing glucocorticoid therapy remains unclear and probably depends on tissue- and/or individual-specific factors. Interestingly, several groups have identified an association between reduced GRα:GRβ ratio and mood disorders such as schizophrenia, bipolar and major depressive disorder [57,58]. In addition, polymorphisms in GRβ have been linked to increased risk of myocardial infarction and coronary heart disease (see the section entitled 'Polymorphisms') [59]. These provocative findings warrant further investigation.

## Translational isoforms

Recently, alternative translation initiation of the mature GR transcript has been recognized as an additional mechanism for generating cellular heterogeneity within human GRα [60,61] (Figure 2b). Internal 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, GRα-C1, GRα-C2, GRα-C3, GRα-D1, GRα-D2 and GRα-D3, respectively [60,61]. Translation initiation from the AUG codon corresponding to methionine 1 yields the full-length classic GR protein that is now referred to as GRα-A (Figure 2a). The translational isoforms only differ in the length of the N terminus, which contains the AF-1 transactivation domain. Importantly, all GRα translational isoforms are expressed in various tissues from the rat and mouse, but differences in expression levels are noted [60]. This indicates that alternative translational initiation operates *in vivo* and gives rise to tissue-specific GRα-isoform-expression patterns. Additional complexity and diversity in GR-isoform expression is implicated by the potential for the similar alternative translation initiation of GRβ transcripts (Figure 2b).

To determine whether functional differences exist between these newly identified GRα isoforms, U-2 OS osteosarcoma cells stably expressing the individual translational forms were generated [60]. All receptor isoforms resided in the cytoplasm in the absence of glucocorticoids

and translocated to the nucleus in a ligand-dependent manner, with the exception of GRα-D isoforms, which were constitutively localized to the nuclear compartment [60]. GRα-D proteins were further distinguished by their relatively low transcriptional activity in reporter assays when compared to the other isoforms [60]. GRα-C3 displayed the highest transcriptional activity, whereas GRα-A, GRα-B, GRα-C1 and GRα-C2 were intermediate [60]. In agreement with these data, large-scale gene expression profiling analysis revealed a similar hierarchy of glucocorticoid-induced endogenous gene regulation by these receptor isoforms; however, isoform-specific genes were also identified [60]. Interestingly, expression of the more active GRα-C3 correlated with increased sensitivity to glucocorticoid-induced apoptosis, and the relatively inactive GRα-D3 was associated with resistance to glucocorticoid-induced apoptosis [39]. Because glucocorticoid-induced apoptosis is a double-edged sword, being beneficial for the treatment of malignancies while promoting the development of osteoporosis [9], it will be valuable to determine whether alterations in expression among the GR translational isoforms contribute to tissue sensitivity and adverse side-effects of glucocorticoids.

## Polymorphisms

Polymorphisms in the gene encoding for GR have been associated with variations in GR function and have the potential to explain individual-specific differences in health, disease and response to glucocorticoid therapy. The ER22/23EK polymorphism that occurs in ~3% of the population is found in exon 2 and results in an arginine (R) to lysine (K) change at position 23 (R23K) within the N terminus [62] (Figure 2c). ER22/23EK is associated with decreased GR transcriptional activity in reporter assays and decreased expression of endogenous genes when compared to wild-type GR [63]. Thus, it has been suggested that the ER22/23EK polymorphism might be associated with glucocorticoid insensitivity. Indeed, early studies identified a link between ER22/23EK and relative glucocorticoid resistance and, more recently, Russcher *et al.* [64] uncovered an association between the ER22/23EK polymorphism and increases in the ratio of GRα-A to GRα-B. When compared to wild-type GR, the ER22/23EK polymorphism facilitated the expression of GRα-A but had no effect on the expression of GRα-B [64]. Because some studies have shown that GRα-A is less transcriptionally active than GRα-B, it has been hypothesized that glucocorticoid insensitivity in individuals harboring the ER22/23EK polymorphism is due to decreased relative levels of GRα-B [64]. It is important to note that the ER22/23EK polymorphism was not associated with glucocorticoid resistance in children with ALL [65]. However, 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 [66]. Furthermore, elderly men carrying the ER22/23EK polymorphism had better 4-year survival rates and lower levels of C-reactive protein, which has been implicated in cardiovascular disease [66,67]. Thus, these data indicate that ER22/23EK carriers might be relatively insensitive to glucocorticoids and, as a result, have a more favorable metabolic profile.

