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Nuclear Receptor Function through Genomics, Lessons from the Glucocorticoid Receptor

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Abstract

Unlocking the therapeutic potential of the glucocorticoid receptor (GR) has motivated a search for small molecules that selectively modulate GR's ability to activate or repress gene transcription. Recently, breakthrough studies in the field of genomics have reinvigorated debate over long-standing transcriptional models explaining how GR controls tissue-specific gene expression. We highlight these genomic studies with the dual goals of advancing understanding of nuclear receptor-mediated transcription and stimulating thought on the development of anti-inflammatory and immunosuppressive ligands for GR that have reduced harmful effects on metabolism.

Keywords

Glucocorticoid receptor; nuclear receptor; transcription; functional genomics; cistromics

GR: a nuclear receptor with widespread physiological impact

GR, or **NR3C1** (see Glossary), is a transcription factor (TF) that regulates gene expression in nearly every cell of the body. A member of the **nuclear receptor (NR) superfamily**, its ligand-binding domain confers transcriptional regulation by endogenous and synthetic lipophilic molecules [1]. Glucocorticoid (GC) activates GR [2]. A class of adrenal cortex steroid hormones named for its glucose regulating properties, site of production and compound structure, i.e. **gluco**se + **cort**ex + ster**oid**, it was first linked to metabolism upon determining that removal of the adrenal gland in diabetic animals lowered glucose levels in the blood [3].

Whereas GR is expressed ubiquitously, cortisol and corticosterone, the major GC hormones in human and mouse, respectively, elicit tissue-specific effects (Figure 1). Consistent with its naming, GC stimulates glucose production in liver. It also affects energy homeostasis by inhibiting insulin-dependent glucose uptake in muscle and adipose, promoting the release of

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amino acids and free fatty acids from muscle and adipose tissue breakdown, and inhibiting insulin release from pancreatic β cells [4]. Systemic metabolic changes from GCs ultimately increase blood glucose levels. GCs also reduce inflammatory responses of immune cells, affect cardiovascular function in coronary arteries and alter mental and emotional states through action in the central nervous system (CNS) [5]. These actions are especially important during periods of acute stress, when elevated release of GC into the bloodstream helps to mobilize stored fuel for the 'fight or flight' response, maintain a ready supply of energy from glucose for the brain, and maintain vascular stability to prevent potentially life-threatening hypotension. It is noteworthy that some of the actions of GCs may result from cross talk between tissues. For example, GCs promote lipid catabolism in adipose tissue, yet whether they drive lipolysis by directly or solely acting on adipocytes remains unclear [6–8]. It should also be mentioned that GCs exert rapid effects occurring in minutes that are insensitive to inhibitors of DNA transcription and protein synthesis [9]. These non-genomic effects are thought to regulate the early stages of the stress response, though more study is needed to clarify this and their potential on impact physiology.

Genomic studies of GR have provided new insights into the tissue-specific effects of GCs. GR targets genes for transcriptional regulation by recognizing and binding to a particular DNA-sequence motif. Although widely expressed, GR occupies only a subset of its genomic motifs in any given cell type because most are buried in repressive chromatin structure that renders them inaccessible [10]. As chromatin structure is organized differently for each cell-type [11], the genomic occupancy and transcriptional output of GR are cell-type specific. How GR interacts with the native genome to regulate gene expression is the main focus of this review.

Health issues arising from defects in GC signaling to GR

Serious health issues result from abnormal GC signaling that can lead to death if untreated, underscoring the importance of GR in human physiology. Primary adrenal insufficiency or **Addison's disease** arises from adrenal gland problems that cause production of cortisol and possibly aldosterone, the other major adrenal cortex steroid hormone and important regulator of blood pressure, to become too low to meet the body's needs. Adrenal damage from the body's immune system and infection in the developing world are often to blame. More common is secondary adrenal insufficiency. Characterized by disrupted signaling through the hypothalamic-pituitary-adrenal (HPA) axis, the pituitary gland fails to produce enough adrenocorticotropin hormone (ACTH) to stimulate cortisol synthesis and secretion. Replacing the absent hormones treats weakness, hypoglycemia, hypotension and other potential symptoms that are affiliated with adrenal insufficiencies [12]. Opposite of too little cortisol, **Cushing's disease** commonly arises when a tumor causes too much cortisol to be produced. It can reside in the adrenal gland itself or a secondary ectopic site usually in the brain that leads to overproduction of ACTH or corticotropin-releasing hormone (CRH) by the hypothalamus. In addition to hyperglycemia, osteoporosis and muscle atrophy, a hallmark of Cushing's disease is obesity from a redistribution of adipose tissue that drives central adiposity at the expense of peripheral fat [13].

