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Review

Epigenetic Mechanisms of the Glucocorticoid Receptor

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The glucocorticoid receptor (GR) has been shown to be important for mediating cellular responses to stress and circulating glucocorticoids. Ligand-dependent transcriptional changes induced by GR are observed across numerous tissues. However, the mechanisms by which GR achieves cell and tissue-specific effects are less clear. Epigenetic mechanisms have been proposed to explain some of these differences as well as some of the lasting, even transgenerational, effects of stress and glucocorticoid action. GR functions in tandem with epigenetic cellular machinery to coordinate transcription and shape chromatin structure. Here, we describe GR interactions with these effectors and how GR acts to reshape the epigenetic landscape in response to the environment.

Highlights

Post-translational modification of epigenetic effectors regulates dyadic ligand-dependent glucocorticoid receptor transcriptional changes.

Noncoding RNAs regulate glucocorticoid receptor expression and function.

Tissue- and cell type-specific effects of glucocorticoids are conferred by chromatin-modifying and chromatin-state responsive protein interaction with the glucocorticoid receptor.

Introduction

The glucocorticoid receptor (GR) is one of two primary receptors responsible for mediating cellular responses to circulating corticosteroids. GR is encoded by the *NR3C1* gene and belongs to the nuclear hormone receptor superfamily. In this capacity, GR has been widely described as a ligand-dependent transcriptional regulator. The GR has been observed in most mammalian tissues [*Tissue Expression of NR3C1 - Summary* (https://www.proteinatlas.org/ENSG00000113580-NR3C1/tissue#gene_information)]. Yet, the transcriptional effects of glucocorticoids are tissue- and cell type-specific [1]. Transcriptional specificity is largely conferred by differences in chromatin landscape across tissues and physiological state directly through interactions between GR and local chromatin and indirectly through recruitment of histone- and DNA-modifying enzymes. The purpose of this review is to summarize recent key findings of the interactions between GR and epigenetic writers or erasers (chromatin-modifying enzymes) and readers (proteins recruited to specific epigenetic marks). We also offer a unique perspective on the role of the deep genome on mediating the complex actions of GR [2].

Interactions with Histone-Modifying Enzymes

Histone Methyl Transferases

Histone-modifying enzyme expression and function are affected by GR activation, leading to downstream changes in transcription. In the central nervous system and periphery, the search for GR target genes in corticosteroid sensitive cells has been a focus of molecular endocrinology research for some time. Several binding motifs have been associated with transcriptional signatures in the brain and in cultured neural cells [3–5]. Acute glucocorticoid challenge elicits differential changes in gene expression in the hippocampal formation in rodents exposed to chronic stress compared with rodents without history of chronic stress. Of note, stress history affects the hippocampal expression of several chromatin-modifying genes: ***Ash1l*** (see Glossary), ***Asf1***, ***Hist1h3f***, and *Tp63* [6]. *Ash1l* has been well characterized as a histone methyltransferase, while *Asf1* and *Hist1h3f* regulate histone turnover and *Tp63* may confer specificity of transcription factor recognition to binding sequences. Therefore, the overarching differences across tissues in transcriptional profiles related to stress history may in part be explained by the expression of chromatin-modifying genes.

Acute stress regulates heterochromatin and induces **Suv39h2** expression in the rat hippocampal formation [7]. This induction is correlated with increased H3K9me3 in the hippocampal formation, accumulating at repetitive elements in gene deserts [7,8]. This response seems to regulate transposon expression in the hippocampal genome [9–11]. These findings suggest a broad and complex set of GR-mediated regulatory interactions with hippocampal chromatin that differ with accumulated stress experience in coding and noncoding genomic elements.

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The prefrontal cortex is particularly sensitive to glucocorticoid treatment and stress. For instance, chronic stress impairs prefrontal cortex-dependent cognitive function in rodents. Wei and colleagues found in repeatedly stressed rodents, GR occupancy was enriched at the ***Hdac2*** promoter glucocorticoid response element and facilitates HDAC2 upregulation [12]. Inhibition and knockdown of HDAC2 reversed stress-induced impairments in prefrontal cortex synaptic transmission and rescued prefrontal-dependent cognitive function. The cognitive impairments of stress were regulated by ubiquitination of AMPA subunits by **NEDD4**, ultimately determining the availability of AMPA-glutamate receptors in the prefrontal cortex. **G9a** and **GLP** are both transcriptional cofactors with histone methyltransferase capacity. In the prefrontal cortex of chronically stressed rodents, HDAC2 downregulates G9a, facilitating a loss of repressive histone methylation at the *Nedd4-1* promoter and transcriptional activation of NEDD4. These findings suggest that chronic stress downregulates AMPA receptors through GR-dependent regulation of epigenetic writers, which induce expression of ubiquitin ligase NEDD4. These proteins may be therapeutic targets for attenuating cognitive deficits associated with chronic stress and/or glucocorticoid exposure (see Boxes 1 and 2).

In renal duct epithelia, **ENaC** is canonically regulated by aldosterone-dependent activities of the mineralocorticoid receptor (MR), in part through ligand-dependent interactions with hormone response element within the *ENaC* promoter. Murine models have suggested an MR-independent pathway, as MR-depleted animals do not display the salt-wasting phenotype characteristic of ENaC-depleted animals. **Dot1a** appears to facilitate compensatory expression of renal ENaC in MR-depleted conditions. **Sgk-1** disrupts Dot1a-**Af9** and histone deacetylase (HDAC) Dot1a-**Sirt1** complexes. Disruption of these complexes appears to facilitate chromatin accessibility, which is permissive for transcriptional de-repression of ENaC. GR may function through Sgk-1 with aldosterone-dependent MR signaling to alter Dot1a methyltransferase activity, thereby relaxing local chromatin at relevant loci to regulate renal salt retention [13].

