Molecular mechanisms of glucocorticoid action

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Glucocorticoids act on target cells via two isoforms of intracellular glucocorticoid receptors (GRs) called GRα (hormone-binding form) and GRβ which does not bind any glucocorticoids. A cycle of Hsp90 chaperone and co-chaperones maintains the functional status of GRs and their recycling. The binding of a glucocorticoid hormone to GRα leads to its activation and translocation from the cytoplasm to nucleus. Glucocorticoids influence activities of genes of target cells using two different types of mechanism. In type-1 mechanism, the hormone-bound GRα undergoes homodimerization (GRα/GRα) or heterodimerization (GRα/GRβ). A homodimer binds directly to a glucocorticoid response element (GRE) and activates gene transcription. Binding of a heterodimer (GRα/GRβ) to GRE induces only partial transactivation, because GRβ inhibits the hormone-induced, GRα-mediated stimulation of gene expression by specifically inhibiting GRE-mediated transcription. In some cases, a hormone–GRα complex binds to a negative GRE (nGRE) and suppresses transcription (transrepression). In type-2 mechanism, a hormone-GRα complex binds to other transcription factors (TFs) and inhibits the transcription of target genes stimulated by the TFs. GRα-induced transactivation and transrepression of genes may also involve several transcription co-activators and dominant negative inhibitors respectively. The present article provides critical information on structure and types of GRs, GR gene, chaperone cycle, and details of different types of mechanism of glucocorticoid actions.

The adrenal gland secretes corticosteroids and catecholamine hormones, which play a vital role in general adaptation to ever-changing environment and emergency situation, and help vertebrates in their successful survival. The adrenal cortex produces a large number of corticosteroids that are released into the circulation in response to a wide range of stressful stimuli. Based on their biological effects, these hormones are classified as glucocorticoids (e.g. cortisol, corticosterone, cortisone, etc.) and mineralocorticoids (e.g. aldosterone). Glucocorticoids are involved in the regulation of carbohydrate metabolism, protein metabolism, lipid metabolism, oxidative metabolism, electrolyte balance, anti-inflammatory actions, immunosuppression, anti-tumour promoting activity, apoptosis, general adaptation, reproduction, growth, etc. (Figure 1). Since glucocorticoids are essential for normal physiology and successful survival of vertebrates, it is essential to understand the molecular mechanism of their actions. During the last one decade, significant progress has been made in understanding the molecular basis of glucocorticoid action. In this article, an attempt has been made to summarize the recent findings related to the structure of glucocorticoid receptor (GR), types of GR, role of chaperones in GR cycle, activation and translocation of GR and the details of mechanisms of glucocorticoid action at the DNA level.

Glucocorticoid receptor

Glucocorticoid hormones produce their effects by activating specific cytoplasmic GRs. A GR is a ~ 94 kDa intracellular phosphoprotein, which belongs to a phylogenetically conserved super-family of steroid hormone receptors. In the ligand-bound state, these receptors specifically bind to and modulate the activity of target gene promoters. On the basis of the amino acid similarities in different portions of the protein, a typical glucocorticosteroid receptor can be divided into six regions – A, B, C, D, E and F. All members of the super-family share three characteristic functional and structural domains, which were first described for GR (Figure 2). The most variable N-terminal domain (A/B region) contains sequences responsible for activation of target genes and interacts with the components of the basal transcription machinery or with other transcription factors. Two highly conserved α-helical loops called ‘zinc fingers’ in the central portion of the receptor molecule constitute the highly conserved 86-amino acid DNA-binding domain (C region). The DNA-binding domain also participates in receptor dimerization, nuclear translocation, and transactivation.