Rare mutations can impair GR's molecular function and alter tissue sensitivity to GCs in humans, resulting in primary generalized GC resistance (PGGR) and hypersensitivity (PGGH) [14]. Familial and sporadic PGGR, or Chrousos Syndrome, is characterized by general and partial insensitivity of tissues to GC and compensatory hyperactivation of the HPA axis. PGGH represents the opposite, with GC hypersensitivity and consequent hypoactivation of the HPA axis. As the cost of whole genome sequencing declines, it is becoming feasible to examine the relationship between mutations and polymorphisms in the GR gene and the considerable variation between patients in their response to GC treatment.

GR ligands are commonly prescribed drugs

GCs and their synthetic analogs are among the most widely prescribed drugs in the world. Their anti-inflammatory and immunosuppressive properties are important for the treatment of rheumatioid arthritis, cerebral edema, asthma and other allergic reactions, and the prevention of organ transplant rejection and graft-versus-host disease [15,16]. GCs are more potent than nonsteroidal anti-inflammatory drugs (NSAIDS, e.g. aspirin), yet up to a third of patients with severe asthma may be unresponsive to them [17]. GC resistance is likely a result of complex interactions between an individual's genetic makeup and environment. In addition, GCs cause undesirable metabolic effects that can prematurely end treatment. Features of metabolic syndrome including obesity, dyslipidemia, insulin resistance and type 2 diabetes mellitus are prevalent, as well as osteoporosis and muscle atrophy [4].

Metabolic dysfunction arising from the chronic use of GC medication, termed **Cushing's syndrome**, is related to Cushing's disease, but more common in western societies that frequently prescribe GC drugs. Though many of the problems stemming from chronic GC excess can be explained by the acute effects of GC to induce insulin resistance, raise plasma glucose and increase protein catabolism, the underlying molecular mechanism(s) explaining how prolonged activation of GR causes weight gain is poorly understood. Indeed, GCs acutely induce lipolysis in human adipose tissue [13], and consistent with their catabolic effects, cause rats to lose weight over time [18,19]. A possible explanation for this paradox may involve depot-specific responses to excess GC that simultaneously trigger the breakdown and growth of limb and abdominal adipose tissue, respectively [13]. GCs facilitate adipocyte differentiation [20,21], though whether this contributes to the obesity of Cushing's syndrome is unknown. While more work is needed to resolve the paradox, it serves as a reminder of the limitations of rodent models to provide insight into human physiology.

Tissue-specific function for GR

Intense effort has been dedicated to understanding how ubiquitously expressed GR elicits complex tissue-specific effects by controlling distinct gene programs in different cell types. Not surprisingly, mechanisms involving both GC availability and GR function provide answers. Cell-type-specific GC availability is modulated by a pair of hydroxysteroid 11-beta dehydrogenase (HSD11B) enzymes that control intracellular cortisol/cortisone levels. HSD11B1 converts the inert GC cortisone to cortisol, while HSD11B2 catalyzes the reverse reaction. Through distinct tissue-specific expression profiles, they amplify or mute responses

to the circulating cortisol level set by the HPA axis [22]. HSD11B2 is found in kidney, lung, colon, salivary glands and HSD2 neurons, all of which are responsive to aldosterone, an activating ligand for the mineralocorticoid nuclear receptor (MR). Because cortisol also activates MR, HSD11B2 prevents its illicit activation by decreasing intracellular cortisol concentrations. HSD11B1 is expressed in key metabolic tissues such as adipose to amplify GC signaling. For example, HSD11B1 overexpression in mouse BAT decreases BAT thermogenic activity, while pharmacological HSD11B1 inhibition or knockdown enhances its function [23], consistent with corticosterone inhibition of BAT in rodents [24], and demonstrating that local GC levels matter. Of note, local levels of GCs may also be affected by extra-adrenal GC synthesis. For example, local GC production by the intestinal epithelium may regulate intestinal immune responses [25].