H3K9-specific methyltransferase transcriptional repression has been well described, but G9a is also recruited to euchromatin as a coactivator in cultured cells [14]. G9a is a transcriptional coactivator for GR through direct interaction with **GRIP1**. G9a also functions as a molecular scaffold for transcriptional coactivators for GR target genes [15]. Counterintuitively, G9a appears to tune cellular responses to GR-induced gene expression rather than mediate repression of target genes. Poulard *et al*. (2017) first showed post-translational modification of G9a and GLP regulates their association with **HP1γ** and subsequent formation of a ternary complex with GR at a subset of glucocorticoid inducible genes in A459 cells [16]. For this subset of genes, transcriptional induction requires both site-specific methylation of G9a and GLP and is prevented by site-specific phosphorylation on either G9a or GLP. This molecular switch regulates G9a/GLP association with HP1γ and glucocorticoid-inducible expression of the subset of G9a/GLP requiring genes (Figure 1). GR recruits histone methyltransferases G9a and GLP as necessary cofactors and implying convergent cellular signals may act on post-translational modification of histone methyltransferases to confer coactivator or corepressor function to GR-recruited cofactors. Poulard and colleagues have gone on to posit a functional role for these histone methyltransferases in glucocorticoid-dependent treatment of acute lymphoblastic leukemia. Inhibition of H3K9 demethylases increased G9a/GLP/HP1γ occupancy at GR target genes, which subsequently enhanced cell death and attenuated cell migration [17]. When demethylase inhibition was coupled with GR activation, caspase activation was markedly enhanced. Therefore, H3K9-specific demethylase inhibitors may bolster traditional corticosteroid treatments of leukemias.

GR is known to regulate transcription in the liver. **MLL3** is downregulated in mice during cholestasis [18]. MLL3 downregulation also resulted in loss of recruitment of other transcriptional cofactors at relevant loci. In the liver, Insig2a increases during fasting, attenuating lipid synthesis. Roqueta-Rivera and colleagues showed Insig2a is GR-**SETDB2** dependent [19]. INSIG2 negatively regulates SREBP. Acute glucocorticoid treatment facilitates SREBP reduction. Transcriptional repression of SREBP by glucocorticoids was prevented by transient knockdown of SETDB2 or INSIG2. Here, epigenetic writers deterministically govern ligand-dependent association of GR with target genes either through direct interaction with the receptor or indirectly through modification of histone methylation valence or recruitment of other transcriptional cofactors.

Glossary

Af9: ALL1-Fused Gene from Chromosome 9 Protein, binds histone H3 acetylated at Lys-9 (H3K9ac).
Asf1: Anti-Silencing Function 1A Histone Chaperone, a key component of the histone donor complex that functions in nucleosome assembly.
Ash1l: ASH1-Like Histone Lysine Methyltransferase, methylates Lys-36 of histone H3 (H3K36me).
ATF-2: Activating Transcription Factor 2, transcription factor that forms a complex with c-Jun and stimulates cyclic AMP responsive element (CRE)-dependent transcription, acetylates histone H2B and H4 subunits.
CBP: CREB Binding Protein, transcriptional enhancer that binds to cyclic AMP binding protein, histone acetyltransferase.
DMAP1: DNA Methyltransferase 1 Associated Protein 1, interacts with HDAC2 and may help to facilitate histone deacetylation.
DNMT1: DNA Methyltransferase 1, transfers methyl groups to cytosine nucleotides of genomic DNA, responsible for maintaining methylation pattern during replication.
DNMT3A/B: DNA Methyltransferase 3 Alpha/Beta, DNA methyltransferase implicated in embryonic development, imprinting, and X-inactivation.
Dot1a: murine homology of human Dot1L, methylates Lys-79 of histone H3 (H3K79me).
ENaC: epithelial Na^+ channel subunit, essential role in salt retention.
EZH2: Enhancer of Zeste 2 Polycomb Repressive Complex 2 subunit, methylates H3K9 and H3K27.
G9a: euchromatic histone lysine methyltransferase 2, methylates histone H3 at Lys-9 (H3K9me1/H3K9me2).
Gas5: Growth Arrest Specific 5, noncoding RNA upregulated in growth arrested cells and contains a pseudo-mimic GRE.
GLP: G9a-Like Protein, methylates histone H3 at H3K9 (H3K9me1/H3K9me2).
GRIP1: Glucocorticoid Receptor-Interacting Protein-1, transcriptional coactivator protein with histone acetyltransferase activity.

