Chapter 2

# Twenty-First Century Glucocorticoid Receptor Molecular Biology

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**Abstract**

Glucocorticoids are central to homeostasis as a function of the circadian cycle, temporally preceding circulating adrenaline concentration circadian fluctuations. Virtually, all cell types express the glucocorticoid receptor (GR). GR is a transcription factor that activates gene expression by binding to enhancers. Intriguingly, not all cell types respond to GR stimulation in the same fashion at the molecular level. This indicates that GR activity is subject to epigenetic control. We discuss the molecular basis for epigenetic control of GR action at the genomic level, including the concept of topologically associating domains which may restrain the roaming range of distal enhancers. Furthermore, much evidence indicates that GR can repress gene expression programs. We therefore discuss current concepts of the molecular basis of GR-mediated gene expression repression, including non-genomic mechanisms that involve mRNA destabilization.

**Keywords:** glucocorticoid receptor, glucocorticoid response element, chromosome conformation, epigenetics, non-genomic action, RNA decay

## 1. Introduction

Glucocorticoids (GCs) are steroids derived from cholesterol that are mainly produced in the adrenal cortex, under the control of the hypothalamic–pituitary–adrenal axis. Due to their lipophilic nature, GCs can traverse cellular membranes and thus enter any cell. Physiologically, GCs show circadian oscillations in man, peaking at 06:00 before we wake up and then dropping until 00:00, when their levels start to rise again. Adrenaline, a catecholamine that is produced by the adrenal medulla, follows this trend with a lag of about 2 hours [1]. Ontogenetically, GC levels increase during the final weeks of human gestation and

in the post-natal period. This not only stimulates gluconeogenesis, but also perinatal lung maturation [2] and many other physiological processes [3–5]. Furthermore, GCs are part of an emotion (stress, fear, and arousal) processing pathway in the brain that impacts memory and aspects of behavior that are controlled by the central nervous system [6–8].

Importantly, from a medical point of view, GCs and their synthetic analogues have strong immunosuppressive properties. Because of this, synthetic GCs belong to the top 50 World Health Organization essential medicines. Prednisone, dexamethasone, and triamcinolone are used to treat a wide range of (auto)inflammatory conditions as well as hematopoietic malignancies. The anti-inflammatory effect of GCs is due to regulation of cell survival and immune signaling molecules such as chemokines, interleukins, and cytokines such as TNF$\alpha$ [9, 10]. GCs are often well accepted as a long-term treatment, making them irreplaceable for medical use. Nevertheless, synthetic glucocorticoid (over)use has a number of side effects that usually involve homeostasis and tissue maintenance [11, 12]. To mitigate such side effects, a detailed understanding of the molecular mode of action of GCs is a necessity. Hence, understanding the molecular mechanisms through which GCs exert their biological function has been a highly active research field in the past century.

The glucocorticoid receptor (NR3C1, abbreviated here as GR) is a sequence-specific DNA-binding transcription factor that is expressed in virtually every human cell type. Hence, almost every tissue is potentially responsive to GCs through gene expression modulation. Since the molecular responses to GCs of given tissues are different, it is thought that epigenetic programming during cellular differentiation underlies the cell-specific GC responses [13]. Below, we will review recent developments in epigenetic research relevant to cell-specific GC response mechanisms. In the last section of this chapter, we will review recent research results that support the notion that non-genomic effects of GCs may be very important too.

## 2. Chromosome architecture and epigenetic control of glucocorticoid responses: DNA accessibility

Eukaryotic transcription factors (TFs) bind to regulatory DNA elements commonly called "*cis*-acting elements" to modulate the transcription rates of their target genes. *Cis*-acting elements can be located at (i) gene promoters, where mRNA transcription starts, or (ii) at enhancers, which can be located hundreds of thousands of nucleotides away from their target gene promoters, or (iii) at boundary elements that flank chromosome domains and function to restrict enhancer activity within individual topologically associated chromosome domains [13].

In order to determine the locations where TFs bind on chromosomes, a technique called chromatin immunoprecipitation (ChIP) was developed in the 1990s. ChIP is based on formaldehyde crosslinking of TFs to DNA, followed by DNA co-immunoprecipitations using antibodies directed against the TF protein [14]. Initially, PCR was used to analyze the co-immunoprecipitated DNA, using the enrichment of putative TF target sites relative to "control" chromosomal regions. Nowadays, the co-immunoprecipitated DNA fragments are prepared as DNA libraries that can be sequenced on next-generation sequencing (NGS) platforms, followed by computational mapping of the obtained reads to a reference genome [15].