Although encoded by a single gene, GR displays considerable heterogeneity through the combined effects of alternative mRNA splicing, alternative translation initiation, and complex post-translational modification. Differential expression of the various GR isoforms may contribute to tissue-specific functions [26,27]. GRβ, the best characterized splice variant, uses of an alternate exon 9 that disrupts the structure of helices 11 and 12 in the ligand-binding domain, a region required for co-regulator recruitment [28]. Constitutively localized in the nucleus, GRβ cannot activate gene transcription in response to GC, suggesting that it acts as an endogenous dominant negative of GRα, the classic GR referred to in most studies. Though generally expressed at lower levels than GRα, cellular signals affecting the expression ratio of GRα to GRα show clinical associations with GC sensitivity, autoimmune disease, and lipid metabolic profiles [26]. Amino-terminal truncations of GR occur through the use of seven alternative translation initiation sites. While none directly prohibit DNA or ligand binding, they can alter GR conformation in a manner that affects its subcellular localization and transcriptional activity [29]. Furthermore, extensive phosphorylation of the N-terminal domain of GR adds to the complexity. Up to six serine residues in human GR are phosphorylated in vitro by either mitogen-activated protein kinases (MAPKs), cyclin-dependent kinases (CDKs) or glycogen synthase kinase 3 (GSK3) [26]. Phosphorylation of Ser-211 by p38 is associated with co-activator recruitment and transcriptional activation [30,31], whereas phosphorylation of Ser-226 by c-Jun N-terminal kinases (JNKs) [32] or CDK5 [33] impairs transcriptional activity [30,34]. Intriguingly, ChIP with phospho-specific GR antibodies suggests differential recruitment [34], however the affects of phosphorylation and other post-translational modifications on genome-wide binding for GR remain to be tested. Indeed, a future challenge is the development of molecular tools that can distinguish between the different isoforms to determine their genomic functions.

Ultimately, a more complete understanding of GR and its complex tissue-specific functions requires insight into how chromatin structure influences its ability to access genomic sites for gene regulation. A detailed discussion describing how GR interacts with the native genome follows, with a goal of dispelling two widespread models for negative regulation by GR.

GR occupies open chromatin through sequence-specific binding

Upon binding ligand in the cytoplasm, GR moves to the nucleus to target genes for transcriptional regulation (Figure 2A). It binds as a homodimer, and possibly as a homotetramer [35], to a palindromic DNA sequence roughly approximated as g/aGnACAnnnTGTnCt/c. GR binding in vivo has been dramatically informed by the advent of genome-wide approaches [36,37]. Early chromatin immunoprecipitation with deep sequencing (**ChIP-seq**) studies set the stage for new and surprising determinants for GR binding in diverse cell types and tissues obtained from mice and humans [10,38–44]. For a field focused on proximal gene promoters, the discovery that most GR-binding sites (GBSs) reside outside of these regulatory regions was eye opening. Also unforeseen was that GBSs vastly outnumber GC-regulated genes. The identification of thousands to tens-of-thousands of binding sites in any particular cell type surpasses the number of gene targets by an order of magnitude or more, with the caveat that the number of targets may increase if GR-regulated non-coding RNAs are discovered in large numbers. This difference is partly explained by findings showing that GC-regulated genes are enriched for multiple GBSs. However, an unexpectedly large number of sites do not co-localize with the histone modifications, chromatin remodelers and transcriptional cofactors associated with active enhancers [45]. It is tempting to consider these as experimental noise or artifact, but they are enriched for the GR motif, suggesting sequence-specific binding. While the size and complexity of the GR **cistrome** is greater than seemingly needed, the existence of sites without transcriptional characteristics may be understood by principles of constructive neutral evolution [46].