The GRβ polymorphism A3669G that is located within the 3′ untranslated region (Figure 2c) results in increased stability of *GRβ* mRNA and the enhanced expression of GRβ protein. By some accounts, stabilization of the dominant-negative GRβ is associated with favorable metabolic parameters [68]. Approximately 27.6% of the European population investigated were heterozygous for A3669G that was linked to decreased incidence of central obesity in women and decreased total cholesterol concomitant with increased high-density lipoprotein in men [68]. This observation is controversial because it was not reported in other similar studies or in A3669G carriers from South Asia [68,69]. Thus, A3669G might not be a reliable predictor of metabolic profiles across all ethnic groups. Interestingly, A3669G is less capable of *trans*-repressing the NFκB-regulated gene IL-2 than wild-type GRβ [69]. Moreover, A3669G is associated with reduced immunosuppression. For example, individuals harboring A3669G have a higher incidence of the autoimmune disease rheumatoid arthritis and a reduced risk of

*Staphylococcus aureus* nasal infection [70]. In a more recent study, homozygous carriers of A3669G were associated with a pro-inflammatory phenotype that included an increased risk of myocardial infarction and coronary heart disease [59]. Therefore, the GRβ polymorphism A3669 might prove to be an important risk factor for disease.

The N363S polymorphism that occurs in ~4% of individuals is located within the GR N terminus and is encoded for by exon 2 [62] (Figure 2c). N363S results in modest increases in GR transcriptional activity [63] and is associated with generalized increases in glucocorticoid sensitivity [66]. Interestingly, microarray analysis revealed a unique, polymorphism-specific pattern of gene regulation for N363S when compared to wild-type GRα [71]. Moreover, some reports indicate that N363S is associated not only with glucocorticoid hypersensitivity but also with an increased body mass index [72], coronary artery disease [73] and decreased bone mineral density [66]. However, the link between N363S and certain parameters of disease remains controversial [66,74].

The *Bcl*I variant is a restriction fragment length polymorphism that is located within intron 2 (Figure 2c) and occurs in ~37% of individuals [62]. *Bcl*I is associated with glucocorticoid hypersensitivity, a lower susceptibility to developing a severe form of an autoimmune disorder of the eye, and abnormalities in metabolic parameters [66,75]. Although both the N363S and *Bcl*I variants contribute to increased glucocorticoid sensitivity, they are not thought to offer any therapeutic advantage in individuals undergoing systemic glucocorticoid therapy for hematological malignancies [65]. Furthermore, the effect of N363S and *Bcl*I variants on dose-limiting side-effects of glucocorticoid therapy, such as osteoporosis, has not been thoroughly investigated. Controversial data exist regarding the effect of *Bcl*I on metabolic parameters (see following paragraph) [66], but individuals harboring both the *Bcl*I and N363S polymorphism tend to have higher blood pressure and cholesterol levels [72].

In a population-based study in northeast England, heterozygotes for another polymorphism within intron 2, rs2918419 (T→C), were identified in ~25.8% of subjects (Figure 2c) [76]. Individuals with the rs2918419 variant allele also had variant alleles at the *Bcl*I locus, and the dual polymorphism was associated with insulin resistance in men but not women [76]. Interestingly, the previously reported detrimental effect of the *Bcl*I polymorphism was not observed in this study in the absence of rs2918419 [76]. Therefore, the association of *Bcl*I with insulin resistance and obesity might crucially depend on the presence of rs2918419. Similarly, a *TthIII*I polymorphism that is located within the GR promoter (Figure 2c), 3807 base pairs upstream of the transcriptional start site, was only associated with glucocorticoid resistance and healthy metabolic profile in the presence of ER22/23EK [66,77]. Even though these data paint a complex picture regarding the role of GR polymorphisms in determining individual-specific responses to endogenous and synthetic glucocorticoids, they imply that the primary sequence of the GR gene might be an indicator of disease, prognosis and side-effects.