Currently, more than 20 human and mouse genome-wide GR occupation profiles are available. These reveal a high degree of GR-binding variability [16]. Grøntved et al. showed that a majority (83%) of GR-DNA binding sites in mouse liver cells are liver cell-specific, while only 0.5% of events are shared between all analyzed cell-types [17]. This suggests that there is a complex and dynamic epigenetic component to GR binding that underlies the differences in GR-mediated transcriptional regulation across cell types.

The first level of epigenetic regulation is rather well defined by DNA being wrapped, or not, around histones to form nucleosomes every ~190 bp [18, 19]. Low nucleosome occupancy can be measured as DNaseI hypersensitivity, because accessible free DNA is more prone to DNaseI endonuclease cleavage than DNA wrapped around nucleosomes [20, 21].

DNA accessibility is an important indicator for GR binding. Early studies indicated that GR binding increases DNA accessibility to DNaseI [22, 23] and it was therefore concluded that GR "opens up" chromatin. Although this is true, more recent research indicates that the a majority of chromosomal GR-binding sites coincide with pre-existing hypersensitive DNA stretches, whose DNaseI accessibility profile is further modulated by GR activity, as first reported on a genome-wide level by John et al. [24–27] (**Figure 1**). Grøntved et al. indicated that 62% of glucocorticoid receptor-binding sites are occupied by the transcription factor C/EBP in mouse liver tissue and that C/EBP maintains chromatin accessibility before GC treatment [17]. Furthermore, it was shown that in HeLa cells, 88% of GR-binding sites are already occupied by the lysine acetyltransferase p300 transcription co-activator prior to GC treatment

