Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Pharmacology

Anti-inflammatory glucocorticoid action: genomic insights and emerging concepts

Afzal Pasha Syed[1,3], Franziska Greulich[1,3], Suhail Akhtar Ansari[1] and Nina Henriette Uhlenhaut[1,2]

Glucocorticoids (GCs) are widely used immunomodulators. They regulate gene expression by binding and activating the Glucocorticoid Receptor (GR), but underlying transcriptional mechanisms remain enigmatic. This review summarizes recent findings identifyingspecific GR-bound DNA sequences whose configuration may affect transcriptional output. Additional factors affecting GR's anti-inflammatory actions, including different chromatin states such as DNAse hypersensitive regions and histone marks will be discussed, together with the relevant transcriptional co-regulators and promoter/enhancer features. Furthermore, the involvement of non-coding RNAs such as lncRNAs, miRNAs and eRNAs adds another level of regulation to the GR's transcriptional activity. Characterizing and understanding these multiple mechanisms will be crucial for developing more targeted immunomodulatory therapies with reduced adverse effects such as obesity, diabetes and osteoporosis.

Addresses
[1] Molecular Endocrinology, IDC, Helmholtz Diabetes Center (HMGU) and German Center for Diabetes Research (DZD), Ingolstaedter Landstr. 1, 85764 Neuherberg, Munich, Germany
[2] Metabolic Programming, TUM School of Life Sciences Weihenstephan, Gregor Mendel Str. 2, 85354 Freising, Germany

Corresponding author:
Uhlenhaut, Nina Henriette (henriette.uhlenhaut@helmholtz-muenchen.de)
[3] These authors contributed equally to this work.

Current Opinion in Pharmacology 2020, **53**:35–44

This review comes from a themed issue on **Immunomodulation**

Edited by **Tamás Röszer**

https://doi.org/10.1016/j.coph.2020.03.003

Introduction

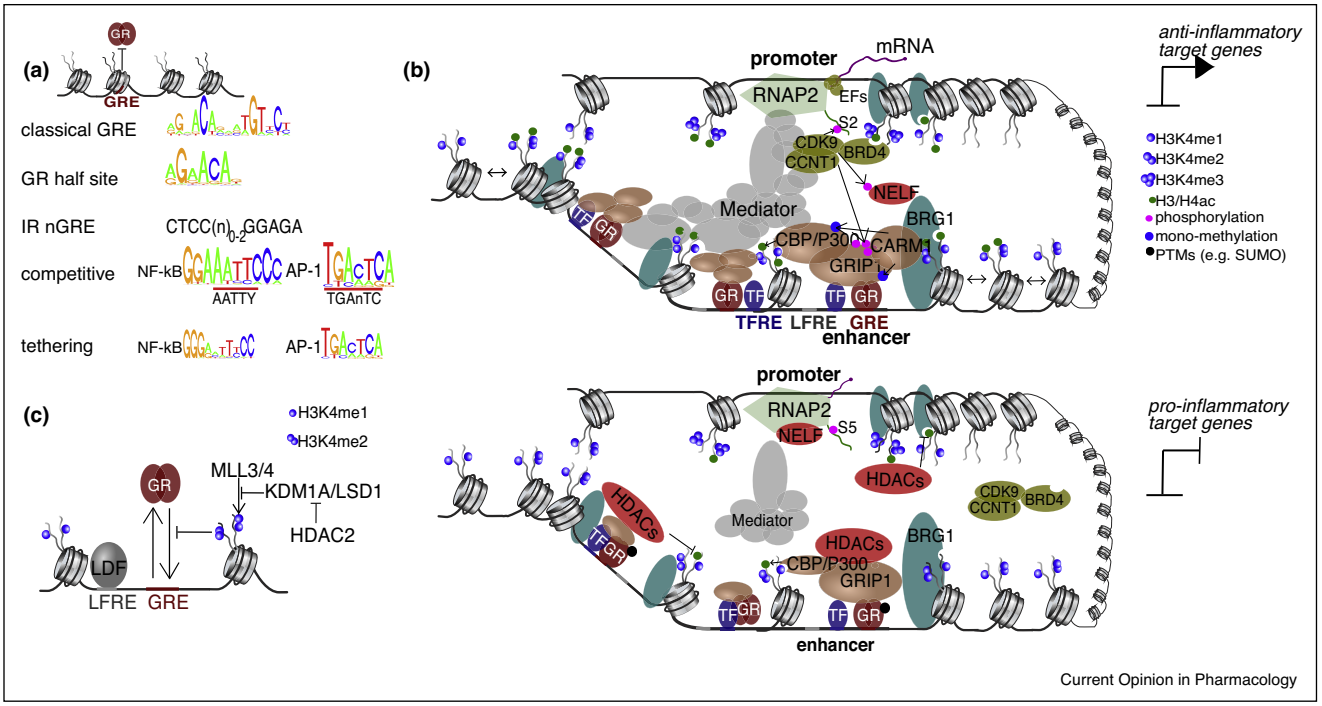
Glucocorticoids (GCs) are steroid hormones secreted by the adrenal gland in a diurnal fashion and in response to various types of stress [1,2]. The potent immunomodulatory activities of synthetic GCs are of great interest in biomedical research. Despite multiple undesirable side effects, GCs remain at the forefront of clinical therapeutic strategies to combat various inflammatory and allergic disorders due to their unmatched efficacy [3,4].

Mechanistically, the counter regulatory activities of GCs during immune responses are primarily stimulated through binding to the ubiquitously expressed GR, a member of the nuclear hormone receptor superfamily of ligand gated transcription factors [5]. Upon ligand binding, a cascade of molecular events triggers GR translocation from the cytoplasm to the nucleus. In a typical scenario, pharmacological doses of GCs potently curb inflammation by direct interference with expression of genes encoding pro-inflammatory mediators, and by increased expression of anti-inflammatory genes [6,7]. GR thus elicits transcriptional changes by binding to promoters and enhancers comprised of specific DNA sequences, termed glucocorticoid response elements (GREs) [8–10]. GREs are generally defined as 15bp palindromes with the consensus sequence AGAACANNNTGTTCT that are bound by GR homodimers in a head-to-head conformation (Figure 1a). Furthermore, depending on the individual sequence composition, the GRE motifs themselves can influence transcriptional output by GR [11].

Moreover, the control of selective gene networks by GR is typically dependent on particular cellular and physiological contexts. These may be attributed to distinct transcriptional activities of multiple GR isoforms, post-translational modifications (PTMs), subcellular localization and cell type-specific recruitment of co-regulators and other proteins by GR [12,13]. In part, GR specificity relies on the dynamic binding of GR-interacting co-regulatory factors that remodel the surrounding chromatin and affect the transcription apparatus. Target gene activation by GR is thought to occur primarily by direct binding to GREs, and subsequent recruitment of co-activators and histone acetyltransferases [14]. In contrast, trans-repression of GR target genes was thought to be mediated by GR tethering to DNA-bound transcription factors via protein-protein interactions [15]. However, recent findings have questioned the trans-repression model and suggest that direct GR binding to DNA, not tethering, is necessary for pro-inflammatory gene repression [7,16–18].