## Concluding remarks

Levels of circulating cortisol are controlled systemically by the HPA axis and locally by the action of 11β-HSD enzymes. This dual regulation ensures the maintenance of glucocorticoid homeostasis. Perturbations of either of these systems contribute to the development of diseases of metabolic origin. Heterogeneity within the GR is emerging as an additional mechanism for modulating endogenous and exogenous glucocorticoid responses. Alternative processing of the GR gene has the potential to explain tissue-specific glucocorticoid responses. Individual-specific heterogeneity manifests as polymorphisms within the GR and might predispose individuals to disease in addition to influencing their responses to glucocorticoid therapy. A more in-depth understanding of the molecular diversity of GR will aid in the development of tailored, individualized glucocorticoid therapies.

## Acknowledgements

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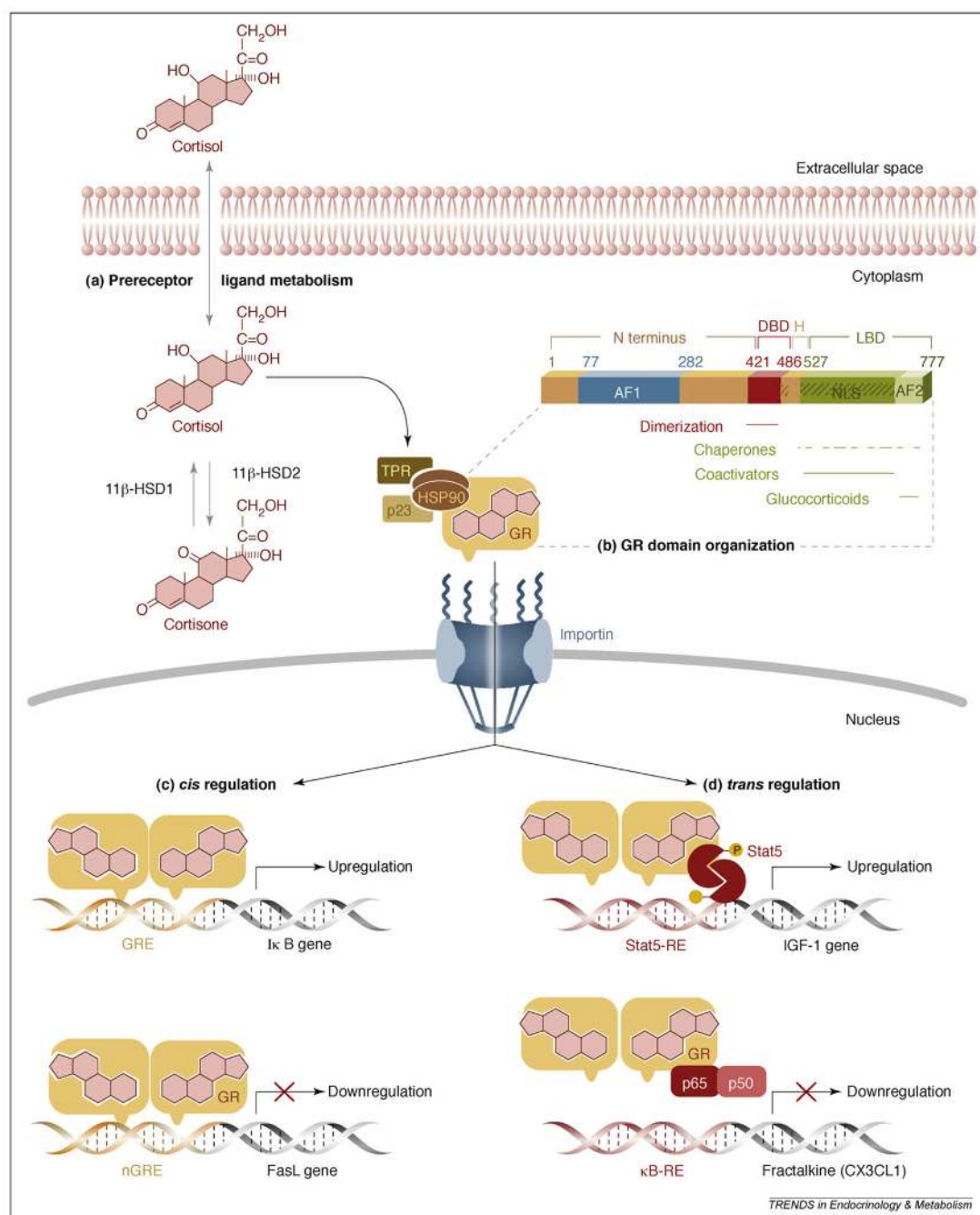
**Figure 1.**
The GR signal transduction pathway. (**a**) Corticosteroid-binding globulin-free cortisol diffuses into the cell, where it is either inactivated by 11β-HSD2 or bound by the cytosolic GR heterocomplex to initiate signal transduction. (**b**) GR is a modular protein that is composed of an N-terminal transactivation domain (AF-1), a central DNA-binding domain (DBD), a hinge region (H) and a C-terminal ligand-binding domain (LBD). A ligand-dependent transactivation domain (AF-2), nuclear localization signals (NLS) and protein–protein interaction domains are also found within the LBD. The GR heterocomplex contains the chaperone heat-shock protein 90 (hsp90) that is associated with co-chaperones such as p23 and one of several tetratricopeptide repeat (TPR) domain-containing proteins [28,29]. Ligand binding induces