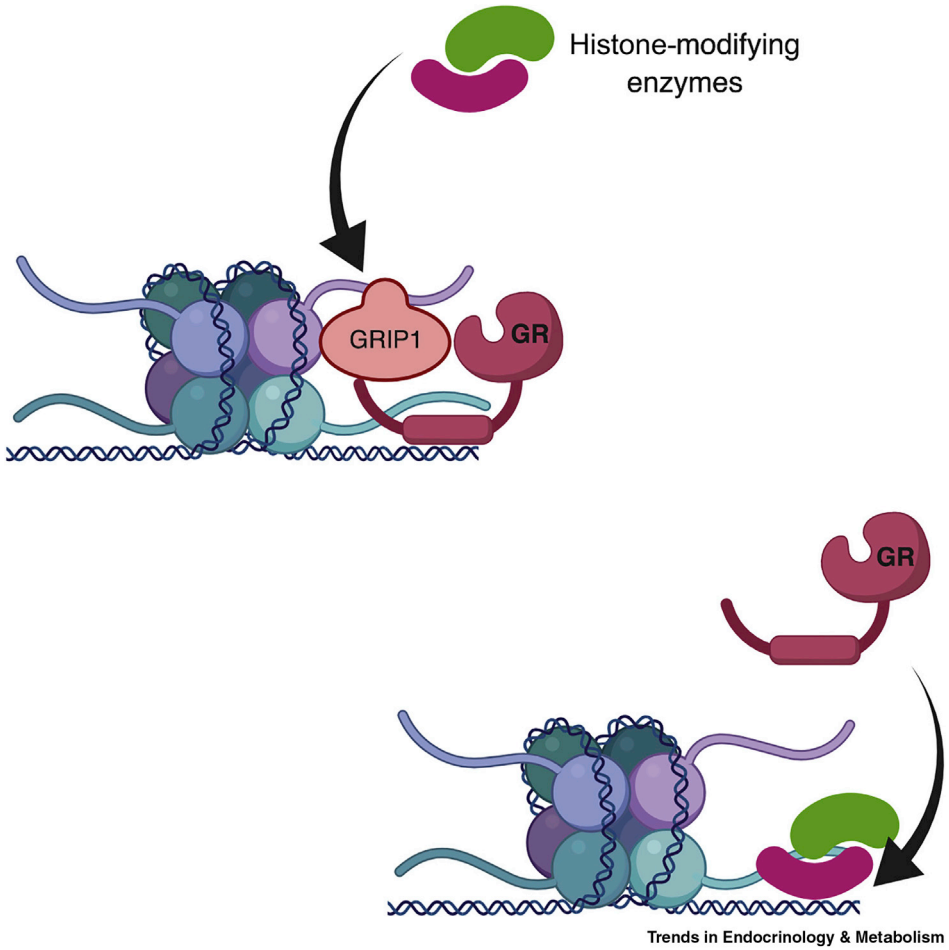

Figure 1. Post-translational Modification of Histone-Modifying Enzymes Functions as a Switch for Glucocorticoid Enhancement or Repression of Target Genes.

Modification of specific residues govern GLP/G9a association with HP1γ and glucocorticoid receptor (GR) at target genes. Site-specific methylation of GLP/G9a promotes association with HP1γ/GR complex and is permissive for transcriptional induction (left). Site-specific phosphorylation of GLP/G9a prevents association with HP1γ/GR complex and facilitates transcriptional repression of target genes (right). This figure was created using BioRender (https://biorender.com/).

Several histone methyltransferases related to GR function as epigenetic writers bear clinical relevance for a variety of cancer. In prostate cancers, GR can promote resistance to antiandrogens. Shah *et al.* show GR-mediated antiandrogen resistance is reversible by down regulation of GR expression [20]. Androgen receptor and **EZH2** association with the GR locus silence expression. BET bromodomain inhibition prevents GR antiandrogen insensitivity. Similarly, a subset of non-Hodgkin lymphoma patients carry EZH2 mutations associated with constitutive expression; this is a cancer for which corticosteroids are often prescribed. Knutson *et al.* found that when an EZH2 inhibitor was combined with classic corticosteroid treatment (in culture and mouse models) or dexamethasone, non-Hodgkin lymphoma cell death was dramatically increased compared with corticosteroid treatment alone [21]. In addition, expression of GR target genes was markedly increased when receptor agonists were combined with EZH2 inhibitor compared with agonist alone. A recent study found more than a quarter of blastic plasmacytoid dendritic cell neoplasm (an aggressive form of leukemia) patients carried a deletion of *NR3C1* [22]. *NR3C1* haploinsufficiency was unsurprisingly associated with reduced GR expression and poor survival. Likewise, *NR3C1* haploinsufficiency was correlated with corticosteroid

HDAC1/2: Histone Deacetylase 1/2, deacetylates N terminal region of core histones.
HDAC6: Histone Deactylase 6, deacetylates N terminal region of core histones, regulates HSP90 activity.
Hist1h3f: Histone Cluster 1 H3 Family Member F, a replication-dependent histone H3-isoform.
HP1γ: heterochromatin protein 1 gamma, binds to histone H3 methylated at Lys-9 facilitating epigenetic repression.
HSP90: heat shock protein 90, chaperone protein that complexes with unliganded steroid hormone receptors and regulates steroid hormone nuclear translocation.
JMJD3: Jumonji Domain Containing 3, histone demethylase that demethylates histone H3 at H3K27.
LSD1: Lysine Demethylase 1A, histone demethylase that demethylates histone H3 at H3K4 and H3K9.
MeCP2: Methyl CpG Binding Protein 2, binds to genomic methylated cytosine nucleotides.
MLL3: Mixed-Lineage Leukemia Protein 3, histone methyltransferase methylates histone H3 at Lys-4 (H3K4me), indispensable at promoters for GR-dependent induction of bile transporter related genes.
NEDD4: neural precursor cell expressed, developmentally down-regulated 4; E3 ubiquitin-ligase.
p300: E1A Binding Protein P300, histone acetyltransferase in complex with CREB-Binding Protein (CBP).
PCAF: P300/CBP-Associated Factor, acetylates histone H3 and H4 subunits.
SETDB2: SET Domain Bifurcated 2, histone methyltransferase that tri-methylates H3K9.
Sgk-1: serum glucose kinase 1, protein-kinase induced by glucocorticoids.
Sirt1: sirtuin 1, deacetylates H3K9ac.
SRC-1: steroid receptor co-activator 1, histone acetyl-transferase with nuclear receptor interacting domains.
Suv39H2: suppressor of variegation 3-9 homology 2, trimethylates Lys-9 of histone H3 (H3K9me3).
TDG: Thymine DNA Glycosylase, binds to 5-formylcytosine (5fC)

Box 1. Stress, Epigenetics, and the Molecular Basis of Fear Memories

Sweatt and colleagues first showed within the contextual fear conditioning paradigm that histone H3 acetylation was associated with long-term potentiation within the hippocampus and histone deacetylase inhibition enhanced fear memory consolidation [70]. Since then, DNA methylation of the *Bdnf* promoter was shown to be associated with changes in local chromatin and fear memory consolidation [71,72]. Many other epigenetic readers and writers have been implicated in the molecular basis of contextual fear memories (for review, see [73]). These findings have been extended to the amygdala as well, suggesting a role in local chromatin in Pavlovian cue-based fear memory consolidation [74]. Behavioral modifications that change local chromatin may prove useful for treatment of neuropsychiatric disorders such as post-traumatic stress disorder [75].

and 5-carboxylcytosine (5caC) to facilitate DNA demethylation.
TET1: Ten-Eleven Translocation 1, catalyzes the conversion of 5-methyl cytosine to 5-hydroxymethylcytosine (5hmC) mediating the conversion of 5hmC to 5fC.
Tip60: Tat Interacting Protein, 60 kDa, histone acetyltransferase that acetylates histone H4 and H2A subunits.

resistance and EZH2 loss of function. Clinically, targeting of EZH2 may prevent drug resistance or bolster standard treatments.