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Figure 1. Major actions of glucocorticoid in mammals.
Each zinc finger at its base contains a zinc ion, which is tetrahedrally coordinated to four precisely oriented cysteine residues\(^\text{12,13}\). The two zinc fingers exhibit different roles: one of the fingers, called the ‘recognition helix’, projects into the major groove of the target DNA, where it recognizes and binds to the glucocorticoid response element (GRE) sequence, while the other finger mediates binding to another GR to form a receptor homodimer\(^\text{14}\). The function of the moderately conserved C-terminal or hormone-binding domain (D/E/F region) is even more complicated. In addition to specifically binding the hormonal ligand\(^\text{15,16}\), it contains sequences critical for the binding of heat shock proteins\(^\text{17,18}\), nuclear translocation\(^\text{19}\), dimerization, transactivation\(^\text{19,20}\), and sequences for silencing the receptor in the absence of hormone\(^\text{21}\).

**Types of GR**

There are two isoforms of GR generated from a single paternal GR gene. The GR gene contains a total of ten exons and spans \(\approx 110\) kilobases, with a minimum size of 80 kilobases. Exon 1 consists solely of 5’-untranslated sequence, and exon 2 encodes the amino-terminal portion of the receptor. The putative zinc fingers are separately encoded by two exons. A total of five exons combine to form the cortisol-binding domain. A single promoter has been described for the human GR gene. However, expression of the GR gene in mouse seems to be controlled by at least three promoters, resulting in GR transcripts with different 5’-nontranslated exons designated as exons 1A (restricted to T cell line), 1B and 1C (active to different degrees in all tissues analysed so far). GR gene expression is regulated in a complex tissue-specific manner, particularly by early-life environmental events that programme tissue GR levels. Several rat GR mRNAs reportedly encode a common protein, but differ in their 5’-leader sequences as a consequence of alternate splicing of eleven different sequences of exon 1. Alternative splicing of the 3’-end of the primary transcript of the human GR gene gives rise to a hormone-binding splice variant 94 kDa GRα composed of 777 amino acid residues, and the wild-type receptor 91 kDa GRβ having 742 amino acid residues\(^\text{22,23}\). Compared to GRα, GRβ is widely expressed in adult and foetal human tissues, but differs from GRα in that it is localized in the cytoplasm as well as in the cell nucleus, independent of the presence of ligand\(^\text{24,25}\).

These two receptor isoforms have the first 727 amino acids in common, and thus, both possess the transactivation and the DNA-binding domains. GRβ is identical to GRα through the first 727 amino acids, but differs from GRα only in its C-terminus, with replacement of the last 50 amino acid residues having unique additional 15 non-homologous amino acid sequence. This variation renders GRβ unable to bind glucocorticoids, to get translocated to the nucleus in the absence of GRα, and to be transcriptionally active on a glucocorticoid-responsive enhancer.

However, GRβ is capable of binding to GR α and forms homodimers as well as heterodimers with GRα. GRβ can also associate with the heat shock protein (Hsp90), although with lower affinity than GRα.\(^\text{27}\) Further, glucocorticoid treatment enhances GRα, but not GRβ binding to DNA. Recent studies using various mammalian tissues and cell lines have revealed an additional, slightly smaller, second hGR protein (molecular mass = 91 kDa) that is not recognized by antibodies specific for the transcriptionally inactive and dominant negative, non-hormone-binding hGRβ isoform. When a single GR cDNA is transfected in COS-1 cells, or transcribed and translated in vitro, two forms of the receptor are observed, similar to those seen in cells that contain endogenous GRα. On the basis of these findings, it has been suggested that two forms of the hGRα are produced by alternative translation of the same gene and are termed hGR-A and hGR-B. Further, functional analyses of hGR-A and hGR-B under various glucocorticoid-responsive promoters have revealed that the shorter hGR-B is nearly twice as effective as the longer hGR-A species in gene transactivation, but not in transrepression.