Here we summarize current insights into transcriptional regulation by GR in response to inflammatory signals. We focus on recent advances in understanding the specific roles of GR co-regulators, epigenetic modifiers, and interactions with other factors that cumulatively define its transcriptional repertoire. Finally, we review the latest

Figure 1



Context-specific gene regulation by the GR.
(a) GR binding depends on the accessibility of GREs, its binding site and the individual motif sequence. Variants of different GRE motifs are listed. Position-Weight matrices were obtained from JASPAR [93] (MA0113.2; MA0105.1; MA0099.2), HOMER (GSE23622), SwissRegulon (GR half site, http://swissregulon.unibas.ch). Competitive binding sites for NF-kB [29] and AP-1 [16] are marked with a red line. **(b)** The binding site motif sequence and H3K4 methylation status of the enhancer determines GR binding kinetics. **(c)** Transcriptional output upon GR binding is mediated by locus-specific recruitment and displacement of co-regulators. CBP/p300 mediates enhancer histone tail acetylation, required for recruitment of chromatin remodelers like BRG1, which in turn open the surrounding chromatin. CARM1 stimulates p300/CBP activity by mono-methylation. GRIP1 is required for CARM1 recruitment and additionally recruits p-TEF/CDK9 that phosphorylates GRIP1 at activating enhancers. p-TEF/CDK9 at the promoter phosphorylates NELF and the C-terminal domain of RNAP2 at Serine-2 (S2) for pause-release and productive elongation. Recruitment of mediator by co-activators and GR facilitates enhancer–promoter interactions. At repressive enhancers, co-repressors containing HDACs are recruited to counteract p300/CBP acetylation of histone tails via histone deacetylation [7]. At the same time, loss of CARM1 reduces p300/CBP activity and recruitment, leading to decreasing acetylation levels at repressive enhancers and release of BRG1. GRIP1 is bound to repressive enhancers, but can act as a co-repressor in its unphosphorylated state. However, multiple different locus-specific, signal-specific and cell type-specific mechanisms have been postulated, and the molecular machinery distinguishing between activation and repression remains to be identified. At repressed promoters, p-TEF/CDK9 is displaced and NELF is bound to RNAP2, which stays in its paused state (Serine-5 phosphorylated (S5)). LFRE — lineage factor response element; TFRE — transcription factor response element. Red: co-factors associated with repression; green: co-factors associated with gene activation.

evidence on the involvement of non-coding RNAs, adding another layer of complexity to GR-regulated gene expression networks.

Transcriptional regulation by the GR

Identifying *cis*-regulatory features

The basis of context-dependent gene regulation by GR depends on availability of its DNA binding element [19]. DNA is normally wrapped tightly around histone octamers forming chromatin fibers, with accessibility controlled by ATP-dependent chromatin remodeling enzymes [20]. GR binding sites (GBSs) are largely determined by DNA accessibility (Figure 1a) [21,22]. Exploiting tissue-specificity of GR binding has emerged as a potential therapeutic strategy to retain specific beneficial effects of GC therapy like immunomodulation, while preventing adverse effects such as obesity, diabetes, and osteoporosis.

However, GR binding to DNA is necessary, but not sufficient for transcriptional regulation, since only 13% of GR binding sites in the human lung cancer cell line A549 as assayed by chromatin-immunoprecipitation (ChIP) are able to activate reporter constructs [23]. Interestingly, most activated sites contain classical GREs (Figure 1a) [23], suggesting that the GR motif strongly contributes to target gene activation. Indeed, the GR motif was found to be the main predictor for stable GR binding and gene activation by GR at p300-occupied enhancer regions in A549 cells [24••]. Furthermore,

introducing a classical palindromic GRE upstream of the transcription start site (TSS) of a non-responsive gene was sufficient to turn it into an activated target in the human osteosarcoma cell line U2OS [25]. Palindromic GREs are mostly enriched in *cis*-regulatory regions associated with GC-induced gene expression [26,27]. Its activation potential might depend on flanking nucleotides that either stabilize a GR dimer conformation or destabilize it [28]. Taken together, several studies suggest that GR target gene activation is predominantly mediated by direct GR-DNA interactions at accessible enhancers containing GRE motifs (Figure 1a).

However, functional GREs have also been found in the enhancers of repressed inflammatory target genes in murine macrophages [7,26]. These GREs mediate gene repression, as confirmed by reporter gene assays [7]. Additional sequence motifs associated with gene repression were recently identified. For instance, cryptic GREs were found within NF-kB [29] and AP-1 [16] binding sites in several cancer cell lines, indicating a competition between GR and AP-1 or NF-kB residency (Figure 1a). These findings challenge the classical tethering model, in which DNA-independent genomic interactions occur via protein-protein interactions between GR and NF-kB or AP-1 [30–32]. Rather, they suggest one should proceed with caution when interpreting overlapping transcription factor GBSs from independent experiments, or motifs identified by ChIP-seq from heterogeneous cell populations as evidence of co-occupancy of different transcription factors. These assays do not measure actual co-binding to the same *cis*-regulatory elements in time and space.

Negative GREs (nGREs) have been identified in several gene promoters of repressed GR target genes, and conservation between mice and humans was shown for the inverted repeated (IR) nGREs (Figure 1a) [17,33]. However, evidence of direct binding of GR to these motifs *in-vivo* is still lacking. Since there is no unifying model explaining GR's repressive mechanisms, especially regarding locus-specific features, and the involvement of different sequence motifs is incompletely understood, future studies are needed to dissect these scenarios.

A view beyond the DNA

In addition to the DNA sequence itself, the chromatin environment also influences transcriptional output. Gene activation by GR upon treatment with synthetic GR ligand like dexamethasone (Dex) is generally associated with increased DNAse I hypersensitivity (DHS) at GBSs, as well as other interacting enhancer elements in different cell types [24••,27,34]. These observations might be explained by decompaction of the entire region, as observed in A549 cells [35], yet the molecular mechanism remains unknown. On the other hand, GBSs associated with repression mostly appear to lack significant changes in DHS [27].

Chromatin remodeling complexes are required for enzymatic nucleosome repositioning and changes in DHS. Among them, the BRG1-containing family of SWI/SNF chromatin remodelers is known to interact with GR [36]. GR recruits BRG1 to a subset of its enhancers in different cell types, and is accompanied by the formation of regularly spaced nucleosome arrays and increased accessibility at those GBSs [34,37] (Figure 1c). BRG1 knockdown experiments in the human melanoma cell line A1-2 showed that BRG1 was mostly required for GR-mediated gene activation, but also for gene repression [37]. However, an indirect effect for the negative regulation cannot be excluded. Interestingly, GBSs that *de novo* recruit BRG1 are H3K27ac-negative, whereas GR enhancers with pre-existing BRG1 are H3K27ac-positive, indicating a crosstalk between remodeling and these active enhancer features, as suggested in other cellular contexts [38].

The activation-associated H3K27ac mark is deposited by the two paralogous histone acetyl transferases, CBP and p300 [39]. CBP has been shown to interact directly with GR. p300/CBP are recruited to GBSs associated with gene activation upon ligand binding, but lost at GBSs associated with gene repression or transient GR occupancy, as observed in several cellular contexts [7,24••,27,40,41]. Interestingly, GR-dependent gain of p300 at enhancers is a feature of enhancers that bind GR persistently [24••]. How these enhancers are selected for stable GR binding and p300 recruitment, remains to be investigated.

Recently, it was shown in a mouse hepatoma cell line that histone deacetylases targeting the histone demethylase KDM1A/LSD1 are required for GR-mediated repression [42]. Furthermore, in A549 cells locus-specific interaction of GR with KDM1A/LSD1 suggests that GR binding can be modulated on an epigenetic level by H3K4me2 at activating GBSs [43]. This observation adds another layer of complexity to the control of GR target genes (Figure 1c).