Histone Demethylases

Far less has been done to investigate the interactions between GR and histone demethylases. Patrick *et al*. (2017) found class I HDAC inhibitors prevented ligand-associated GR transcriptional repression [23]. This repression was independent of nucleosome occupancy or histone acetylation and did not require GRIP1 scaffolding. Rather, glucocorticoid and HDAC cotreatment induced reduction in H3K4me2 at unrepressed promoters (while unchanging local H3K4me3 occupancy), implicating **LSD1**. Further, knockdown of LSD1 rescued ligand-induced repression of target genes, suggesting LSD1 was indispensable for repression for these target genes.

Another recent finding showed GR activation suppresses **JMJD3** histone demethylase expression in endothelial cells found in blood–brain barrier tight junctions [24]. Ligand-activated GR and N-CoR are recruited to the negative GRE upstream of the JMJD3 transcription start site in endothelial cells treated with TNFα. Corticosteroid-attenuated JMJD3 was associated with suppressed MMP-2, -3, and -9. This implies glucocorticoids may support endothelial tight junctions during inflammatory challenge indirectly via regulation of JMJD3 histone demethylase. Therefore, transient repression of histone demethylases by GR may bear importance in a variety of inflammation-related neuropathologies, including schizophrenia, autism spectrum disorder, and mood disorders (for a review of blood–brain barrier function as it relates to psychiatric disorders, see [25]).

Histone Acetyl Transferases (HATs)

GRs interact with several HATs. GRIP1 interacts directly with several class I nuclear receptors, including GR [26,27]. Through GRIP1, GR can associate with the SAGA histone-modifying complex. GR recruits the SAGA complex to chromatin to facilitate histone acetylation through GCN5 activity [26]. Likewise, **Tip60** is recruited to chromatin by GR as a transcriptional coactivator [28]. Ligand-bound GR readily recruits GRIP1 [29]. In addition to intrinsic HAT activity, GRIP1 appears to serve as a molecular scaffold for recruitment of additional cofactors, including histone acetyltransferases **PCAF** and **p300**, for transcriptional coactivation of GR target genes (Figure 2) [30]. These foci can

Box 2. Circadian Rhythm: GR as a Transcriptional Cofactor

The relation between GR and p300 histone acetyltransferase has been ubiquitously described. In short, GR interacts with the nuclear receptor interaction domain of the p300. p300 recruitment is necessary for transactivation at some known positive GREs (e.g., DUSP1) [76]. GR also directly interacts with histone acetyltransferase CLOCK [77] (for review, see [78]). The epigenetic control of the circadian rhythm via the molecular clock has been well described (for review, see [79]). Circulating levels of glucocorticoids follow a circadian rhythm in mammals. In addition to recruitment of clock proteins, GR can also influence the expression of several clock genes (e.g., *Per1*) [80]. In this capacity, GR serves as both a cofactor for p300 and CLOCK HATs and can indirectly feedback on CLOCK activity via induction of Per1 to influence the molecular clock.

act in concert with other transcription factors (e.g., Stat3 or TR2) to enhance corticosteroid-induced transcriptional coactivation or exchange cofactor subunits [31,32]. Active-Stat3 acts synergistically with GR transcriptional coactivators **SRC-1**, PCAF, **CBP**, and GRIP1, enhancing transactivation [31]. Activation of TR2 orphan receptor facilitates exchange of PCAF at GRIP1 for RIP140 to facilitate transrepression of *TR2* promoter [32]. Interestingly, this suggests transcriptional negative feedback can utilize 'coactivator' complexes for transrepressive processes, which may ultimately depend on GR recruitment and underlie some of the biphasic effects of glucocorticoids on transcription. When antagonized, GR still associates with GRIP1, suggesting cofactor recruitment may be distinct from the capacity of these factors to alter transcription [33]. Alternatively, these findings may support the duality of GRIP1 function as a transcriptional coactivator and corepressor [34].

Ligand-activated GR inhibits **ATF-2** activation via attenuated cJun phosphorylation [35]. GR-dependent inhibition of cJun phosphorylation appears to be induced by post-translational modification of the receptor by TNFα, but ATF-2 could be used as an alternative substrate [36]. In CD4+ cells, knockdown of ATF-2 is required for corticosteroid-induced transactivation, raising the question of the role of ATF-2 in chronic inflammatory diseases for which corticosteroids are commonly prescribed for treatment [37]. Foxf1(+/-) mice have reduced GR and ATF-2 expression in the lungs and perturbed lung development resulting in lethality, perhaps indicating an indispensable role for ATF-2 in lung development [38]. Glucocorticoid treatment in prostate cancer cells suppresses proliferation and results in attenuated ATF-2 expression, suggesting a putative mechanism for GR tumor suppression via epigenetic effectors [39].