Transactivation and transrepression activities of glucocorticoids are mediated by GRα\(^\text{28}\). Formation of transcriptionally impaired GRα/GRβ heterodimer is an important component of the mechanism responsible for the inhibitory effects of glucocorticoids\(^\text{25,29}\) (Figure 3). Transfection experiments with MMTV–CAT reporter plasmid (pGMCS) and either pCMV5, pCMVhGRα or pCMVhGRβ in COS-1 cells and CV-1 cells have revealed that in the absence of GRα, GRβ is transcriptionally inactive on the glucocorticoid-responsive MMTV enhancer\(^\text{26}\). CAT expression remained unchanged in the hGRβ-containing COS-1 cells treated with dexamethasone. Similarly, in GRα receptor negative CV-1 cells transfected with hGRβ, CAT expression was not changed following treatment with dexamethasone. However, in HeLa S1 cells (which contain approximately 20,000 hGRα receptors per cell) transfected with pGMCS alone, dexamethasone treatment resulted in 50-fold induction of CAT activity. When these cells were cotransfected with

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**Figure 2.** Schematic line diagram of glucocorticoid receptor. A/B, Transactivation domain; C, DNA-binding domain; D, E/F, Hormone-binding domain; NLS1, Nuclear localization signal 1; NLS2, Nuclear localization signal 2.

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increasing amounts of pCMVhGRβ, the glucocorticoid-induced, hGRα-mediated activation of the MMTV enhancer was repressed in a dose-dependent manner. But transfection of a 2-fold molar excess of the CMV-vector backbone (pCMV5) had no effect on hGRα-mediated induction of CAT expression, indicating that hGRβ was responsible for the repression of MMTV enhancer. Further, the findings of experiments on HeLa S2 cotransfected with pGRE2CAT and various amounts of pCMVhGRβ strongly suggest that GRβ represses the function of GRα by specifically inhibiting GRE-mediated transcription. The negative activity of GRβ probably resides within its unique carboxyl-terminal 15 amino acid residues. A recent report suggests that over-expression of the glucocorticoid receptor splice variant GRβ in inflammatory cells might contribute to steroid insensitivity in diseases such as asthma.

GR and the chaperone cycle

GR is kept in a functional state by a cycle of chaperones and co-chaperones. A schematic representation of chaperone cycle in relation to GR is depicted in Figure 4. In the absence of a glucocorticoid hormone, GR exists in the cytosol as a part of a multi-chaperone heterocomplex, which exhibits a cycle of association and dissociation of chaperones and co-chaperones. Recent reports suggest that Hsp90 and Hsp70 are sufficient to interact with and activate the GR. However, Hop, Hsp40, p23 and immunophilin act as nonessential but contributive co-chaperones that optimize the assembly of Hsp90, Hsp70 and GR as a heterocomplex.

Hsp70 is a molecular chaperone containing ATPase activity. Hsp70 interacts with Hsp40 (DnaJ, which regulates many functions of Hsp70) and a co-chaperone called Hsp70 interacting protein (Hip or p48). Hip plays a critical role in the regulation of ATPase activity of Hsp70 (ref. 35), and prevents inhibition of heterocomplex assembly by BAG-1 as well as opposes BAG-1-induced reduction of steroid binding activity. Hsp40- and Hip-bound Hsp70 binds to a 60 kDa co-chaperone called Hsp organizer protein (Hop). Hop acts as an adapter protein, which binds both Hsp70 and a dimer of Hsp90 (ref. 37).

Figure 3. Schematic representation of type-1 mechanism of action of glucocorticoid hormones. After binding a glucocorticoid, hormone-bound GRα becomes activated and translocated to nucleus where it undergoes dimerization, leading to formation of a homodimer (GRα-GRα) or a heterodimer (GRα-GRβ). Binding of a homodimer (GRα-GRα) to GRE activates transcription of a gene. However, binding of a heterodimer (GRα-GRβ) to GRE leads to suppression of GRα-stimulated gene transcription. GRβ represses the stimulatory function of GRα by specifically inhibiting GRE-mediated transcription. In contrast, binding of a hormone-GR complex (without undergoing dimerization) to a nGRE inhibits gene transcription. GRα, α isoform of glucocorticoid receptor; GRβ, β isoform of glucocorticoid receptor; GRE, Glucocorticoid response element; H, Hormone; nGRE, Negative glucocorticoid response element.