In addition to the enhancer status, co-activators and co-repressors like the p160 family SRC-1/2/3 are essential for transcriptional regulation by GR [44,45]. SRC-1/2/3 are well known nuclear hormone receptor co-activators. They provide an interaction platform for nuclear receptors, as well as other transcription factors and co-factors [46].

SRC-2, also known as GRIP1, has both co-activator and co-repressor functions [47]. GR directly interacts with GRIP1 [48] and recruits it to GBSs of activated and repressed genes in murine macrophages [7,47]. As co-activator, GRIP1 recruits enzymes like p300/CBP and the

arginine methyltransferase CARM1 to modify histones at activating enhancers [49]. Interestingly, GRIP1 is required for both GR-dependent gene activation and repression, and in macrophages the switch between co-repressor and co-activator function requires its phosphorylation by CDK9 [7,50]. Thereby, transcriptional elongation at enhancers is coupled to the stable induction of certain GR target genes in a feed-forward loop, providing another level of signaling input for transcriptional control upon GR binding (Figure 1b) [50].

Like the SRC co-regulators, GR function is also controlled by PTMs. Several PTMs have been shown to affect GR transcriptional activity (see Ref. [12] for review). For example, SUMOylation of GR at Lys293 increases recruitment of an NCOR1/SMRT/HDAC3 complex to inflammatory enhancers in fibroblasts [51]. Of note, SUMOylation of GR at Lys293 does not influence GR binding to DNA, but rather recruitment of the co-repressor complex.

In addition to locus-specific co-factor interactions, recent evidence has shown cooperation between adjacent GR enhancers mediating gene regulation, rather than single GBSs. GBSs may form clusters within the genome, and those clusters are usually marked by similar changes in the binding of co-factors such as p300 [23,24••,52]. In the adenocarcinoma cell line 3134, GR enhancers are found clustered in tightly connected chromatin domains that can regulate several target genes in a similar manner, thereby establishing domains of GR-mediated gene activation and repression in 3D [27]. These observations are in line with the transcriptional condensate model of gene regulation, where clustered *cis*-regulatory elements locally enrich the co-regulators required for enhancer activation, enhancer-promoter interactions and transcription [53•,54]. Ligand-dependent enhancer interaction and condensate formation was also recently reported for the related estrogen receptor [55••].

Another level of complexity is obtained by combinations of enhancer-promoter pairs. For instance, in mouse macrophages GR-dependent repression of RNA polymerase 2 (RNAP2) paused genes like *Tnf* and *Ccl2* depend on NELF recruitment, whereas non-paused targets like *Il1a* and *Il1b* do not [40]. However, the contribution of different core promoter types [56] in regulating transcriptional outputs of GR requires more systematic evaluation.

In summary, ligand-activated GR is recruited to accessible binding sites in the genome, and its transcriptional activity depends on the DNA sequence and GBS density. The DNA sequence is the key parameter to tweak GR transcriptional activity in a signal-dependent way by the co-bound transcription factors, co-regulators and the chromatin environment. GBS density on the other hand, is a multiplier required for stable gene regulation. The complex interactions of GR with its co-factors establishes context-specific positive and negative feedback loops that contribute to distinct expression dynamics [57]. Further studies analyzing the dynamic timing of events are required for a deeper understanding of GR's molecular circuits. Genome-wide multi-omics approaches coupled with unsupervised machine learning could be used to systematically categorize these GR regulatory events, contributing to a deeper understanding of the wide variety of context-specific genomic GR functions.

Non-coding RNAs in inflammation and GC signaling

While many direct GR target genes have been discovered using genomics techniques described above, functionally assigning binding sites to a target TSS (whether nearby or distally) is still a major challenge. Many GBSs have not been linked to clear mRNA targets, and could play a role in non-coding transcription. Non-coding RNAs (ncRNAs) do not code for proteins, exist as small and long transcripts. Small ncRNAs, such as microRNAs (miRNAs), mainly regulate gene expression post-transcriptionally, whereas different subclasses of long non-coding RNAs (lncRNAs) can act at both transcriptional and post-transcriptional levels [58,59]. The inter-species conservation of ncRNAs is low, therefore, search for novel ncRNAs mainly depends on the sequence homology, structure, function and expression from syntenic loci. Recent studies have linked ncRNAs to inflammation, GC signaling [60] and pathological conditions [61]. However, the specific involvement of ncRNAs and in particular lncRNAs, in GR's transcriptional mechanisms has not yet been fully explored. In principle, these ncRNAs might affect GR transcriptional polarity, or act as transcriptional co-regulators. Characterizing the functional importance of specific ncRNA species in controlling inflammation and GC action could pave a path towards alternative GC therapies.

Long non-coding RNAs regulate inflammation and modulate GR function

Recently, high-throughput expression profiles showed that inflammation induces striking expression changes of numerous lncRNAs [62,63]. For example, treatment of human macrophages with LPS revealed more than 200 differentially expressed lncRNAs, with many regulating the expression of nearby protein coding genes. In particular, the highly conserved lncRNA-ROCKI was identified as a negative regulator of *MARCKS*. The lncRNA-ROCKI forms a structural complex with APEX1 protein to recruit histone deacetylase HDAC1 to the *MARCKS* promoter to suppress its transcription [64••]. The MARCKS protein serves as a modulator of immune responses, as its expression was regulated by TLR1/2, TLR2/6, 3, 4, 5, and 7, and loss of function studies showed an effect on inflammatory gene expression.

In addition, lncRNA-NKILA and the abundantly expressed lncRNA-MALAT1 were shown to exert anti-inflammatory actions in atherosclerosis. The lncRNA-MALAT1 is abundant and highly conserved. It was shown to be downregulated by Dex in human primary osteoblasts cells [65], and acts as a miRNA sponge in murine macrophages to fine tune the expression of miR-503-5p. This miRNA is known to impair post-ischemic reparative angiogenesis and is involved in macrophage differentiation processes [66]. In contrast, in response to inflammatory stimuli in human endothelial cells, the cytoplasmic lncRNA-NKILA releases NF-κB. NF-κB then translocates into the nucleus to recruit DNMT3A at the promoter of the anti-inflammatory transcription factor KLF4. DNMT3A then methylates CpG sites in the *KLF4* promoter, which subsequently downregulates its expression [67]. The lncRNA-NEAT1 resides upstream of lncRNA-MALAT1 and promotes Dex resistance in multiple myeloma patients. The lncRNA-NEAT1 is highly expressed in patients with multiple myeloma and linked to poor prognosis. The lncRNA-NEAT1 acts as a sponge to miR-193a, which in turn targets the anti-apoptotic protein MCL1 in multiple myeloma [68].

Mechanistically, *cis*-acting lncRNAs can regulate the expression of nearby genes by interacting with regulatory components present in gene promoters, or dependent on the sequence and structure of the RNA [59]. For instance, the three lncRNAs ROCKI, BCAT1 and BAIAP2 control the expression of nearby protein coding genes by DNA sequence dependent mechanisms [64••]. Other possible mechanisms by which lncRNAs affect their targets are competition with endogenous RNAs sharing a common response element, or by contending with other ncRNAs to regulate mRNA expression either directly or indirectly [69].