A recent study showed splice variants of SRC-1 histone acetyltransferase in the mouse brain conferred GR transcriptional responsiveness in a gene-specific manner [40]. Meijer and colleagues observed

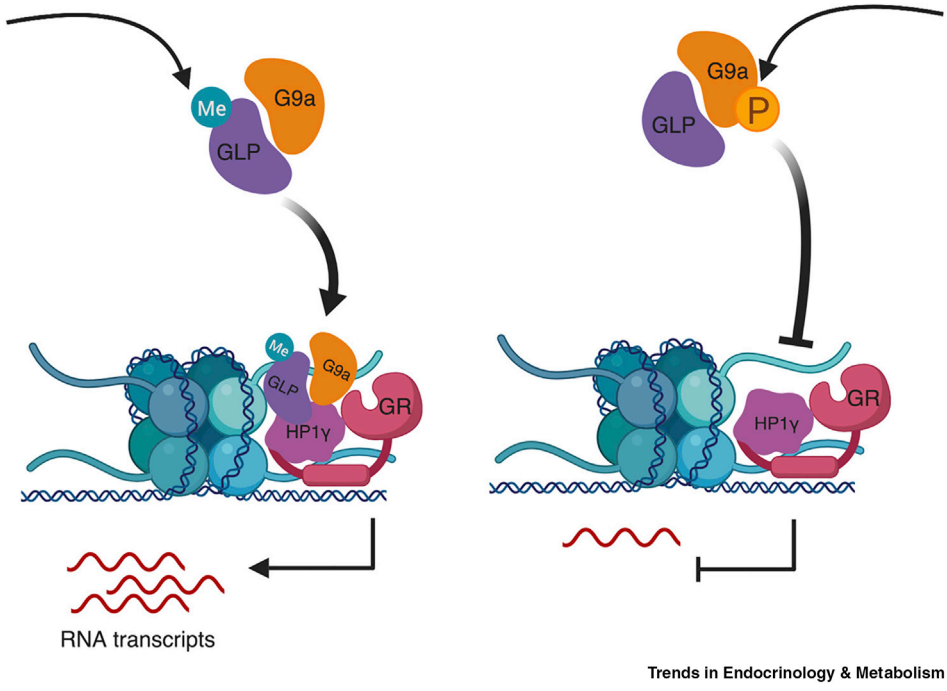

Figure 2. The Glucocorticoid Receptor (GR) Recruits Histone-Modifying Enzymes and Can Be Recruited by Enzyme Complexes.
The glucocorticoid receptor is shown recruiting histone-modifying enzymes through GRIP1 scaffolding at local chromatin (top left). The glucocorticoid receptor is shown recruited to histone-modifying enzymes at local chromatin (bottom right). This figure was created using BioRender (https://biorender.com/).

that the splice variant of SRC-1 determined GR-dependent transcription of Crh mRNA but did not affect the ligand-dependent induction of *Fkbp5* expression [40]. These findings suggest two important points. First, structural variations may affect the 'intrinsic' properties of epigenetic writers. Secondly, these splice variations introduce layered specificity to GR transcriptional cofactors.

Despite redundancy in function SRC-1, -2, and -3, HATs recruitment by GR is not interchangeable [41,42]. SRC-1-deficient mice exhibit increased adrenal SRC-2 and SRC-3 expression [43]. This SRC-2 and -3 dysregulation was associated with increased adrenal sensitivity to ACTH and reduced glucocorticoid-mediated negative feedback in the hypothalamic–pituitary–adrenal axis. SRC-3-deficient mice exhibit increased sensitivity to lipopolysaccharide-induced toxic shock, suggesting GR-dependent anti-inflammatory effects may be mediated through these transcriptional cofactors [44]. Under co-agonism, ERα and GR crosstalk requires GRIP1 and SRC-3, suggesting regulation of epigenetic landscape mediates complex multihormonal transcriptional changes [42]. Given degeneracy within hormone response elements, epigenetic enzyme complexes could confer tissue- or cell type-related crosstalk effects of during multihormonal signals, determining preferential binding of hormone receptors to loci [45].

HDACs

In addition to regulating chromatin acetylation through HATs, GR can also regulate chromatin acetylation indirectly by changing HDAC expression and directly by mediating HDAC activity. Bronchial biopsies of asthma patients have higher HDAC1 expression compared with controls [46]. Low levels of GRβ, the dominant negative isoform of GRα, were observed in severe asthma patients. Low levels of GRβ may reduce the ability of corticosteroids to repress HDAC1 expression and point to a potential mechanism of corticosteroid resistance in asthma patients. **HDAC6** appears to be required for GR-dependent gluconeogenesis in hepatic cells [47]. Dexamethasone-treated HDAC6 knockout mice show attenuated transcriptional responses for GR target genes *Dusp1* and *Sgk1* compared with wild type mice. Knockout mice also had reduced ligand-dependent GR nuclear translocation, implicating HDAC6 in translocation of nuclear receptors.

Other roles have been identified for HDAC6 in the prefrontal cortex. Acute stress potentiates prefrontocortical glutamatergic transmission through GR induction of *Sgk1*. Lee *et al.* (2012) showed that knockdown or inhibition of HDAC6 attenuated glutamatergic synaptic potentiation in the prefrontal cortex [48]. HDAC6 inhibition prevented ligand-dependent transcription of *Sgk1*. Similar results were produced when GR chaperone **HSP90** was targeted, suggesting that HDAC6 may function through HSP90 to alter synaptic function in the prefrontal cortex in response to acute stress. Evidence suggests that HDAC6 regulates HSP90 association with GR in the brain [49]. Selective knockout of HDAC6 in dorsal raphe neurons reduced the acute anxiogenic effects of glucocorticoids in mice. In mice exposed to chronic social defeat, HDAC6 depletion in serotonergic neurons prevented social avoidance and midbrain hypo-excitability of neurons expressing the serotonin receptor, 5-HT1A. HDAC6 depletion was associated with reduced interaction between HSP90 and GR and, subsequently, a loss of GR translocation during social defeat (during which glucocorticoids are elevated). The effects of HDACs may be multimodal and extend beyond intrinsic deacetylase activity by regulating GR coactivator complexes.