Figure 4. Schematic representation of the cycle of Hsp90 chaperone, co-chaperones and GR. This cycle is responsible for maintaining the structure of GR and making it capable of hormone-binding. First, Hsp70 in association with Hsp40 and Hip binds to GR. Then a dimer of Hsp90 binds to Hsp70-bound GR. Simultaneously, Hop acts as a docking protein and binds to both Hsp90 and Hsp70, and completes the formation of an intermediate complex. BAG-1 promotes dissociation of Hop-bound Hsp70 complex from the intermediate complex, and simultaneous association of p23 and IP to GR-Hsp90 complex, leading to formation of the mature complex. Then p23 facilitates dissociation of IP and GR capable of hormone-binding from the mature complex. Finally, CHIP stimulates dissociation of p23 from the Hsp90 dimer. The functional GR either binds a glucocorticoid, otherwise it re-enters the chaperone cycle in order to maintain its 3-dimensional functional structure. BAG-1, Bcl-2-associated gene product-1; CHIP, Carboxy-terminal of Hsp70-interacting protein; GR, Glucocorticoid receptor; GRE, Glucocorticoid response element; H, Hormone; Hop, Hsp70-interacting protein; Hop, Hsp organizer protein; Hsp40, Heat shock protein 40; Hsp70, Heat shock protein 70; Hsp90, Heat shock protein 90; IP, Immunophilin; p23, Phosphoprotein 23.
The binding of Hsp70 to Hop facilitates binding of Hsp90. The N-terminal domain of Hop interacts with the C-terminus of Hsp70, while its C-terminal domain interacts with C-terminal of Hsp90 (ref. 38). Hop also enhances the rate of complex assembly. It is noteworthy that Hsp70 and its co-chaperones are the first to approach the receptor molecule. In the first step, ATP- and Hsp40-dependent binding of Hsp70 to GR primes the receptor for its subsequent ATP-dependent binding and activation by Hsp90 (refs 40 and 41). During the priming step, ATP-bound Hsp70 is converted to GR-bound Hsp70 that is approximately one-third in the ADP- and approximately two-thirds in the ATP-dependent conformation. This conversion initiates the opening of the hydrophobic steroid binding pocket so that it can now accept the hydrophobic binding form of Hsp90, which in turn must be converted to its ATP-dependent conformation for the pocket to be accessible by glucocorticoids. In the second step, Hsp90, which is provided in the non-nucleotide-bound state, is converted to GR-bound Hsp90 in the ATP-dependent conformation. GR-Hsp90-Hsp70-Hop heterocomplex constitutes an intermediate complex.

After formation of the intermediate complex, another co-chaperone called Bcl-2-associated gene product 1 (BAG-1) competes with Hop for binding to ATPase domain of Hsp70 (ref. 43). BAG-1 binds to the ATPase domain of Hsp70 and modulates its chaperone activity by acting as an ATP-triggered substrate discharging factor for Hsp70 (ref. 44). The interaction of BAG-1 with Hop leads to dissociation of Hsp70 machinery and Hop from the intermediate complex. Simultaneously, the Hsp90–GR complex gets associated with a large immunophilin (IP) and a co-chaperone called p23 (ref. 45), and becomes a mature complex. Thus, the mature complex contains GR, a dimer of Hsp90, IP and p23. The co-chaperone p23 binds directly to Hsp90 homodimer, and this interaction requires an ATP- and temperature-dependent conformational change in Hsp90 (ref. 46). In the receptor heterocomplexes, the large TPR-containing immunophilin (e.g. cyclophilin (Cyp-40), FKBP51, and FKBP52/Hsp56) targets a single TPR acceptor site on Hsp90, and acts coordinately with Hsp90 to modulate receptor activity. p23 couples the ATPase activity of Hsp90 to polypeptide dissociation and, thus, functions as a substrate release factor for Hsp90 from its substrate and co-chaperones. Due to the activity of p23, GR capable of corticoid binding and IP are released from the mature complex with simultaneous release of Hsp90–p23 complex. Then, an interaction partner of Hsp70 called carboxyl terminus of Hsp70–interacting protein (CHIP) interacts directly with a tetra-tricopeptide repeat acceptor (TPR) site of Hsp90, and negatively regulates chaperone function by eliciting release of the regulatory cofactor p23 (ref. 50).