Genomic studies have afforded new insight into the influence of chromatin structure on GR binding. An important clue came from genome-wide mapping of chromatin structure using DNase I hypersensitive site sequencing (DNase-seq), and comparing the locations of open chromatin to GBSs [10]. DNase-seq in the absence and presence of ligand revealed that up to 95% of GR occupancy is directed to preexisting regions of accessible chromatin. Similar results were gathered in vivo with an adrenalectomized mouse model [39], suggesting that GR on its own cannot efficiently counteract histone-mediated repression to access DNA in nucleosomes. As this is a distinguishing feature of pioneer TFs [47], the ability of GR to serve a pioneering role on its own is limited [48]. Yet, GR binding further remodels chromatin to increase accessibility for other TFs through a mechanism termed assisted loading, originally advanced to describe how TFs targeting the same DNA motifs can stimulate, rather than inhibit, each other’s occupancy via dynamic TF-DNA interaction [49]. The model depends on the ability of TFs to trigger chromatin remodeling [50], and provides insight into the collaborative binding widely observed at neighboring cis-regulatory sequences [51–55], and conversely, helps to explain why enhancers typically contain several closely-spaced TF-binding sites.

Along with open chromatin, it is important to emphasize that the palindromic GR motif is a critical determinant of genomic occupancy. Prior to the emergence of genomics, characterization of GR’s recognition sequence had been primarily determined by in vitro approaches. Thus, it was reassuring that motif analyses of GR ChIP-seq data revealed robust enrichment of the palindromic sequence in vivo. However, due to the limited resolution of

ChIP-seq, strong enrichment of additional co-localized motifs was also observed, confounding a definitive interpretation of GR's genomic substrate. This was resolved by ChIP with lambda exonuclease digestion and sequencing (**ChIP-exo**), a technique designed to unequivocally identify bound motifs through unbiased, high resolution mapping of TFs to the genome [56]. With ChIP-exo, several groups demonstrated GR binding at the expected palindromic sequence in native chromatin [57–59].

GR monomers occupy the genome sequence-specifically and through tethering

Binding sites lacking the expected motif are present as a minor fraction in most TF ChIP-seq datasets, but can still account for thousands of sites in some cistromes. While experimental artifact such as antibody cross reactivity with chromatin-bound proteins other than GR may partially explain these, it is possible that TF binding in the absence of a motif may be biologically relevant. Of interest to GR, bound sites lacking the motif may represent protein-protein interaction between GR and other DNA-bound TFs to form so called tethered sites [60,61]. Indirect DNA binding is thought to explain GR's effects on the expression of reporter genes lacking GR motifs, but unambiguous determination of the interacting protein(s) is challenging because presumed tethered sites often reside at composite enhancers bound by multiple TFs. With its superior resolution, ChIP-exo has identified tethering partners for GR monomers [58,59]. GR monomers were also found to interact sequence-specifically with the GR half-site motif, consistent with earlier in vitro studies [62–64]. It is intriguing that tethered sites strongly co-localize with monomeric, genomic sites, suggesting that transient contacts between monomers and nearby TFs are promoted and/or stabilized by GR interaction with half-site motifs. This idea, termed half-site-facilitated tethering, designates DNA as the primary recruiter of monomeric GR (Figure 2A), and stably bound TF neighbors as targets for subsequent protein-protein interaction through GR tracking on chromatin [62,65]. As the evidence for TF tethering mounts in vivo [66], better understanding of its prevalence and role(s) is needed.

GR is a transcriptional activator

How GR activates some genes while repressing others remains unsettled after decades of study. While significant controversy remains regarding GR-mediated repression, a consensus model for transcriptional activation has emerged: GR activates transcription through sequence-specific binding to the genome at palindromic motifs. This is strongly supported by genomic studies. Dimeric sites preferentially associate with ligand-activated gene expression on a genome scale [57,58,63], and become occupied by transcriptional cofactors, activating histone modifications and RNAPII in response to GR binding during cell differentiation [67,68]. While these data are correlative, self-transcribing active regulatory region sequencing (**STARR-seq**) has been used to directly examine the transcriptional regulatory properties of GBSs. The assay interrogates enhancer function in a direct, quantitative, and high-throughput manner by placing DNA fragments from any source downstream of a minimal promoter and introducing the reporter library into cells [69]. Analysis of GR ChIP DNA by STARR-seq revealed that 95% of the fragments conferring

GC regulation increased reporter gene expression in response to exogenous GC [70]. Moreover, sequence analysis identified the GR palindromic motif as the sole predictor of GC regulation, and ChIP-exo revealed a characteristic dimeric profile at the GC-regulated regions, demonstrating that GC-induced enhancers encode dimeric sites for GR.