Nonspecific HDAC inhibitors like valproic acid have been used to treat neurological disorders and have been linked to increased visceral fat load. Kuzmochka *et al.* investigated the role of specific HDACs on GR-dependent preadipogenesis. Knockdown and loss-of-function mutagenesis of HDAC1 demonstrated that HDAC1 is indispensable for suppressing glucocorticoid-mediated adipogenesis by preventing receptor accumulation at the C/EBPβ promoter [50]. Converging research shows that HDACs function as coregulators to mediate nuclear hormone receptor occupancy of DNA loci. A recent studied showed specific HDACs determine occupancy of the androgen receptor and GR at the same hormone response element [51]. In this paradigm, HDAC2 occupancy prevented androgen receptor association with the response element and instead promoted GR association. HDAC2 acted as a transcriptional enhancer, suggesting distinctive roles for HDACs in foci recruitment and coactivation or repression. HDAC2 also appears to have Trpv1-dependent effects of

chronic stress [52]. Tprv1(-/-) mice display reduced hippocampal GR-induced HDAC2 expression and are resilient to unpredictable chronic stress. Both transcriptional and behavioral effects in Tprv1(-/-) mice are reversed by overexpression of HDAC2, suggesting HDAC2 as a link between this ion channel and the stress response. HDAC1 appears indispensable for ligand-dependent GR-mediated transcription of gluconeogenesis related genes. In the rat liver, knockdown of HDAC1 reduced acetylation and transcriptional induction of GR target genes [53]. HDAC inhibition also reduced blood pressure and ion regulator expression in the kidneys in ACTH-stimulated rats, suggesting HDAC activity may facilitate hypertension and hyperglycemia in this context.

Interactions with DNA-Modifying Enzymes

DNA Methyltransferases (DNMTs)

DNA-modifying enzymes can regulate GR function and, reciprocally, GR can regulate DNA-modifying enzyme expression and function. For example, DNMT activity appears to regulate hepatic GR expression. Fasting is associated with increased corticosteroid secretion and studies have found associations between nutritional deprivation and changes in DNA methylation. Feeding a protein-restricted diet to pregnant rats results in hypomethylation of specific gene promoters in the offspring. In hepatic cells of protein-restricted offspring, GR methylation was reduced to nearly a third of normal levels and receptor expression was nearly double that of control offspring [54]. Promoter hypomethylation was also associated with the accumulated transcriptional-enhancing histone modifications and reduction in DNMT1 expression. The same group found DNMT1 expression predicted GR promoter methylation in the orthologous human region [54]. Therefore, a similar mechanism of metabolic programming of hepatic GR sensitivity by maternal diet may be conserved across mammals. These findings suggest that the epigenetic regulation of hepatic GR expression and associated metabolic phenotype may be due to reduced DNMT1 expression.

Tissue-specific expression of DNMTs has been observed across species [Tissue Expression of DNMT1 - Summary (https://www.proteinatlas.org/ENSG00000130816-DNMT1/tissue)] [55,56]. In chickens, early-life food deprivation appears to pattern DNMT expression in the brain and liver [56]. Kang and colleagues found **DNMT3A/B** expression was elevated in the anterior pituitary and **DMAP1** and DNMT3A expression was elevated in liver in food-deprived animals compared with controls [56]. GR expression was increased in liver and persisted for at least 6 weeks, suggesting early-life metabolic stress may program hepatic function through epigenetic regulation of GR. Importantly, this highlights the capacity for DNMTs to function in tandem with GR to regulate the effects of early-life nutritional stress. In maternal malnutrition models, offspring from dams receiving low-protein diet or dexamethasone have upregulated *Agtr1a* mRNA in the paraventricular nucleus of the hypothalamus. Expression of *Agtr1a* is crucial for renin–angiotensin system function, such that knockdown prevents salt-induced hypertension. Upregulated *Agtr1a* expression appears to be associated with reduced DNMT3a expression, reduced DNMT3a occupancy at the *Agtr1a* locus, and reduced *Agtr1a* DNA methylation [57]. In both low-protein diet or dexamethasone-treated offspring, salt loading increased blood pressure. In dexamethasone-treated Agtr1a-deficient mice, DNMT3a was downregulated but salt loading did not increase blood pressure. In all, prenatal glucocorticoid exposure, including through maternal malnutrition, regulates DNMT3a expression and, hence, epigenetically programs angiotensin signaling in offspring and contributes to lifetime susceptibility to hypertension.

In the brain, glucocorticoids downregulate hypothalamic CRH expression. Sharma and colleagues have demonstrated a putative mechanism for this negative feedback. GR associates with *Crh* promoter in a ligand-dependent manner. GR occupancy was associated with HDAC1, **MeCP2**, and DNMT3b, suggesting a potential repressive complex for associated changes in local chromatin in the *Crh* promoter [58]. Acute stress downregulates GR expression in the rat hippocampal formation. Mifsud and colleagues showed that downregulated GR expression was associated with increased promoter methylation following forced swim stress [59]. Increased *Nr3c1* promoter methylation was associated with increased DNMT3a accumulation. There is no difference in GR binding to nGRE upstream of the *Nr3c1* gene between stressed and control animals, implying transient changes in expression were likely due to epigenetic regulation of the locus.

DNA Demethylases

Few studies have looked specifically at DNA demethylase interactions with GR. Hippocampal GR expression is altered by maternal care in the first few weeks of life, which persists into adulthood and has consequences for anxiety-like behaviors [60]. Around birth, the *Nr3c1* promoter is methylated and subsequent high maternal licking and grooming behavior during the first postpartum week leads to demethylation specifically at the NGFI-A consensus motif in the 1–7 region of the promoter [61]. This increases NGFI-A binding and acetylated H3K9 occupancy and confers adult hippocampal GR expression, acute stress reactivity, and anxiolytic behavior [60]. Importantly, targeting hippocampal histone acetylation or supplementation with a methyl donor can reverse the adult phenotype [62]. The molecular mechanism by which maternal care demethylates the *Nr3c1* promoter is still unknown. Understanding demethylation is clinically relevant, as hypermethylation of the *NR3C1* promoter has been observed in suicide victims with a history of childhood abuse [63,64].