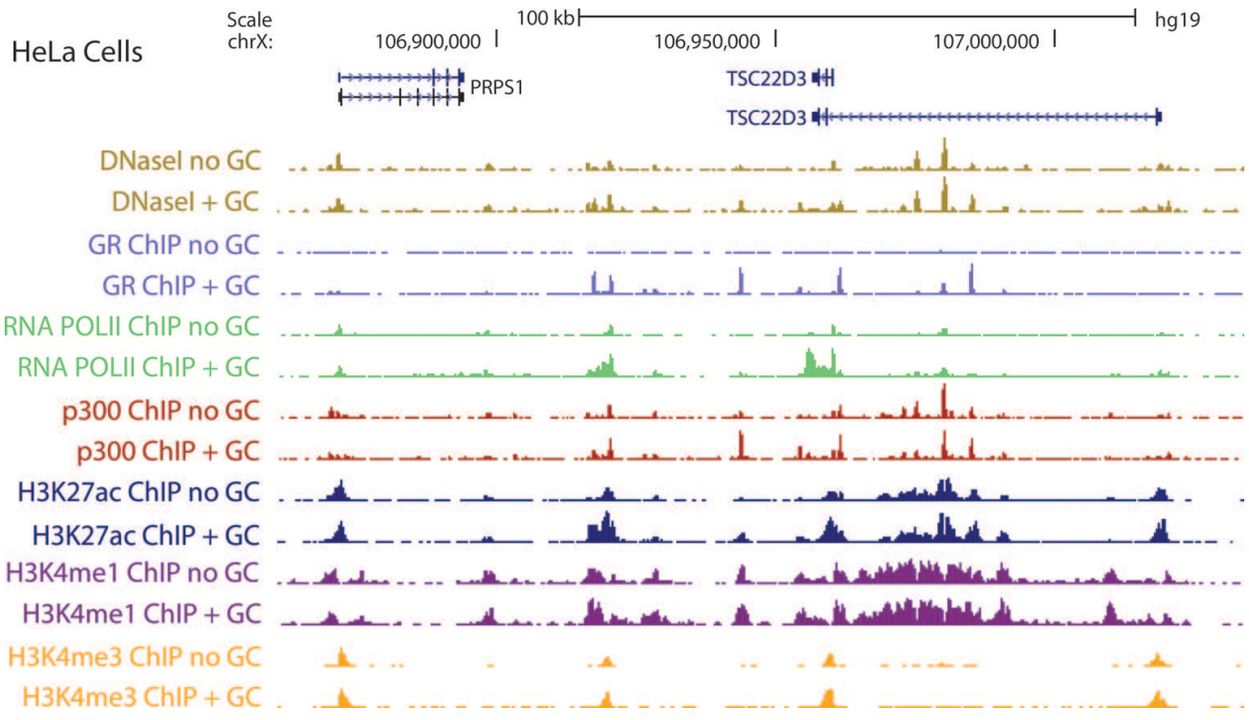


**Figure 1.** GR binds to GREs at several DNaseI hypersensitive locations within the *TSC22D3/GILZ* locus on human chromosome X. This can increase p300 histone acetyltransferase occupancy, H3K27ac marking, and DNaseI hypersensitivity. Notably, occupancy by RNA polymerase II is dramatically increased upon 4 hours of GC treatment, indicating transcription activation. Histone H3 lysine modifications are indicated (H3K27 acetylation, H3K4 mono-methylation, H3K4 tri-methylation). Data are from HeLa cells, Rao et al. [27].

[27, 28]. Altogether, the available evidence indicates that GR-mediated transcriptional control is dependent on other TFs that establish baseline chromatin accessibility profiles in a cell-type specific manner, as exemplified by FoxA1 [29]. However, one single pioneer TF is unlikely to be the sole key to differential use of GR response elements by different cell lineages, or by the same cell type under different conditions. Rather, combinations of DNA sequence-specific transcription factors may act together as "reciprocal pioneers" in an environmentally cued fashion [30–33].

In summary, in a given cell type, GR generally binds to a predetermined set of nucleosome free regions within enhancers that are marked by lineage determining TFs, and GR only rarely binds at sites with very low initial levels of DNA accessibility (**Figure 1**) [28, 34]. Intriguingly, GR appears to associate for rather short times with its cognate sites in vivo, with reported DNA residence times in the order of seconds [35–39]. GR binding usually results in increased histone acetylation [27] (**Figure 1**).

## 3. Chromosome architecture and epigenetic control of glucocorticoid responses: topologically associated domains

Over the last decade, a lot of effort was invested in mapping active *cis*-regulatory enhancer elements to susceptible promoters. This is especially relevant in GR-mediated transcriptional regulation, because the majority of GR-bound *cis*-acting DNA elements are enhancers that are located many kilobases away from the promoters of GR-responsive genes [17, 24]. An important contributor in the identification of enhancer-promoter interactions was the development of nuclear proximity-based chromosome conformation capture (3C) technology in 2002 [40]. In brief, interacting DNA regions are fixed by formaldehyde through DNA-protein-DNA cross-links. The crosslinked chromatin is then digested using restriction enzymes and the digested ends are ligated to obtain DNA circles that harbor sequences from interacting DNA regions. In the original 3C protocol, which is considered a "one-to-one" approach, interactions between two defined genomic loci are assessed by quantitative polymerase chain reaction (RT-qPCR) using locus-specific primers. Circularized Chromatin Conformation Capture (4C), is a "one-to-all" approach that implements a second round of restriction and ligation to obtain small DNA circles which are suitable for inverse PCR amplification to identify the genome-wide DNA interactions of one defined viewpoint locus with any other chromosomal loci [41, 42]. The most recent technical development in 3C technologies is the establishment of chromosome capture followed by high-throughput sequencing (Hi-C) [43]. Crosslinked DNA is digested, labeled with biotin, and re-ligated resulting in a biotin-labeled 3C library. Ligated circles are sheared, purified, and subsequently analyzed using NGS. Hi-C is an "all-to-all" approach because it potentially identifies all possible genome-wide DNA interactions. Capture Hi-C is a further modified version of Hi-C that uses immobilized custom DNA probes and DNA hybridization to enrich for specific loci interactions present in a Hi-C library [44].

A fascinating feature of nuclear chromosome organization is its hierarchical character, containing several layers of compartmentalization. Analyses of Hi-C contact matrices confirm the existence of a first level of organization, namely the occurrence of chromosome territories [45]

that were previously described in microscopy-based studies [46]. At the next level, individual chromosomes are partitioned into multi-megabase "A" and "B" compartments that have a propensity to cluster separately. "A" compartments tend to display a euchromatin profile, being gene-rich, transcriptionally active, and accessible. "B" compartments are generally gene-poor with a tendency to be more heterochromatic, transcriptionally inactive, and less accessible. Hi-C maps with improved resolution, mainly obtained through increased sequencing depth and the use of different restriction enzymes, reveal the partitioning of A and B compartments into so-called sub-Mb-sized topologically associated domains (TADs) [47]. TADs are defined by their tendency to favor internal rather than external DNA interactions. Hence, the TAD hypothesis states that TADs are flanked by left and right boundaries and that enhancers mainly interact with promoters and enhancers within their TAD, but not outside of it. It is currently thought that TADs consist of dynamic sub-Mb chromatin fiber loops that undergo continuous remodeling, among others through RNA polymerase II passage.