Most lncRNAs can either activate or repress transcription of target genes by directly binding to DNA and forming triple helices [70,71]. The most studied lncRNA in the context of GR is GAS5, which was discovered in human cell lines to bind the DNA binding domain (DBD) of the GR protein itself to suppress its transcriptional activity [72]. A recent study showed that the GR-DBD can bind to RNA hairpin motifs in a structure-specific mode. The binding affinity with which the GR-DBD binds to RNA appears to be similar to that of GR occupying DNA [73•,74••]. This *in vitro* study suggests the physiological possibility of interactions between GR and ncRNAs. It has been shown that human GR acts as an RNA binding protein to regulate *CCL2* and *CCL5* mRNA levels, but this ability of GR has to be fully characterized on a broader level [75]. Clinically, GR's anti-inflammatory potential can be subject to GC resistance, which could partly be attributed to lncRNA-GAS5 [76,77]. For example, GAS5 expression is upregulated specifically in GC resistant children with inflammatory bowel disease. Both GAS5 and GR can accumulate in the cytoplasm, indicating that GAS5 blocks GR activity [78]. In a clinical study on multiple sclerosis, GAS5 expression was correlated with GR expression. These studies show that lncRNAs act as potential modulators of GR function in pathological conditions. Conceivably, GC therapies might be augmented by reducing GAS5 expression [78,79].

Finally, GR regulates an immune related lncRNA, PSORS1C3, that has long and short splice variants arising from alternative promoters. Upon GC treatment in the human A549 cell line, both variants are differently expressed. GR inhibits the promoter activity of the long variant, which recruits inflammatory transcription factors like SP1. In contrast, GR induces the short variant which in turn upregulates *OCT4*. This effect of lncRNA-PSORS1C3 on OCT4 expression might affect OCT4 function during stress responses [73•]. Overall, the scope and functions of lncRNAs interfering with GR's transcriptional activity have not been fully explored. Characterizing novel lncRNAs and their associations with GR may assist in deciphering the mechanisms of GR's immunomodulatory potential.

Interactions between miRNAs and GR in inflammation

MiRNAs are small ncRNAs, and are beginning to emerge as novel players in the context of GR and inflammation. For instance, LPS treatment alters the expression of numerous miRNAs in human monocytes [80]. One of the most studied miRNAs in inflammation is miR-155, whose expression is induced in most immune cell types by a variety of inflammatory stimuli. Dex treatment in LPS stimulated RAW 264.7 cells decreases miR-155 expression in exosomes [81]. During inflammation, miR-155 expression is primarily upregulated by NF-κB, whereas GR suppresses miR-155 expression to control acute inflammation [69]. In human monocytes, miR-511-5p selectively targets LPS induced inflammatory responses. In addition, this miRNA partly mediates GR's anti-inflammatory effects by suppressing expression of *Tlr4*, the key LPS receptor and signal transducer [82].

GC treatment has also been shown to induce miR-708 expression. Induction of miR-708 inhibits IKKβ expression, which in turn suppresses NF-κB activity. Therefore, GCs influence NF-κB signaling via the induction of miR-708, which may inhibit the proliferation of human breast cancer cells [69,83•]. In turn, miRNAs can also enhance the efficacy of GCs. For example, TNF-α induces miR-146a expression in human airway epithelial cells, which is repressed by GR. miR-146a acts as a feedback inhibitor of NF-κB signaling to control inflammation. However, overexpression of miR-146a in GC treated A549 cells, had greater anti-inflammatory effects due to increased efficacy of GCs, this in turn increased the inhibition of cytokine production [84]. It is important to

note that miRNAs not only inhibit GR action, but that targeting certain miRNAs might make GR more effective.

Finally, miRNAs play a role in GC biosynthesis and influence endogenous ligand availability by targeting several enzymes that are necessary for steroid production. Many of these miRNAs mainly target cytochrome P450 enzymes, for example, miR-24 and miR-10b negatively regulate the expression of *CYP11B1*, and miR-320a-3p negatively regulates the expression of *CYP11A1* and *CYP17A1*. In general, synchronized actions of miRNAs involved in GC biosynthesis could negatively impact the availability of cortisol (but in this case not affecting synthetic ligands) [85].

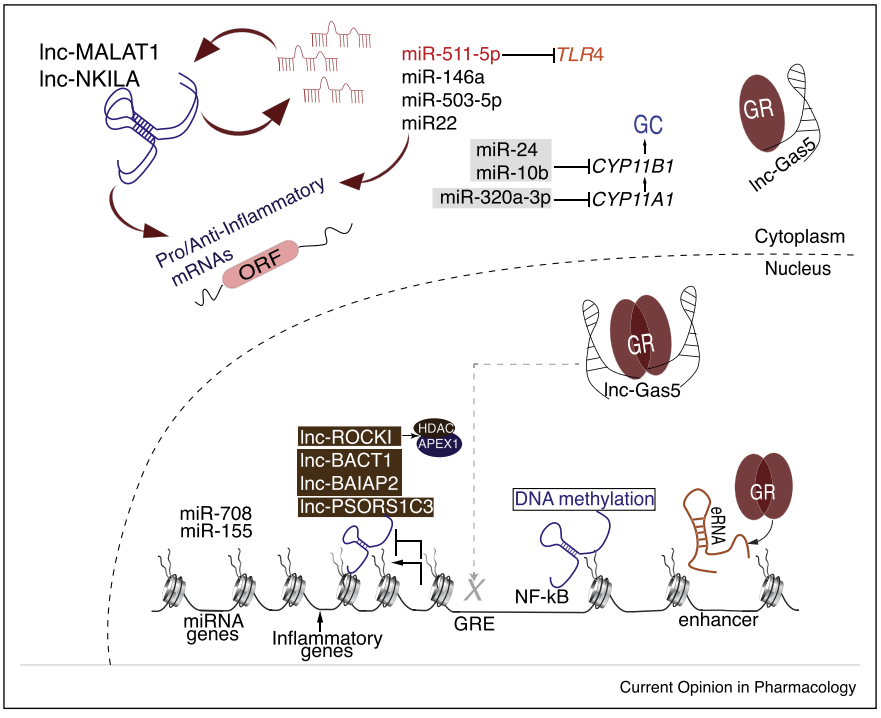
The modes of action for lncRNAs and miRNAs are linked, therefore using genome-wide transcriptomic data integrated with chromatin profiles to construct lncRNA-miRNA-mRNA regulatory networks will identify putative GR co-regulators in the future [86,87]. Furthermore, characterizing ncRNA profiles in the secretome of GC treated immune cells might open up new avenues for the discovery of molecular mechanisms or therapeutic intervention [81].

Unexplored non-coding RNA potential in immune responses

In addition to lncRNAs and miRNAs, enhancer RNAs (eRNAs) transcribed from *cis*-regulatory, transcription factor-bound regions, are often associated with open chromatin and H3K27ac marks. These eRNAs may regulate the expression of their corresponding mRNA target genes [88]. One elegant example of an eRNA regulating inflammatory genes is the *IL1β*-eRNA, that regulates the expression of its cognate pro-inflammatory target *IL1β* in human monocytic THP-1 cells [89].