DNMT1, BDNF, GR, and the DNA demethylase **TET1** are upregulated in corticolimbic areas of patients with schizophrenia [65]. Expression in peripheral blood lymphocytes showed similar upregulation of epigenetic effectors. These findings suggest DNA methylation enzymes working to methylate/demethylate and GR may be aberrantly expressed in brain regions associated with schizophrenia. A recent study found ligand-dependent recruitment of the androgen receptor to the hormone response element in

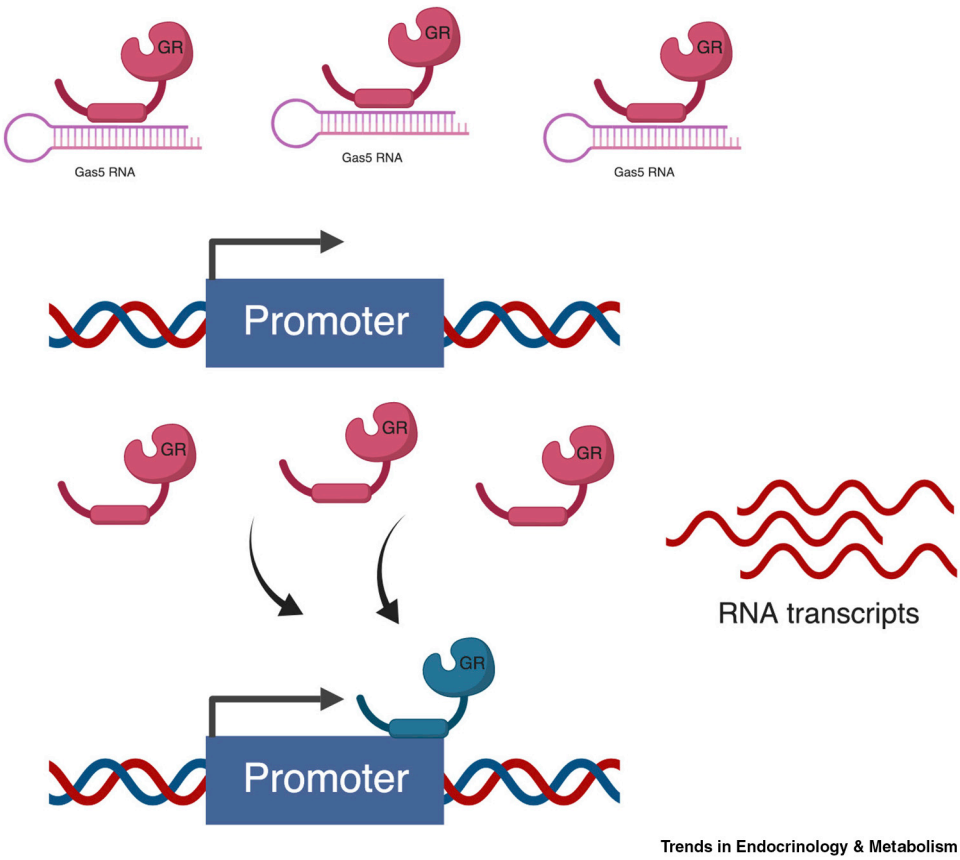

Figure 3. Noncoding RNA Gas5 Represses Glucocorticoid Receptor (GR) Induction of Target Genes.
Gas5 RNA contains a pseudo-glucocorticoid response element and binds the activated glucocorticoid receptor. This serves to sequester ligand-activated receptors preventing binding to target gene promoters and target gene induction (above). In the absence of gas5 RNA ligand-activated glucocorticoid receptors bind to target gene promoters and facilitate transcription. This figure was created using BioRender (https://biorender.com/).