molecular rearrangement of the GR complex and importin-mediated nuclear translocation. (**c**) *Cis* regulation of gene expression involves a direct interaction between ligand-activated GR homodimers and DNA. GR induces inhibitory κB (IκB) gene expression by interacting with glucocorticoid response elements (GREs) and represses Fas ligand (FasL) gene transcription by interacting with negative GREs (nGREs) [36]. (**d**) *Trans* regulation of gene expression involves the tethering of GR to other transcription factors. Ligand-activated GR interacts with DNA-bound Stat5 to co-activate insulin-like growth factor-1 (IGF-1) transcription from the Stat5-response element (Stat5-RE) [33]. However, ligand-activated GR interacts with the p65 subunit of NFκB [37] and reduces recruitment of p65 to the κB-response element (κB-RE), thereby inhibiting NFκB-mediated fractalkine (CX3CL1) gene transcription [35].
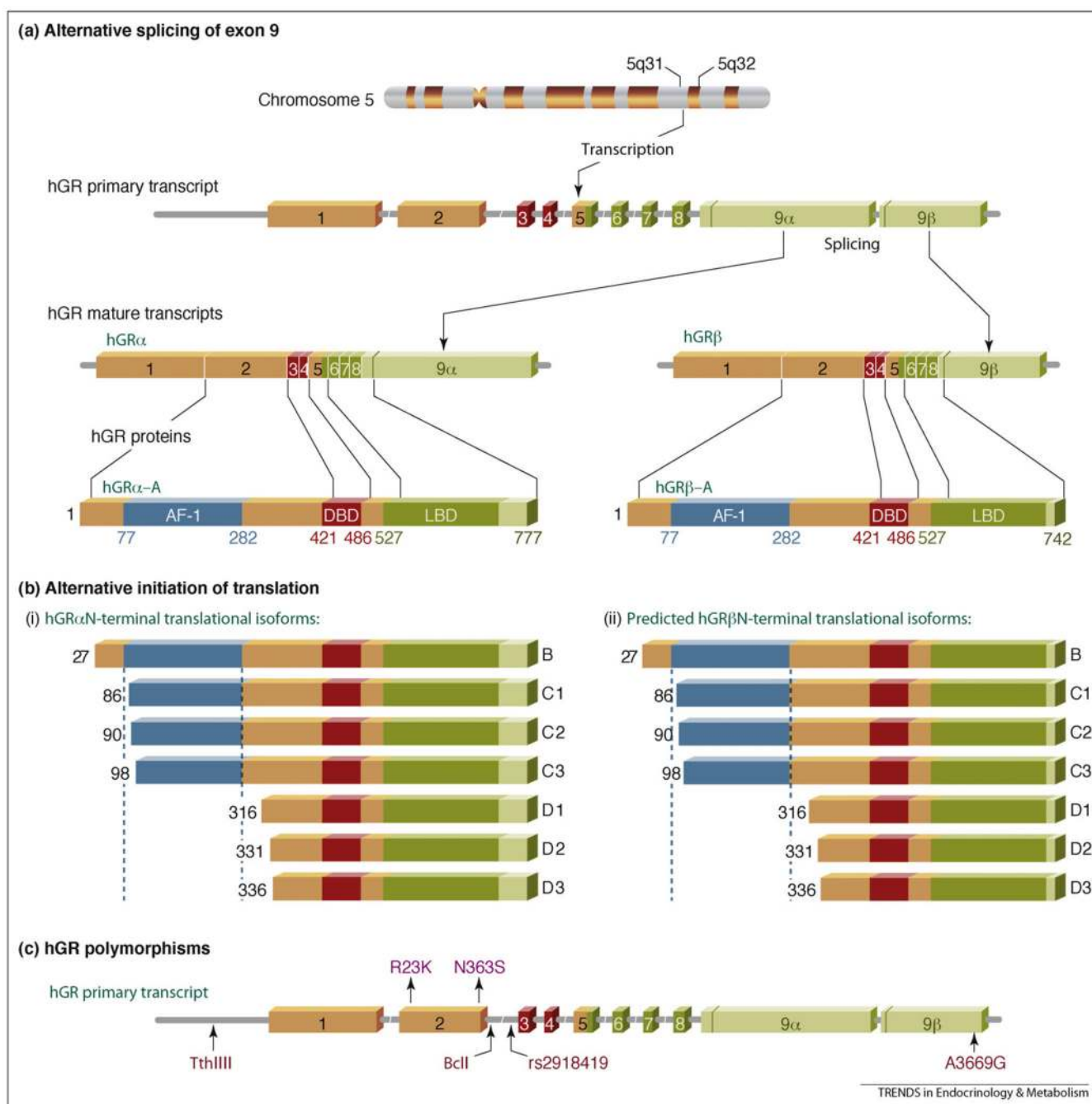
**Figure 2.**
Molecular heterogeneity of GR. (**a**) Human GR (hGR) is located on chromosome 5 (5q31–32). Alternative splicing of the primary GR transcript yields the GRα-A and GRβ-A protein forms, which differ only at the extreme C terminus. (**b**) Alternative initiation of translation of GRα and GRβ. (i) Initiation of translation at internal AUG codons gives rise to the N-terminal translational isoforms GRα-B, GRα-C1, GRα-C2, GRα-C3, GRα-D1, GRα-D2 and GRα-D3. (ii) Alternative translation initiation is predicted to occur for GRβ as well. (**c**) Positions of GRα and GRβ polymorphisms are shown. Polymorphisms that do not result in amino acid changes are shown below the primary transcript, whereas the polymorphisms that are located in exons and result in amino acid changes are shown above the primary transcript. The

corresponding amino acid substitutions are indicated. This figure was adapted, with permission, from Ref. [78].