Integration of nascent transcriptomic data with ChIP-seq profiles and chromatin accessibility assays showed that GR suppresses TNF-α induced inflammatory eRNAs. Conversely, in the human BEAS-2B cell line, certain

Figure 2



The diverse actions of non-coding RNAs in inflammation and GR function.
NcRNAs regulate gene expression in the nucleus and the cytoplasm. In the nucleus, lncRNAs interfere with transcription to regulate the expression of the corresponding genes. For example, they may act at promoters and modify chromatin elements to modulate transcription, like the lncRNA-ROCKI. On the other hand, in the cytoplasm, lncRNA-NKILA modulates NF-κB activity and lncRNA-GAS5 directly binds to cytoplasmic GR to block its nuclear entry and regulation of target genes. Some miRNAs, such as miR-155 and miR-708, are induced by NF-κB in response to inflammatory stimuli to activate pro-inflammatory genes. In that case, GR suppresses the expression of such miRNAs. GR also represses eRNAs that are induced by inflammatory stimuli, and at the same time, GR increases the activity of anti-inflammatory eRNAs. In the cytoplasm, the lncRNA-MALAT1 acts as a miRNA sponge to miR-503-5p. Moreover, GR regulates the expression of miR-511-5p, which controls the expression of the key innate immune receptor TLR4 to eventually affect its downstream inflammatory targets. Finally, miRNAs might also be involved in the regulation of glucocorticoid biosynthesis and ligand availability by cytochrome P450.

eRNAs that control anti-inflammatory genes are increased by GR in cooperation with NF-κB, which highlights again the transcriptional crosstalk between GR and NF-κB [90••]. These eRNAs could therefore be another subset of ncRNAs involved in GR transcriptional mechanisms. The availability of new types of genome wide data now enable the identification of eRNAs and other RNA species that influence GR function [90••]. These novel ncRNAs in inflammation and GC signaling are summarized in Figure 2.

Conclusion

Context-specific GR mediated regulation of anti-inflammatory gene expression is very complex. Despite significant advancement in our understanding of the molecular basis of this complex mechanism, the lack of a unifying mechanism poses many challenges in the pursuit of novel anti-inflammatory therapies with less adverse effects. Major bottlenecks involve cell type-specific GC responses and inter-species differences. Therefore, achieving tissue-specific drug delivery may boost the efficiency and safety of GC therapies. As the new scientific concepts and technologies evolve, novel players of GR mediated gene regulation are continuously identified. Recent advancements in concepts such as co-activator condensates, 3D genome topology and application of high-throughput machine learning approaches may create promising novel therapeutic targets. Apart from these, a need to develop selective GR agonists that modulate preferential binding of GR or that favor recruitment of specific co-regulators, still exists.

Furthermore, miRNAs have emerged as potential therapeutic agents and are currently being tested in clinical trials for different pathological conditions like cancer and hepatitis [91]. Based on the studies mentioned in this review, targeting certain miRNAs might improve the efficacy of GCs, but the underlying mechanisms are yet to be studied. Similarly, the identification of lncRNAs and eRNAs affecting GR's anti-inflammatory potential has only just begun [92]. The potential interactions between ncRNAs and GR can be inferred from the recent study [74••], which shows that the DBD of GR has the ability to bind to RNAs with hairpin loop structures. Overall, the factors mentioned above collectively may open new avenues for the development of safer GC therapies.

Funding

This work was supported by grants from the ERC (ERC StG 638573 SILENCE) and the DFG (SFB1064 chromatin dynamics) to NHU, as well as Entzuendungsprozesse GR 5179/1-1 to FG.

Conflict of interest

None declared.

Acknowledgements

We thank Kenneth A. Dyar for critical reading of this work. We are thankful for the contributions of S. Regn and I. Guderian. We apologize to all authors whose work could not be cited due to space constraints.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Miller WL, Auchus RJ: **The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders**. *Endocr Rev* 2011, **32**:81-151.
2. Munck A, Guyre PM, Holbrook NJ: **Physiological functions of glucocorticoids in stress and their relation to pharmacological actions**. *Endocr Rev* 1984, **5**:25-44.
3. Vandewalle J, Luypaert A, De Bosscher K, Libert C: **Therapeutic mechanisms of glucocorticoids**. *Trends Endocrinol Metab* 2018, **29**:42-54.
4. Barnes PJ: **Therapeutic strategies for allergic diseases**. *Nature* 1999, **402**:B31-8.
5. Evans RM, Mangelsdorf DJ: **Nuclear receptors, RXR, and the big bang**. *Cell* 2014, **157**:255-266.
6. Ronchetti S, Migliorati G, Bruscoli S, Riccardi C: **Defining the role of glucocorticoids in inflammation**. *Clin Sci* 2018, **132**:1529-1543.
7. Uhlenhaut NH, Henriette Uhlenhaut N, Barish GD, Yu RT, Downes M, Karunasiri M, Liddle C, Schwalie P, Hübner N, Evans RM: **Insights into negative regulation by the glucocorticoid receptor from genome-wide profiling of inflammatory cistromes**. *Mol Cell* 2013, **49**:158-171.
8. Sacta MA, Chinenov Y, Rogatsky I: **Glucocorticoid signaling: an update from a genomic perspective**. *Annu Rev Physiol* 2016, **78**:155-180.
9. Meijsing SH, Pufall MA, So AY, Bates DL, Chen L, Yamamoto KR: **DNA binding site sequence directs glucocorticoid receptor structure and activity**. *Science* 2009, **324**:407-410.
10. Kassel O, Herrlich P: **Crosstalk between the glucocorticoid receptor and other transcription factors: molecular aspects**. *Mol Cell Endocrinol* 2007, **275**:13-29.
11. Bain DL, Yang Q, Connaghan KD, Robblee JP, Miura MT, Degala GD, Lambert JR, Maluf NK: **Glucocorticoid receptor–DNA interactions: binding energetics are the primary determinant of sequence-specific transcriptional activity**. *J Mol Biol* 2012, **422**:18-32.
12. Weikum ER, Knuesel MT, Ortlund EA, Yamamoto KR: **Glucocorticoid receptor control of transcription: precision and plasticity via allostery**. *Nat Rev Mol Cell Biol* 2017, **18**:159-174.
13. So AY-L, Chaivorapol C, Bolton EC, Li H, Yamamoto KR: **Determinants of cell- and gene-specific transcriptional regulation by the glucocorticoid receptor**. *PLoS Genet* 2007, **3**:e94.
14. Muzikar KA, Nickols NG, Dervan PB: **Repression of DNA-binding dependent glucocorticoid receptor-mediated gene expression**. *Proc Natl Acad Sci U S A* 2009, **106**:16598-16603.
15. Reichardt HM, Tuckermann JP, Göttlicher M, Vujic M, Weih F, Angel P, Herrlich P, Schütz G: **Repression of inflammatory responses in the absence of DNA binding by the glucocorticoid receptor**. *EMBO J* 2001, **20**:7168-7173.
16. Weikum ER, de Vera IMS, Nwachukwu JC, Hudson WH, Nettles KW, Kojetin DJ, Ortlund EA: **Tethering not required: the glucocorticoid receptor binds directly to activator protein-1 recognition motifs to repress inflammatory genes**. *Nucleic Acids Res* 2017, **45**:8596-8608.

17. Surjit M, Ganti KP, Mukherji A, Ye T, Hua G, Metzger D, Li M, Chambon P: **Widespread negative response elements mediate direct repression by agonist-liganded glucocorticoid receptor**. *Cell* 2011, **145**:224-241.