The Hsp90 heterocomplex undergoes constant cycle of dissociation and ATP- and Hsp-dependent association in the absence of corticosteroids. This cycle takes several minutes, and is completely independent of the hormone. Interestingly, Hsp90 partner proteins exist independent of the presence of the hormone. Apparently, the main function of the GR–Hsp complex is to keep the receptor in a transcriptionally inactive, yet in the high affinity glucocorticoid binding state.

The GR released from the mature complex binds to a glucocorticoid molecule and forms a hormone–GR complex, which binds to GRE or negative GRE (nGRE) leading to activation or inactivation of gene(s) respectively. However, in the absence of a glucocorticoid, the GR re-enters the chaperone cycle in order to maintain its 3-dimensional functional structure (Figure 4). Further, the steroid-binding activity and transactivation potential of the receptor are abolished by CHIP. CHIP also causes ubiquitylation of GR and its degradation by proteasomal enzymes.

Activation and translocation of GR

As lipophilic steroids, glucocorticoids are able to readily enter the cell by free diffusion across the lipid bilayer of the cell membrane into the cytoplasm to interact with the intracellular GR. Once inside the cell, the glucocorticoid molecule binds the GR and induces a poorly understood process known as receptor activation. Ligand binding induces conformational changes in the GR molecule, that has a number of functional consequences. Furthermore, the partially phosphorylated receptor becomes hyperphosphorylated, mostly at serine residues. Subsequently, nuclear localization signal 1 (NLS1) adjacent to DNA-binding domain and nuclear localization signal 2 (NLS2) that overlaps with the ligand-binding domain of the receptor are unmasked, resulting in the movement of the GR to the nucleus. The rapid nuclear import of GR seems to be mediated by NLS1 and correlates with binding of GR to pentalin/ importin α. NLS2 mediates slow nuclear import of GR and forms an alternative nuclear import pathway. The relevance of GR phosphorylation, whether it has any impact on the GR-mediated regulation of transcription, is not well established, and remains arbitrary. There is a possibility that the phosphorylation status of the GR co-determines its subcellular localization rather than its transcriptional activity. For unidirectional movement to occur, the receptor is attached to a retrograde movement system like the cytoplasmic dynein (a motor protein involved in retrograde transport of vesicles towards the nucleus) in a manner that involves the nuclear localization signal.

The 643-proline of GR ligand-binding domain has been implicated in both stabilizing the receptor-Hsp90 complex and in permitting an efficient nuclear translocation. It, thus, supports the concept that the chaperone is an integral component of the steroid-receptor signalling pathway. Further, under physiological conditions in which the cytoskeleton is intact, the GR utilizes a move-
ment machinery (which is dependent on Hsp90 chaperone) for its rapid movement from the cytoplasm to the nucleus along cytoskeletal tracts. In the chaperone system, FKBP52 specifically facilitates trafficking of glucocorticoid-bound GR and targets retrograde movement of the GR along microtubules by linking the receptor to the dynein motor. The Hsp90 chaperone machinery is also involved in chromatin recycling of GR.