Like ligand, DNA functions as an allosteric regulator of GR by modulating its activity downstream of genomic occupancy to induce a select fraction of the GR transcriptome [64,71–73]. More generally, GR activates gene expression by integrating signals to nucleate the assembly of cofactors and the general transcription machinery. Recruitment of the Mediator Complex [43,68], a cofactor that facilitates long-range chromatin interactions between TFs and the transcription initiation machinery [74,75], implicates GR in the formation of DNA loops that potentially connect GBSs with promoters to activate distant genes. Similarly, DNA looping was invoked to explain the observation that dimeric sites are frequently surrounded by additional GBSs that lack both the palindromic motif and the ability to confer transcriptional regulation in response to GC [70]. The model posits that many ChIP-seq peaks represent chromatin-bound GR tethered to remotely bound TFs via looping, but this requires testing by chromatin confirmation capture techniques. It is also possible that monomeric binding by GR helps to explain the clustered arrangement of GBSs. Monomeric sites are enriched near dimeric sites in liver, yet they outnumber dimeric sites by 5:1 [58]. In comparison with GR dimers, monomers are sub-optimized for DNA binding [62,64] and transcriptional activation [71,76,77]. This may suggest that they are intermediates in the evolution of dimeric sites. However, with the discovery that genomic recognition sequences are sub-optimized for TF affinity to favor tissue specificity at the expense of activity [78], it is also possible that monomeric sites are important for the tissue-specific functions of GR. Consistent with this, chromatin-bound monomers colocalize more frequently with lineage dependent TFs than dimers [58,59], which may be a general property of steroid nuclear receptors [60,79].

Glucocorticoid-mediated repression and GR

About half of the genes affected by GC treatment are down regulated independent of the experimental system under study. This is important given that the immunosuppressive properties of GCs are mediated, at least in part, by transcriptional inhibition of pro-inflammatory genes in immune cells [80]. Unlike transactivation, a clear mechanism for GR transrepression has not emerged from genomic data. What appears clear, however, is that the two prominent models for repression, namely 1) tethering of GR monomers and 2) binding of GR to repressive DNA motifs termed negative glucocorticoid response elements, or nGREs [81,82], are not supported by an unbiased examination of GBSs. ChIP-seq in primary macrophages [83] and liver tissue [39] revealed similar enrichment of GR near both ligand-activated and ligand-repressed genes. Despite expectations, sequence analyses failed to find motifs distinguishing the putatively activating and repressing GBSs, and thus failed to implicate potential TFs mediating GR-dependent repression through tethering. Furthermore, the nGRE was not enriched in these studies nor any other published GR ChIP-seq dataset, challenging the idea that GR binds directly to this motif under physiological conditions. It is striking that STARR-seq failed to identify GBSs that repress reporter gene activity in response to exogenous GC [70]. A technical deficiency is unlikely given that

STARR-seq found sequences conferring negative regulation by steroid hormone in flies [84]. In this case, the repression occurred independently of receptor binding to the regulatory sequence, suggesting an indirect cause. A potential concern of STARR-seq is that current iterations measure reporter activity from plasmids, and substantial differences exist between enhancer activity encoded on episomes versus chromosomes [85]. While it is formally possible that STARR-seq is unable to detect GR-mediated repression requiring a chromosomal context, this is unlikely for tethering, which was originally described using transiently transfected reporter plasmids.