18. Gebhardt JCM, Suter DM, Roy R, Zhao ZW, Chapman AR, Basu S, Maniatis T, Xie XS: **Single-molecule imaging of transcription factor binding to DNA in live mammalian cells**. *Nat Methods* 2013, **10**:421-426.

19. John S, Sabo PJ, Thurman RE, Sung M-H, Biddie SC, Johnson TA, Hager GL, Stamatoyannopoulos JA: **Chromatin accessibility pre-determines glucocorticoid receptor binding patterns**. *Nat Genet* 2011, **43**:264-268.

20. Workman JL, Kingston RE: **Alteration of nucleosome structure as a mechanism of transcriptional regulation**. *Annu Rev Biochem* 1998, **67**:545-579.

21. Grøntved L, John S, Baek S, Liu Y, Buckley JR, Vinson C, Aguilera G, Hager GL: **C/EBP maintains chromatin accessibility in liver and facilitates glucocorticoid receptor recruitment to steroid response elements**. *EMBO J* 2013, **32**:1568-1583.

22. Biddie SC, John S, Sabo PJ, Thurman RE, Johnson TA, Schiltz RL, Miranda TB, Sung M-H, Trump S, Lightman SL *et al.*: **Transcription factor AP1 potentiates chromatin accessibility and glucocorticoid receptor binding**. *Mol Cell* 2011, **43**:145-155.

23. Vockley CM, D'Ippolito AM, McDowell IC, Majoros WH, Safi A, Song L, Crawford GE, Reddy TE: **Direct GR binding sites potentiate clusters of TF binding across the human genome**. *Cell* 2016, **166**:1269-1281.e19.

24. •• McDowell IC, Barrera A, D'Ippolito AM, Vockley CM, Hong LK, Leichter SM, Bartelt LC, Majoros WH, Song L, Safi A *et al.*: **Glucocorticoid receptor recruits to enhancers and drives activation by motif-directed binding**. *Genome Res* 2018, **28**:1272-1284

McDowell *et al.* used regression models and time series studies to shed light on the timing of events at GR bound enhancers, putting P300 recruitment and H3K27ac at the top of the GR-dependent enhancer remodeling cascade.

25. Thormann V, Glaser LV, Rothkegel MC, Borschiwer M, Bothe M, Fuchs A, Meijsing SH: **Expanding the repertoire of glucocorticoid receptor target genes by engineering genomic response elements**. *Life Sci Alliance* 2019, **2**.

26. Lim H-W, Uhlenhaut NH, Rauch A, Weiner J, Hübner S, Hübner N, Won K-J, Lazar MA, Tuckermann J, Steger DJ: **Genomic redistribution of GR monomers and dimers mediates transcriptional response to exogenous glucocorticoid in vivo**. *Genome Res* 2015, **25**:836-844.

27. Portuguez AS, Grbesa I, Tal M, Deitch R, Raz D, Weismann R, Schwartz M, Loza O, Sung M-H, Kaplan T *et al.*: **Gene activation and repression by the glucocorticoid receptor are mediated by sequestering Ep300 and two modes of chromatin binding**. *bioRxiv* 2019 http://dx.doi.org/10.1101/764480. [date unknown].

28. Schöne S, Jurk M, Helabad MB, Dror I, Lebars I, Kieffer B, Imhof P, Rohs R, Vingron M, Thomas-Chollier M *et al.*: **Sequences flanking the core-binding site modulate glucocorticoid receptor structure and activity**. *Nat Commun* 2016, **7**:12621.

29. Hudson WH, de Vera IMS, Nwachukwu JC, Weikum ER, Herbst AG, Yang Q, Bain DL, Nettles KW, Kojetin DJ, Ortlund EA: **Cryptic glucocorticoid receptor-binding sites pervade genomic NF-κB response elements**. *Nat Commun* 2018, **9**.

30. Ray A, Prefontaine KE: **Physical association and functional antagonism between the p65 subunit of transcription factor NF-kappa B and the glucocorticoid receptor**. *Proc Natl Acad Sci U S A* 1994, **91**:752-756.

31. Yang-Yen H-F, Chambard J-C, Sun Y-L, Smeal T, Schmidt TJ, Drouin J, Karin M: **Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein-protein interaction**. *Cell* 1990, **62**:1205-1215.

32. Oh K-S, Patel H, Gottschalk RA, Lee WS, Baek S, Fraser IDC, Hager GL, Sung M-H: **Anti-inflammatory chromatinscape suggests alternative mechanisms of glucocorticoid receptor action**. *Immunity* 2017, **47**:298-309.e5.

33. Escoter-Torres L, Caratti G, Mechtidou A, Tuckermann J, Uhlenhaut NH, Vettorazzi S: **Fighting the fire: mechanisms of inflammatory gene regulation by the glucocorticoid receptor**. *Front Immunol* 2019, **10**.

34. Johnson TA, Chereji RV, Stavreva DA, Morris SA, Hager GL, Clark DJ: **Conventional and pioneer modes of glucocorticoid receptor interaction with enhancer chromatin in vivo**. *Nucl Acids Res* 2018, **46**:203-214.

35. Jubb AW, Boyle S, Hume DA, Bickmore WA: **Glucocorticoid receptor binding induces rapid and prolonged large-scale chromatin decompaction at multiple target loci**. *Cell Rep* 2017, **21**:3022-3031.

36. Hsiao P-W, Fryer CJ, Trotter KW, Wang W, Archer TK: **BAF60a mediates critical interactions between nuclear receptors and the brg1 chromatin-remodeling complex for transactivation**. *Mol Cell Biol* 2003, **23**:6210-6220.

37. Hoffman JA, Trotter KW, Ward JM, Archer TK: **BRG1 governs glucocorticoid receptor interactions with chromatin and pioneer factors across the genome**. *eLife* 2018, **7**.

38. Alver BH, Kim KH, Lu P, Wang X, Manchester HE, Wang W, Haswell JR, Park PJ, Roberts CWM: **The SWI/SNF chromatin remodelling complex is required for maintenance of lineage specific enhancers**. *Nat Commun* 2017, **8**.

39. Jin Q, Yu L-R, Wang L, Zhang Z, Kasper LH, Lee J-E, Wang C, Brindle PK, Dent SYR, Ge K: **Distinct roles of GCN5/PCAF-mediated H3K9ac and CBP/p300-mediated H3K18/27ac in nuclear receptor transactivation**. *EMBO J* 2011, **30**:249-262.

40. Sacta MA, Tharmalingam B, Coppo M, Rollins DA, Deochand DK, Benjamin B, Yu L, Zhang B, Hu X, Li R *et al.*: **Gene-specific mechanisms direct glucocorticoid-receptor-driven repression of inflammatory response genes in macrophages**. *eLife* 2018, **7**.

41. Sheppard K-A, Phelps KM, Williams AJ, Thanos D, Glass CK, Rosenfeld MG, Gerritsen ME, Collins T: **Nuclear integration of glucocorticoid receptor and nuclear factor-κB signaling by CREB-binding protein and steroid receptor coactivator-1**. *J Biol Chem* 1998, **273**:29291-29294.

42. Patrick NM, Griggs CA, Icenogle AL, Gilpatrick MM, Kadiyala V, Jaime-Frias R, Smith CL: **Class I lysine deacetylases promote glucocorticoid-induced transcriptional repression through functional interaction with LSD1**. *J Steroid Biochem Mol Biol* 2017, **167**:1-13.