The nuclear export of GR seems to limit receptor down-regulation and desensitization, resulting in increased efficiency of biological response to secondary hormone challenges. The mechanism of nuclear export of GR is not well established. It has been demonstrated that receptors that accumulate within nuclei upon ligand binding are not statically confined to that compartment, but rather have the capacity to reversibly traverse the nuclear envelope. Further, the ability of various GR mutants to shuttle between nuclei of heterokaryons excludes transcriptional activation and DNA binding as prerequisites for nuclear-cytoplasmic shuttling of GR. In situ biochemical extractions have revealed a distinct subnuclear compartment, which collects GRs that have been released from chromatin and serves as a nuclear export staging area. GRs that release from chromatin do not require transit through the cytoplasm to regain functionality. Since geldanamycin (a hsp90-binding drug) inhibits hormone-binding to recycled nuclear GRs, hsp90 may be required to reassemble the receptor into a form capable of productive interactions with the hormone. Further, geldanamycin also inhibits GR release from chromatin during hormone withdrawal. These findings suggest that hsp90 chaperone function may play multiple roles to facilitate chromatin recycling of GR. In addition to Hsp complex, protein phosphatase 5 (PP5) is also involved in the regulation of GR nucleo-cytoplasmic shuttling, and its inhibition leads to accumulation of GR in the nucleus. Hormone withdrawal leads to dissociation of GR from chromatin. It seems that the 69-amino acid DNA-binding domain (DBD) of GR, which is unrelated to any known nuclear export sequence (NES), is necessary and sufficient for nuclear export of GR. It is important to mention that the DBD is highly related (41–88% identity) in steroid, nonsteroid, and orphan nuclear receptors and DBDs from ten different nuclear receptors reportedly function as export signals. DBD-dependent nuclear export is saturable, and prolonged nuclear localization of the GR increases its transcriptional activity. There are indications that NLS can influence nuclear processing pathways that culminate in efficient nuclear export. It, thus, seems that NLS-mediated import and DBD-mediated export jointly define a shuttling cycle that integrates the compartmentalization and activity of nuclear receptors.

Mechanisms of action of glucocorticosteroids

The glucocorticoid-bound GR can modulate gene transcription in the nucleus in two ways. It can either activate or repress the transcription of target genes by interacting with short, palindromic DNA sequences—called glucocorticoid response elements (GREs). Activation of transcription is usually the result of a direct interaction between the GR and the GRE. However, repression of transcription is either the result of an indirect action of GR mediated by a response element as a result of protein–protein interaction, or by an occlusion mechanism in which GR displaces a general or regulatory transcription factor (TF). In order to simplify the presentation, direct activation or repression of target genes by glucocorticoid-bound GR has been termed as type-1 mechanism, while indirect inhibition of target genes by hormone-activated GR has been termed as type-2 mechanism.

Type-1 mechanism of action

The type-1 mechanism of glucocorticoid action represents the classic model of GR action, and it is characterized by the GR interacting with specific DNA sequences (Figure 3). After activation by glucocorticoids, the activated GRs proceed rapidly to the cell nucleus, and bind to GRE in the promoter region of the glucocorticoid-responsive genes, as a homodimer. The GRE has been reported to have two defined stretches of nucleotides separated by undefined nucleotide creating two half-sites. The consensus sequence of GRE is 5'–AGAACanmn–TGTTCT–3', where n can be any nucleotide. Members of the nuclear receptor superfamily often recognize response elements with similar or even identical half-site sequences, but with different orientations or spacing. For instance, the estrogen response element (ERE) differs from the GRE only by changes in the central two base pairs in their identical 6 bp half-site. Exchange of only two bases at the homologous portion within the 15 bp palindrome, altering the heptanucleotide TGACCT to TGTTCT leads to loss of estrogen response and to induction of glucocorticoid response. The DNA-binding domain of GR binds cooperatively to specifically spaced target half-sites in the DNA, and this specific DNA association induces receptor dimerization. The subunits of GR homodimer then interact with adjacent major grooves of the DNA via their amphipathic-helices. As a result of dimerization, two recognition helices, one from each subunit of the dimer, are symmetrically extended into the two adjacent major grooves of the GRE sequence.

When bound to the GRE, the GR homodimer interacts with components of the basic transcription machinery, either directly (i.e., by physical contact between the GR transactivation domains and basic transcription factors TFII B) or indirectly (i.e., via 'bridging' factors such as steroid receptor co-activator, SRC). Moreover, binding of the GR homodimer has been reported to induce chromatin remodelling in the respective promoter region, thereby allowing other transcription factors to bind to previously inaccessible DNA.
In addition to its function as a ligand-dependent activator of transcription, in some promoters, binding of the activated GR to so-called negative glucocorticoid response elements (nGREs) causes inhibition of transcription. The nGREs are less well-defined than positive GREs, and their gene-repressive function appears to be context-dependent. GR is presumed to bind to the nGRE and interferes with transcriptional activation by transcriptional factors. The mechanism of action for GR on nGRE seems to involve displacement of a positive regulatory protein from the promoter. The prototype of nGRE is located in the pro-opiomelanocortin (POMC) promoter and only slightly resembles the classic GRE. Instead of binding GR homodimer, the nGRE reportedly accommodates three molecules of nGRE. For reasons that are not well understood, the GR–nGRE complex represses transcription of the POMC gene.