Key Figure

Epigenetic Mechanisms of Glucocorticoid Receptor (GR) Signaling

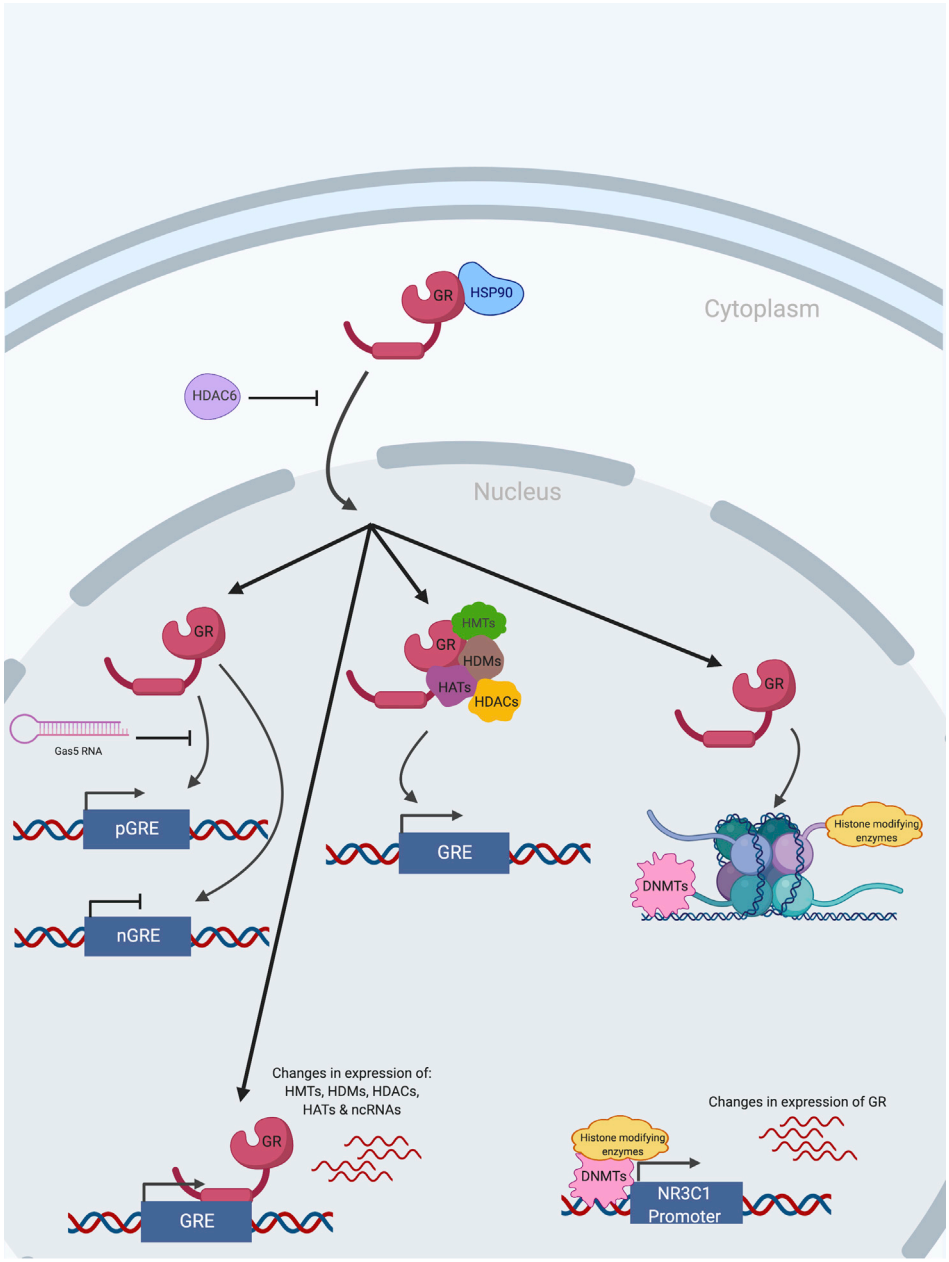

Figure 4. The glucocorticoid receptor translocates into the nucleus following ligand activation, regulated in part by histone deacetylase 6 (HDAC6). The glucocorticoid receptor then binds to positive (pGRE) or negative

(Figure legend continued at the bottom of the next page.)

the *Sgk1* promoter. The androgen receptor formed a complex with RNA Pol II, TET1, and **TDG** [66]. Given the overlap between AREs and GREs, it is possible GR may also act at this locus. Further, the recruitment of both TET1 and TDG demethylases with RNA Pol II suggest a direct role for transient demethylase activity ligand-dependent transcription. Given the importance of DNA methylation in regulating transcription, GR interactions with this machinery have clear implications for its role in modulating the transcriptional landscape in response to environmental inputs.

Interactions with Noncoding RNAs (ncRNAs)

ncRNAs

Several studies have outlined an emerging role for ncRNAs in GR signaling. Notably, the ncRNA **Gas5** has been shown to dynamically regulate GR function [66]. Kino and colleagues found Gas5 RNA directly interacts with ligand-bound GR via the receptor DNA binding domain [66]. Gas5 binding of GR both prevents receptor binding to GREs found in responsive genes, preventing target gene expression (Figure 3). Thus, Gas5 sequesters GR and attenuates transcriptional regulation of GR targets. This study was one of the first, though likely not the last, to show a 'decoy', or endogenous antagonist mechanism for ncRNA-GR interplay. Others have shown interesting interactions with miRNAs and GR (for review, see [67]). Recently, a study described a transcriptional negative feedback loop, whereby ligand-stimulated GR induces miR-29a expression, which targets *Nr3c1* RNA for repression [68]. The mechanism was conserved between mice and humans and appeared to regulate adipogenesis, suggesting a potential mechanism for glucocorticoid resistance that may contribute to a variety of metabolic disorders such as insulin resistance, obesity, and hyperglycemia. Other studies have also examined roles for ncRNAs in regulating GR activity. Future studies examining the direct physical interactions between GR and these RNAs would be useful in characterizing novel mechanisms of GR regulation.

Concluding Remarks

GR has numerous complex interactions with epigenetic machinery. GR can function as a cofactor, recruiting epigenetic readers and writers. As a reader, GR function is altered by histone- and DNA-modifying enzymes and is attenuated by ncRNA (see Figure 4). Some have argued local chromatin does not dictate GR transcriptional activity, but few studies have comprehensively accounted for the full range of chromatin-modifying enzymes or regulatory RNAs [69]. This view does not comport with the wider understanding of transcription factor and chromatin interactions. Future work should continue to specifically define the role of DNA demethylases and ncRNAs on GR function, as well as describe the interactions between GR and the deep genome. However, it is already clear that GR is capable of globally transducing environmental inputs into changes in the epigenetic landscape, which in turn result in changes in both behavior and physiology. A more complete understanding of this role for GR will have significant implications for our understanding of both medical and mental disorders, as well as how both interact within individual patients (see Outstanding Questions).

Outstanding Questions

How do chromatin landscapes near target genes following acute glucocorticoid exposure compare with landscapes following chronic glucocorticoid exposure?

How do glucocorticoid receptors interact with large noncoding swathes of the genome?

In addition to Gas5 and miRNAs, what other noncoding RNAs regulate glucocorticoid receptor activity?

By what mechanism do DNA demethylases act on the *Nr3c1* promoter and on GR-target gene promoters?

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glucocorticoid response elements (nGRE) in the DNA, thereby enhancing or repressing transcription, respectively. Gas5 noncoding RNA (ncRNA) sequesters ligand-bound receptors. The glucocorticoid receptor recruits histone methyltransferases (HMTs), histone demethylases (HDMs), histone acetyltransferases (HATs), and HDACs to promoters. Histone-modifying enzymes and DNA methyltransferases (DNMTs) recruit the glucocorticoid receptor to local chromatin. The glucocorticoid receptor changes the expression of HMTs, HDMs, HDACs, HATs, and ncRNAs. Reciprocally, DNMTs and histone-modifying enzymes act at the *NR3C1* promoter to regulate glucocorticoid receptor expression. This figure was created using BioRender (https://biorender.com/).

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