43. Clark EA, Wu F, Chen Y, Kang P, Kaiser UB, Fang R, Shi YG: **GR and LSD1/KDM1A-targeted gene activation requires selective H3K4me2 demethylation at enhancers**. *Cell Rep* 2019, **27**:3522-3532.e3.

44. Clarisse D, Thommis J, Van Wesemael K, Houtman R, Ratman D, Tavernier J, Offner F, Beck I, De Bosscher K: **Coregulator profiling of the glucocorticoid receptor in lymphoid malignancies**. *Oncotarget* 2017, **8**.

45. Kucera T, Waltner-Law M, Scott DK, Prasad R, Granner DK: **A point mutation of the AF2 transactivation domain of the glucocorticoid receptor disrupts its interaction with steroid receptor coactivator 1**. *J Biol Chem* 2002, **277**:26098-26102.

46. Johnson AB, O'Malley BW: **Steroid receptor coactivators 1, 2, and 3: critical regulators of nuclear receptor activity and steroid receptor modulator (SRM)-based cancer therapy**. *Mol Cell Endocrinol* 2012, **348**:430-439.

47. Rogatsky I, Luecke HF, Leitman DC, Yamamoto KR: **Alternate surfaces of transcriptional coregulator GRIP1 function in different glucocorticoid receptor activation and repression contexts**. *Proc Natl Acad Sci U S A* 2002, **99**:16701-16706.

48. Reily MM, Pantoja C, Hu X, Chinenov Y, Rogatsky I: **The GRIP1: IRF3 interaction as a target for glucocorticoid receptor-mediated immunosuppression**. *EMBO J* 2006, **25**:108-117.

49. Chevillard-Briet M: **Control of CBP co-activating activity by arginine methylation**. *EMBO J* 2002, **21**:5457-5466.

50. Rollins DA, Kharlyngdoh JB, Coppo M, Tharmalingam B, Mimouna S, Guo Z, Sacta MA, Pufall MA, Fisher RP, Hu X *et al.*:

Glucocorticoid-induced phosphorylation by CDK9 modulates the coactivator functions of transcriptional cofactor GRIP1 in macrophages. *Nat Commun* 2017, **8**:1739.

51. Hua G, Ganti KP, Chambon P: **Glucocorticoid-induced tethered transrepression requires SUMOylation of GR and formation of a SUMO-SMRT/NCoR1-HDAC3 repressing complex**. *Proc Natl Acad Sci U S A* 2016, **113**:E635-E643.

52. Kuznetsova T, Wang S-Y, Rao NA, Mandoli A, Martens JHA, Rother N, Aartse A, Groh L, Janssen-Megens EM, Li G *et al.*: **Glucocorticoid receptor and nuclear factor kappa-b affect three-dimensional chromatin organization**. *Genome Biol* 2015, **16**.

53. • Shrinivas K, Sabari BR, Coffey EL, Klein IA, Boija A, Zamudio AV, Schuijers J, Hannett NM, Sharp PA, Young RA *et al.*: **Enhancer features that drive formation of transcriptional condensates**. *Mol Cell* 2019, **75**:549-561.e7
This study shows for the first time how the clustering of transcription factor binding sites drives condensate formation and co-factor recruitment *in vitro*.

54. Cho W-K, Spille J-H, Hecht M, Lee C, Li C, Grube V, Cisse II: **Mediator and RNA polymerase II clusters associate in transcription-dependent condensates**. *Science* 2018, **361**:412-415.

55. •• Nair SJ, Yang L, Meluzzi D, Oh S, Yang F, Friedman MJ, Wang S, Suter T, Alshareedah I, Gamliel A *et al.*: **Phase separation of ligand-activated enhancers licenses cooperative chromosomal enhancer assembly**. *Nat Struct Mol Biol* 2019, **26**:193-203
Nair *et al.* show that the estrogen receptor contributes to phase separation in an eRNA-dependent manner *in vivo.* Furthermore, they show that enhancer dynamics are influenced by liquid-gel condensate transitions that are dynamically distinct from steadily regulated enhancers.

56. Haberle V, Arnold CD, Pagani M, Rath M, Schernhuber K, Stark A: **Transcriptional cofactors display specificity for distinct types of core promoters**. *Nature* 2019, **570**:122-126.

57. Chinenov Y, Coppo M, Gupte R, Sacta MA, Rogatsky I: **Glucocorticoid receptor coordinates transcription factor-dominated regulatory network in macrophages**. *BMC Genomics* 2014, **15**:656.

58. Momen-Heravi F, Bala S: **miRNA regulation of innate immunity**. *J Leukoc Biol* 2018, **103**:1205-1217.

59. Dykes IM, Emanueli C: **Transcriptional and post-transcriptional gene regulation by long non-coding RNA**. *GenomProteom Bioinf* 2017, **15**:177-186.

60. Mueller KL: **Long noncoding RNAs in inflammation**. *Science* 2016, **352**:48-49.

61. Liu F, Gong R, Lv X, Li H: **The expression profiling and ontology analysis of non-coding RNAs in dexamethasone induced steatosis in hepatoma cell**. *Gene* 2018, **650**:19-26.

62. Li Z, Chao T-C, Chang K-Y, Lin N, Patil VS, Shimizu C, Head SR, Burns JC, Rana TM: **The long noncoding RNA THRIL regulates TNF expression through its interaction with hnRNPL**. *Proc Natl Acad Sci U S A* 2014, **111**:1002-1007.

63. Carpenter S, Aiello D, Atianand MK, Ricci EP, Gandhi P, Hall LL, Byron M, Monks B, Henry-Bezy M, Lawrence JB *et al.*: **A long noncoding RNA mediates both activation and repression of immune response genes**. *Science* 2013, **341**:789-792.

64. •• Zhang Q, Chao T, Patil VS, Qin Y, Tiwari SK, Chiou J, Dobin A, Tsai C, Li Z, Dang J *et al.*: **The long noncoding RNA ROCKI regulates inflammatory gene expression**. *EMBO J* 2019, **38**
This study in human macrophages identified 229 lncRNAs and their immune related cognate protein coding genes. Apart from ROCKI-*MARCKS*, several other lncRNA - protein coding gene pairs are validated.

65. Fan J-B, Zhang Y, Liu W, Zhu X-H, Xu D-W, Zhao J-N, Cui Z-M: **Long non-coding RNA MALAT1 protects human osteoblasts from dexamethasone-induced injury via activation of PPM1E-AMPK signaling**. *Cell Physiol Biochem* 2018, **51**:31-45.

66. Cremer S, Michalik KM, Fischer A, Pfisterer L, Jaé N, Winter C, Boon RA, Muhly-Reinholz M, John D, Uchida S *et al.*: **Hematopoietic deficiency of the long noncoding RNA MALAT1 promotes atherosclerosis and plaque inflammation**. *Circulation* 2019, **139**:1320-1334.

67. Zhu X, Du J, Yu J, Guo R, Feng Y, Qiao L, Xu Z, Yang F, Zhong G, Liu F *et al.*: **LncRNA NKILA regulates endothelium inflammation by controlling a NF-κB/KLF4 positive feedback loop**. *J Mol Cell Cardiol* 2019, **126**:60-69.

68. Wu Y, Wang H: **LncRNA NEAT1 promotes dexamethasone resistance in multiple myeloma by targeting miR-193a/MCL1 pathway**. *J Biochem Mol Toxicol* 2018, **32**:e22008.