Some proteins function as transcription co-activators for the GR, and enhance and/or enable the transcriptional activity of ligand-activated GR. Prominent among these co-activators is the 160 kDa protein, named as the steroid receptor co-activator (SRC) family, which consists of SRC-1 (p160), GRIP1 (TIF2/SRC-2), and SRC-3 (RAC3/ACTR/TRAM-1/AIB1/pCIP). These cofactors interact with nuclear receptors in a ligand-dependent manner and enhance transcriptional activation by the receptor via histone acetylation/methylolation and recruitment of additional cofactors. Other examples of proteins recently identified as GR co-activators are CREB binding protein (CBP/p300), HMG-1 proteins, 14-3-3 proteins, STAT5 (ref. 93), COUP-TF1, GRIP, hRPF1, (ref. 96), hPGC-1 (ref. 97), SWI-SNF, RAP-46 (ref. 99), and RIP140 (ref. 100). Detailed scrutiny of SRC family members has led to identification of a conserved motif, LXXLL (where L is leucine, X is any amino acid), termed as the nuclear receptor (NR) box, which is necessary and sufficient to mediate binding of the coactivators to liganded nuclear receptors.

Nuclear receptors activate gene transcription by binding to specific enhancer elements and recruiting co-activators to promoters of target genes. The co-activators in turn enhance transcription by recruiting secondary co-activators, including histone acetyltransferases (e.g. CBP and p300/CBP-associated factor, p/CAF) as well as protein methyltransferases. Binding of the activated receptor to the enhancer region directs modification of the local chromatin structure into a transcriptionally permissive state (derepression), followed by recruitment of TFs to form a preinitiation complex at the promoter. The cell cycle regulator, retinoblastoma protein, is known to potentiate glucocorticoid receptor-activated transcription through the interaction of its pocket domain with the transcription co-activator, hBRM. Recently, it has been reported that GR-induced apoptosis also depends on retinoblastoma protein, and hBRM, p107, and p130.

**Figure 5.** Schematic representation of type-2 mechanism of action of glucocorticoid hormones. In this type of action, a hormone-bound GR binds to a transcription factor (TF) and suppresses the TF-induced transcription. For example, hormone-bound GR binds to a c-Jun–c-Fos dimer and suppresses the gene transcription stimulated by the dimer. In general, a dimer of GRs binds to a GRE and stimulates the transcription of a target gene. AP-1 binding sites, Activation protein-1 binding sites; GR, Glucocorticoid receptor; GRE, Glucocorticoid response element; H, Hormone.
mediated transrepression is reported for nuclear factor NF-kB\(^{14,15}\), the octamer transcription factors Oct-1 and Oct-2 (ref. 116), the Spi-1/Pu.1 (OTF-1) oncoprotein\(^{17}\), hepatocyte nuclear factors (HNFs)\(^{18}\), GATA-1 (ref. 119), eukaryotic co-chaperone BAG-1\(^{120}\), CAAC and CCAAT box binding factor\(^{121,122}\), and other activated receptors of steroid superfamily (e.g. estrogen receptor and the thyroid hormone receptor)\(^{122}\). In addition, hormone-activated GR\(\alpha\) has also been reported to interact with the glucocorticoid modulatory element (GME)\(^{123}\).

Proteins that interact with steroid and other nuclear receptors, and serve as transcriptional co-repressors, including nuclear receptor co-repressor (N-CoR), and silencing mediators for retinoid and thyroid hormone receptors (SMRT) have also been identified\(^{124}\). These nuclear factors, which interfere negatively with GR-mediated transactivation, are referred to as dominant negative inhibitors. These factors seem to represent the most significant endogenous regulators of glucocorticoid responses, and can act through the following mechanism: (i) creation of steric hindrance of the GR by binding to DNA sequences overlapping a GRE, (ii) competition with GR for GRE binding, and (iii) squelching of necessary factors for the interaction of GR with the basic transcription complex\(^{125,122}\).