TADs are highly conserved between different cell lineages [48], indicating that TADs may be universal functional chromosomal units that serve as a platform within which *cis*-regulatory elements are spatially brought together with their susceptible promoter element. The basis of TAD loops is highly enriched for CCCTCF-binding factor (CTCF) [47] (**Figure 2**). CTCF

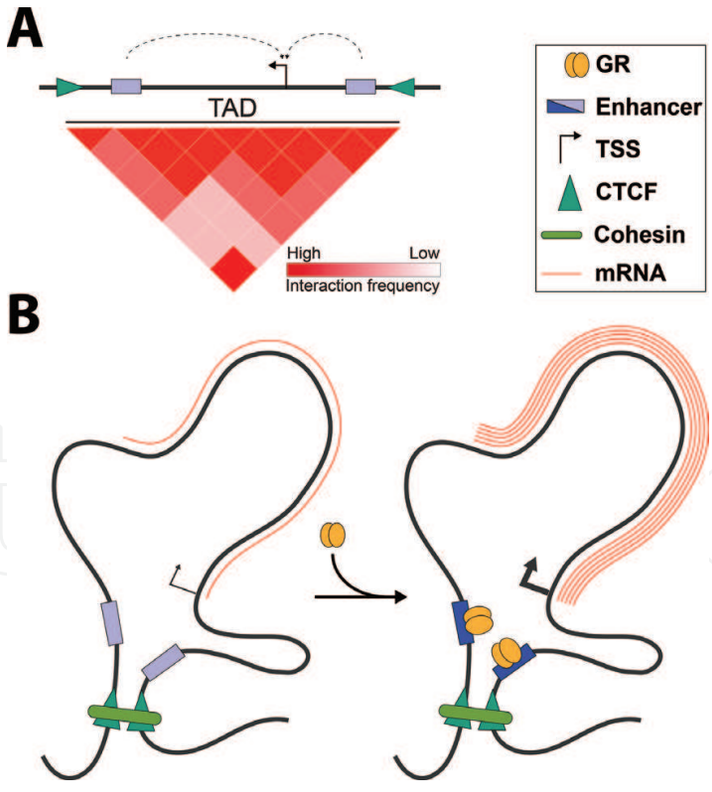


**Figure 2.** A model depicting long-range transactivation after glucocorticoid stimulation. (A) Linear overview of *cis*-acting element organization. Convergent CTCF motifs define TAD boundaries that restrict promoter-enhancer interactions. A schematic contact matrix of a virtual Hi-C experiment is shown as an interaction heatmap. (B) GR induces transcription through binding of a pre-configured locus without affecting its spatial chromosome architecture. Low and high levels of enhancer H3K27 acetylation are depicted by light and dark rectangles, respectively. TAD: topologically associating domain, GR: glucocorticoid receptor, and TSS: transcription start site. See also Ref. [13].

is known as a transcriptional regulator that functionally segregates chromosomal TADs by inhibiting enhancer-promoter interactions [49]. Importantly, the majority of mammalian TAD loops are flanked by a pair of convergent CTCF motifs that mark the TAD's left and right boundaries [50]. Deletion or inversion of CTCF sites can alter TAD architecture and therefore result in dysregulated enhancer-promoter interactions [51]. Moreover, CTCF depletion disrupts TAD boundaries [52] and impacts gene expression [53]. Dysregulation of CTCF is associated with improper gene regulation during development and oncogenesis [54, 55].

The cohesin complex co-localizes with CTCF when assayed by ChIP [56, 57]. Cohesin rings are composed of the core subunits SMC1, SMC3, RAD21, and STAG [58, 59]. Cohesin is most likely loaded onto its chromatid substrate by the NIPBL2/Mau2 cohesin-loading complex, which is enriched at transcription start sites (TSS) [60, 61]. Conversely, cohesin release from chromatids is facilitated by WAPL [62]. Depletion of cohesin leads to altered short-range chromatin interactions, while global TAD organization seemingly persists, suggesting that cohesin and CTCF play different mechanistic roles in TAD formation [63]. Indeed, while inhibiting cohesin loading (by inhibiting cohesin loading factors) inhibits the formation of topologically associated domains, inhibiting cohesin release by inhibiting WAPL restricts loop extension [64]. In the absence of both CTCF and WAPL, cohesin accumulates in up to 70 kilobase-long regions at the 3'-ends of active genes, in particular, if these converge on each other [60, 61]. Cohesin can be moved along chromosomes through RNA polymerase II translocation along its template in yeast and human; this indicates evolutionary conservation of the translocation of Cohesin rings during RNA polymerase II passage.