69. Mahesh G, Biswas R: **MicroRNA-155: a master regulator of inflammation**. *J Interferon Cytokine Res* 2019, **39**:321-330.

70. Kuo C-C, Hänzelmann S, Cetin NS, Frank S, Zajzon B, Derks J-P, Akhade VS, Ahuja G, Kanduri C, Grummt I *et al.*: **Detection of RNA–DNA binding sites in long noncoding RNAs**. *Nucl Acids Res* 2019, **47**:e32.

71. Cao M, Zhao J, Hu G: **Genome-wide methods for investigating long noncoding RNAs**. *Biomed Pharmacoth* 2019, **111**:395-401.

72. Hudson WH, Pickard MR, de Vera IMS, Kuiper EG, Mourtada-Maarabouni M, Conn GL, Kojetin DJ, Williams GT, Ortlund EA: **Conserved sequence-specific lincRNA–steroid receptor interactions drive transcriptional repression and direct cell fate**. *Nat Commun* 2014, **5**.

73. • Azad FM, Malakootian M, Mowla SJ: **lncRNA PSORS1C3 is regulated by glucocorticoids and fine-tunes OCT4 expression in non-pluripotent cells**. *Sci Rep* 2019, **9**
This study shows how GR acts on different promoter sites to differentially regulate the transcription of target genes.

74. •• Parsonnet NV, Lammer NC, Holmes ZE, Batey RT, Wuttke DS: **The glucocorticoid receptor DNA-binding domain recognizes RNA hairpin structures with high affinity**. *Nucl Acids Res* 2019, **47**:8180-8192
In this *in vitro* study, the GR-DBD is shown to have the ability to bind to a variety of RNAs in a structure specific manner.

75. Ishmael FT, Fang X, Houser KR, Pearce K, Abdelmohsen K, Zhan M, Gorospe M, Stellato C: **The human glucocorticoid receptor as an RNA-binding protein: global analysis of glucocorticoid receptor-associated transcripts and identification of a target RNA motif**. *J Immunol* 2011, **186**:1189-1198.

76. Engreitz JM, Pandya-Jones A, McDonel P, Shishkin A, Sirokman K, Surka C, Kadri S, Xing J, Goren A, Lander ES *et al.*: **The Xist lncRNA exploits three-dimensional genome architecture to spread across the X chromosome**. *Science* 2013, **341**:1237973.

77. Chikanza IC: **Mechanisms of corticosteroid resistance in rheumatoid arthritis**. *Ann N Y Acad Sci* 2002, **966**:39-48.

78. Lucafò M, Di Silvestre A, Romano M, Avian A, Antonelli R, Martelossi S, Naviglio S, Tommasini A, Stocco G, Ventura A *et al.*: **Role of the long non-coding RNA growth arrest-specific 5 in glucocorticoid response in children with inflammatory bowel disease**. *Basic Clin Pharmacol Toxicol* 2018, **122**:87-93.

79. Gharesouran J, Taheri M, Sayad A, Ghafouri-Fard S, Mazdeh M, Omrani MD: **The growth arrest-specific transcript 5 (GAS5) and nuclear receptor subfamily 3 group C member 1 (NR3C1): novel markers involved in multiple sclerosis**. *Int J Mol Cell Med* 2018, **7**:102-110.

80. Naqvi AR, Zhong S: **Expression profiling of LPS responsive miRNA in primary human macrophages**. *J Microb Biochem Technol* 2016, **8**.

81. Chen Y, Zhang M, Zheng Y: **Glucocorticoids inhibit production of exosomes containing inflammatory microRNA-155 in lipopolysaccharide-induced macrophage inflammatory responses**. *Int J Clin Exp Pathol* 2018, **11**:3391-3397.

82. Curtale G, Renzi TA, Drufuca L, Rubino M, Locati M: **Glucocorticoids downregulate TLR4 signaling activity via its direct targeting by miR-511-5p**. *Eur J Immunol* 2017, **47**:2080-2089.

83. • Kumar KJS, Senthil Kumar KJ, Gokila Vani M, Hsieh H-W, Lin C-C, Liao J-W, Chueh P-J, Wang S-Y: **MicroRNA-708 activation by glucocorticoid receptor agonists regulate breast cancer tumorigenesis and metastasis via downregulation of NF-κB signaling**. *Carcinogenesis* 2019, **40**:335-348
GR induced miR-708 suppresses NF-κB activity and its downstream targets.

84. Lambert KA, Roff AN, Panganiban RP, Douglas S, Ishmael FT: **MicroRNA-146a is induced by inflammatory stimuli in airway epithelial cells and augments the anti-inflammatory effects of glucocorticoids**. *PLoS One* 2018, **13**:e0205434.

85. Clayton SA, Jones SW, Kurowska-Stolarska M, Clark AR: **The role of microRNAs in glucocorticoid action**. *J Biol Chem* 2018, **293**:1865-1874.

86. Wang G, Zheng X, Zheng Y, Cao R, Zhang M, Sun Y, Wu J: **Construction and analysis of the lncRNA-miRNA-mRNA network based on competitive endogenous RNA reveals functional genes in heart failure**. *Mol Med Rep* 2019, **19**:994-1003.

87. Miao L, Yin R-X, Zhang Q-H, Hu X-J, Huang F, Chen W-X, Cao X-L, Wu J-Z: **A novel lncRNA-miRNA-mRNA triple network identifies lncRNA TWF1 as an important regulator of miRNA and gene expression in coronary artery disease**. *Nutr Metab* 2019, **16**.

88. Natoli G, Andrau J-C: **Noncoding transcription at enhancers: general principles and functional models**. *Ann Rev Genet* 2012, **46**:1-19.

89. Ilott NE, Heward JA, Roux B, Tsitsiou E, Fenwick PS, Lenzi L, Goodhead I, Hertz-Fowler C, Heger A, Hall N *et al.*: **Long non-coding RNAs and enhancer RNAs regulate the lipopolysaccharide-induced inflammatory response in human monocytes**. *Nat Commun* 2014, **5**:3979.

90. •• Sasse SK, Gruca M, Allen MA, Kadiyala V, Song T, Gally F, Gupta A, Pufall MA, Dowell RD, Gerber AN: **Nascent transcript analysis of glucocorticoid crosstalk with TNF defines primary and cooperative inflammatory repression**. *Genome Res* 2019, **29**:1753-1765
The authors have integrated GRO-seq data with ChIP-seq and chromatin accessibility assays to study mechanisms of GR's transcriptional repression. They defined enhancer activity and showed the transcriptional dynamics of GR.

91. Rupaimoole R, Slack FJ: **MicroRNA therapeutics: towards a new era for the management of cancer and other diseases**. *Nat Rev Drug Discov* 2017, **16**:203-222.

92. Lam MTY, Cho H, Lesch HP, Gosselin D, Heinz S, Tanaka-Oishi Y, Benner C, Kaikkonen MU, Kim AS, Kosaka M *et al.*: **Rev-Erbs repress macrophage gene expression by inhibiting enhancer-directed transcription**. *Nature* 2013, **498**:511-515.

93. Fornes O, Castro-Mondragon JA, Khan A, van der Lee R, Zhang X, Richmond PA, Modi BP, Correard S, Gheorghe M, Baranašić D *et al.*: **JASPAR 2020: update of the open-access database of transcription factor binding profiles**. *Nucleic Acids Res* 2020, **48**:D87-D92.