It is increasingly argued that the immunosuppressive effects of GCs are conferred indirectly by GR through the activation of genes encoding proteins that inhibit expression of pro-inflammatory genes [86–88]. Genomic studies offer another mechanism compatible with the idea that GC-mediated repression is a secondary effect of GR function (Figure 2B). Treatment of mice with a GC drug caused liver gene expression changes that associated with a redistribution of GR and RNAPII from monomeric to dimeric GR-binding sites, with lost and gained sites enriched near repressed and induced genes, respectively [58]. This can be explained by expanding the classic squelching model to include the redistribution of TFs in addition to co-activators in response to external stimuli. Consistent with observations showing ligand-stimulated degradation of GR [89], the model proposes that both monomers and dimers are available in limiting amounts so that gain of occupancy at one set of sites leads to loss at another. Furthermore, by attributing GC-repressed transcription to the loss of activating GR monomers, the model maintains the logic of sequence-specific binding for GR genomic function. As squelching has been suggested to explain the transcriptional repression resulting from other NR ligands [90,91], its role in NR function needs further examination.

Concluding remarks and future perspectives

The development of ligands that provide immunosuppressive benefits without unwanted metabolic effects remains as an unmet goal of GR research. Traditionally, this has meant searching for ligands that repress pro-inflammatory genes in immune cells without activating catabolic genes in liver, fat, muscle and bone. While the genomic data do not support a path to such ligands, they raise questions that could increase GR’s therapeutic value if resolved (Outstanding Questions Box). For example, is GC-mediated repression of gene transcription dependent on squelching or an as yet to be determined molecular mechanism? Of particular interest to inflammation, does squelching occur in immune cells to affect expression of pro-inflammatory genes? Are stress-induced releases of cortisol into the bloodstream sufficient to redistribute monomers and dimers on the genome in a similar manner to what has been observed for GC drugs, or relatedly, do ultradian and circadian GC fluctuations alter GR interaction with the genome over the course of a day? Given that GC-mediated repression may result secondarily from GR action, can DNA loops map direct gene targets? Ultimately, a productive therapeutic approach may involve cell-selective delivery of GC drugs to minimize their complex tissue-specific effects.

An overlooked area of inquiry from a genomics viewpoint is the potential interplay between GR and the mineralocorticoid receptor (MR). MR is unique among nuclear receptors

because it can bind to multiple steroid hormones including aldosterone, cortisol and progesterone [92]. This is likely to have physiological consequences [93]. MR and GR bind with high affinities to cortisol, which circulates at concentrations that are 10–1000-fold higher than aldosterone, and HSD11B2 is absent in some tissues with high MR expression such as myocardium and hippocampus. MR can form heterodimers with GR [94,95], and with DNA-binding domains that share 94% similarity, both receptors target the same DNA motif. Unfortunately, genomic data for MR are limited by the lack of good ChIP-grade antibodies, yet how and where it binds the genome may provide new mechanisms of GC signaling that could be leveraged for therapeutic gain.

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GLOSSARY

Addison’s disease
Also called adrenal insufficiency, it is a disorder that occurs when the body produces insufficient amounts of the adrenal gland hormone cortisol and possibly aldosterone. It is a rare condition, only 1 in 100,000 people has it. Affected individuals can lead a normal life with hormone replacement therapy. President John F. Kennedy had the disease

ChIP-exo
An experimental technique that combines ChIP with lambda exonuclease digestion and sequencing. Exonuclease digestion of ChIP DNA before sequencing generates nuclease-protected regions, or footprints, for chromatin-bound proteins. ChIP-exo improves the spatial resolution of ChIP-seq approximately 5–10-fold, facilitating the determination of bound sequences and the identification DNA motifs

ChIP-seq
An experimental technique that couples chromatin immunoprecipitation (ChIP) with deep sequencing (seq) to map the location of histone and nonhistone proteins across the genome in an unbiased manner. It can also be used to interrogate DNA modifications such as methylation. With a resolution of approximately 200 base pairs, it is best suited to determine where, rather than what, a transcription factor binds genome-wide

Cistrome
The set of genomic loci occupied by a particular protein, e.g. GR, or associated with an epigenomic modification, e.g. histone 3 lysine 27 acetylation. It is further specified by cell type, tissue, species, physiological state, etc

Cushing’s disease and syndrome
Both occur when the body is exposed to excessively high levels of glucocorticoid over a prolonged time period, leading to metabolic dysfunction including obesity, diabetes, osteoporosis and muscle wasting. They are sometimes distinguished by the source of glucocorticoid. Cushing’s disease results from the overproduction of endogenous cortisol

most often caused by a tumor residing in the adrenal gland or a secondary ectopic site, usually in the brain, that drives excessive secretion of the hormones that stimulate cortisol production and release. Cushing's syndrome arises from the chronic use of glucocorticoid medication, and is more common than the disease in western societies