Like AP-1, NF-kB has also been shown to activate transcription of genes involved in inflammatory diseases such as rheumatoid arthritis and asthma\(^{125}\). NF-kB has been reported to act synergistically in the induction of some proinflammatory genes in lung epithelium\(^{126}\). In addition, many inflammatory genes that are repressed by glucocorticoids, but do not have nGREs in their promoters, carry sites for AP-1 as well as for NF-kB\(^{127}\). NF-kB functions as a dimeric DNA-binding protein that comprises subunits from a family of related proteins called the Rel family, which includes p65 (Rel A), Rel B, c-Rel, p50/p105 (NF-kB 1), p100/p52 (NF-kB 2), and the Drosophila melanogaster proteins Dorsal and DiI\(^{128}\). The various members of the Rel family can homodimerize or heterodimerize with other Rel proteins to form DNA-binding competent NF-kB factors with different sequence specificity\(^{129,130}\). Reports indicate that GR and NF-kB physically interact\(^{151}\) and function as mutual transcriptional antagonists\(^{17,130,132}\). Since both these transcription factors are known to be potent regulators of the immune system, elucidation of the mechanisms by which GR and NF-kB negatively interact, not only provides the basis for understanding their role in the precise control of normal immune response, but also opens up the possibility of novel therapeutic intervention in immune pathology.

Summary

Glucocorticoids act on target cells through their specific intracellular GRs, and produce stimulatory or inhibitory actions by stimulating or inhibiting gene activity and transcription. GR, which belongs to the superfamily of steroid receptors, is synthesized in two isoforms, GR\(\alpha\) (which binds glucocorticoids) and GR\(\beta\) (which cannot bind hormones). GR\(\alpha\) shuttles between the cytoplasm and the nucleus, and its nuclear import and export are controlled by a complex mechanism. GR\(\beta\) is present predominantly in the cell nucleus. A cycle of Hsp90 and co-chaperones is essential for maintaining the folding of GR protein, and also for making it capable of binding to glucocorticoids. After binding of a glucocorticoid, GR\(\alpha\) becomes activated, hyperphosphorylated and finally translocated to the nucleus. After translocation, the hormone-bound GR alters gene activity and transcription via two types of mechanism, now termed as type-1 mechanism and type-2 mechanism. In type-1 mechanism, hormone-bound GR\(\alpha\) undergoes homodimerization (GR\(\alpha\)-GR\(\alpha\)) or heterodimerization (GR\(\alpha\)-GR\(\beta\)) in the nucleus. The homodimer binds to a GRE and stimulates transcription of gene(s). However, binding of a heterodimer to a GRE induces only partial activation of transcription of a gene (transactivation). nGRE can bind a maximum of three hormone–receptor complexes (without undergoing dimerization), resulting in inhibition of transcription of a gene (transrepression). In type-2 mechanism, the hormone-bound GR\(\alpha\) binds to other transcription factors (TFs) belonging to AP-1 family (e.g. c-Jun, Jun-B, Jun-D, c-Fos, Fos-B, Fos-B2) or activating transcription factor (ATF) family proteins (e.g. Fra-1, Fra-2) and inhibits the transduction of target genes stimulated by the respective TFs. Further, GR\(\alpha\)-induced transactivation may also involve several transcription co-activators (e.g. steroid receptor co-activator (SRC) family composed of SRC-1, GRIP1, and SRC-3, CBP, HMG-1 proteins, STAT 5, COUP-TFI, GRIP, RIP140, RAP-46). There are also dominant negative inhibitors (e.g. nuclear receptor co-repressor, N-CoR and silencing mediator for retinoid and thyroid hormone receptors, SMRT), which interfere negatively with GR-mediated transactivation.

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