GR (NR3C1)

The molecular target of glucocorticoids and a type I nuclear receptor, GR is released from a cytoplasmic chaperone complex upon binding ligand and trans-located to the nucleus, where it regulates gene transcription through sequence-specific interaction with the genome. It modulates numerous gene programs in a cell-type-specific manner to widely impact development and physiology

Nuclear receptor superfamily

With 48 human members, it is class of transcription factors that bind and respond to hormones (steroid and thyroid hormones), vitamins (A and D), metabolic intermediates (e.g. fatty acids, bile acids, sterols) and xenobiotics. Nuclear receptors have a modular design that is typified by an N-terminal AF-1 domain (weak transcriptional activation domain), DNA-binding domain, hinge region, ligand-binding domain (ligand-dependent transcriptional activation), and a variable C-terminal domain. Through sequence-specific DNA binding as momomers, homodimers and/or heterodimers, they regulate gene expression by recruiting additional transcriptional regulators such as co-activator and co-repressor complexes

STARR-seq

An acronym for "self-transcribing active regulatory region sequencing," it is a high-throughput experimental method for evaluating the ability of DNA fragments from any source, e.g. ChIP DNA, to enhance transcription

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OUTSTANDING QUESTIONS BOX

Is GC-mediated repression of gene transcription dependent on squelching or through an undetermined molecular mechanism?

How do the ultradian, circadian and stress-induced releases of cortisol into the bloodstream modulate GR occupancy of the genome?

Does GR drive long-range chromatin interactions, and can DNA loops be used to distinguish primary versus secondary gene targets?

Does cortisol signaling involve interplay between GR and MR?

TRENDS BOX

Glucocorticoids (GCs) are among the most widely prescribed drugs in the world. They target the glucocorticoid receptor (GR) to counteract harmful inflammation associated with autoimmune and allergic reactions and organ transplant rejection, yet their negative effects can halt treatment.

GR, a transcription factor present in nearly every human cell, drives programs of tissue-specific gene expression with widespread physiological impact including effects on energy homeostasis.

Emerging genomic data reveal that GR activates transcription through monomeric and dimeric interaction with specific DNA motifs. Transcriptional inhibition by GCs may be mediated by secondary mechanisms.

GR's genomic function suggests that developing immunosuppressive-selective ligands without unwanted metabolic effects may require targeting delivery of GC drugs to specific cell types to minimize their complex tissue-specific effects.

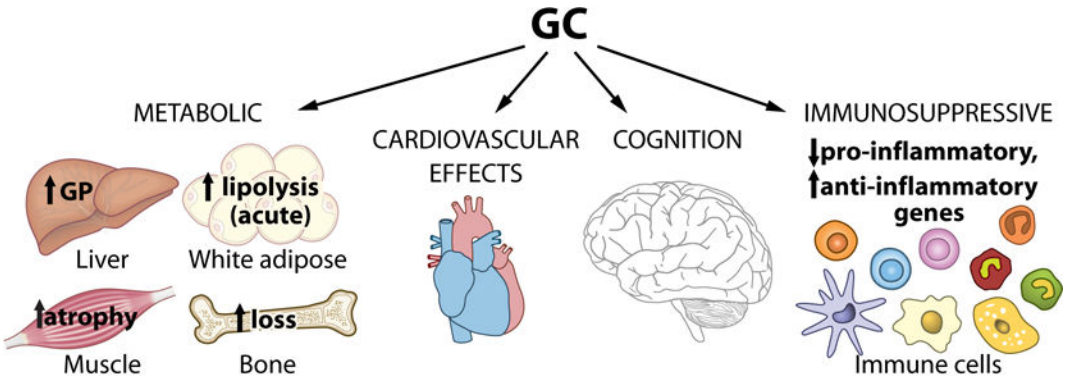

Figure 1. Tissue-specific functions of GC signaling to GR

GCs mediate distinct biological effects in different tissues and cell types to systemically influence metabolism, cardiovascular function, cognition and inflammation. They impact energy homeostasis by increasing glucose production (GP) in the liver and promoting catabolic processes in muscle, adipose and bone. Their immunosuppressive effects are conferred by the repression of pro-inflammatory genes and activation of anti-inflammatory genes in white blood cells.

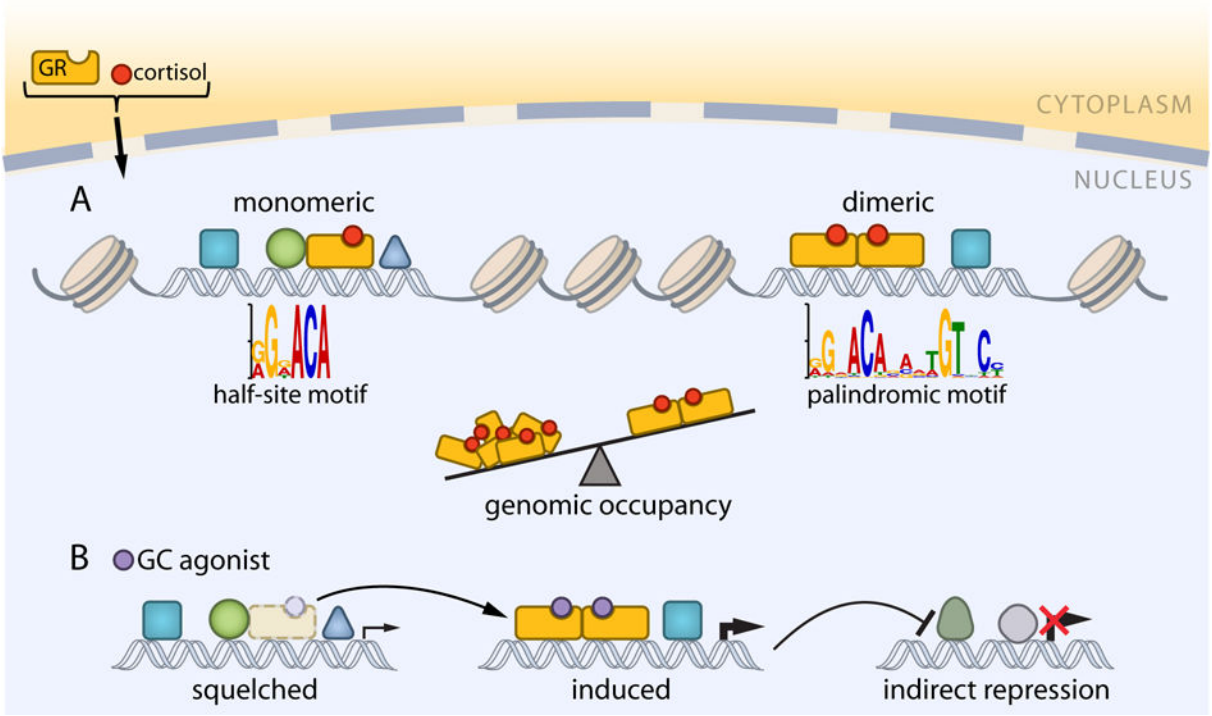

Figure 2. Genomic occupancy of GR monomers and dimers regulates gene transcription

(A) Under normal physiological settings, cortisol enables GR to activate transcription through monomeric and dimeric interaction with half-site and palindromic motifs scattered throughout the genome. Neither monomers nor dimers can efficiently access motifs buried in repressive chromatin. Thus, most of their binding sites reside in open chromatin established by lineage determining TFs. Genomic occupancy is tilted toward monomers given that monomeric sites outnumber dimeric by 5:1 in liver. (B) In response to GC drugs, induced gene expression is associated with increased GR occupancy at dimeric sites, whereas down-regulated and unchanged genes correlate with a concomitant loss of GR at monomeric sites. While the genome-wide balance remains tilted toward monomers, gain of occupancy at one set of sites at the expense of another suggests a squelching mechanism for GC-mediated repression of gene expression. Indirect repression can also result from the primary induction by dimeric GR of genes whose products repress transcription. Genomic data do not support a mechanism for direct GC-mediated repression.