A quantitative model of "chromatin loop extrusion" was proposed that explains the dynamic features of TADs rather well [50, 65, 66]. Very recently, looping was studied in the monocytic leukemia cell line THP1 that can differentiate into macrophage-like cells. About 16,000 chromatin loops were detected in both cell types and, using stringent selection criteria, 217 were found to be "dynamic" [67]. This indicates that although loss and gain of TAD loops can occur naturally as cells adapt their gene expression landscape, it is not an obligate step in gene activation/repression. Indeed, Hi-C results obtained in parallel in eight primary human hematopoietic cell types show high correspondence [68].

In 2009, long-range interactions involving GR-bound *cis*-acting DNA sequences were identified in mouse cells using a modified 3C technique [70]. An interaction that spans 30 kb was detected between a GR-binding site in the *Lcn2* gene and the promoter of the *Ciz1* gene. This interaction may be responsible for GC-mediated *Lcn2* and *Ciz1* transcription induction in mouse mammary epithelial adenocarcinoma 3134 cells [69]. In 2011, the same research group reported that "the predominant hormone-induced changes for *Lcn2*-contacting loci can be attributed to an increased frequency of pre-existing interactions" [70]. More recently, the 4C approach and genome-wide chromatin structure analysis were applied to characterize GR-associated DNA interactions [71]. In the 3134 murine cell line, this showed that activated GR response elements can interact with a downstream enhancer of the *Tsc22d3* transcription repressor gene, whose transcription is strongly upregulated by glucocorticoids. See also **Figure 1** where human *TSC22D3* is shown. However, upon glucocorticoid receptor activation, contact intensities changed two-fold at most [71].

Theoretically, there are two types of models for transcription factor (TF)-mediated gene regulation at the level of chromatin organization and chromosome folding. In the first type, repressed loci reside in a silent and inaccessible chromatin state with a low enhancer-promoter interaction frequency. Binding of TFs to distal *cis*-regulatory elements would then enhance the accessibility of the locus for other TFs to bind the enhancers and promoters, and consequently, increased interaction between promoter and enhancer elements would alter gene expression [72, 73]. In the second type of models, the locus is dynamically pre-configured in 3D through boundary-boundary interactions controlled by CTCF and cohesin dynamics that insure that TFs can rapidly exert stimulatory or repressive effects on transcription [74] (**Figure 2**). In this model, TFs hardly affect enhancer-promoter interaction frequencies, although they do affect the histone-borne epigenetic marks such as H3K27 acetylation (see **Figure 1**). Currently, available data suggest that GR-responsive loci fit the second type of models, since dexamethasone-mediated GR activation does not greatly alter TAD structure [70, 71] (**Figure 2**).

## 4. GR-binding site sequences and GR-mediated transrepression

The oligomerization state and quaternary structure of GR protein on DNA is thought to influence the activity of *cis*-acting GR-binding DNA elements. Experimentally determined glucocorticoid receptor DNA-binding sites have been broadly classified as "simple," "composite" or "tethering." In the "simple" case, homodimers of GR trans-activate genes by binding to canonical GR response elements (GREs) and consequently recruit transcription co-activators [75]. In the composite DNA motif case, repression and activation are both possible outcomes. Finally, "tethering" is a DNA-binding mode whereby GR does not directly bind specific DNA sequences; instead, it is indirectly tethered to DNA by another TF via protein-protein interactions. Tethering was historically proposed to be the main mechanism of GC-induced GR-mediated transcription repression.

Canonical GREs, mineralocorticoid, progesterone, and testosterone receptor-binding sites are virtually identical, being composed of two inverted pseudo-palindromic repeats separated by a spacer sequence of three bases (GRACANNNTGTYC) [76, 112]. Spacer sequence length has been proposed to be important to maintain GR's dimerization state [77, 78]. Furthermore, it has been suggested that allosteric DNA plasticity in the GR recognition sequences influences the conformational state of GR and, thereby, its spatiotemporal regulatory character [79, 80]. However, Presman et al., shone new light on this paradigm as real-time imaging suggests that GR tetramerizes at GREs [81]. Furthermore, in another key publication, Presman et al. used GR point mutations to confirm that trans-repression and transactivation by GR are two functions that can be separated genetically, whereby loss of transactivation potential though impaired homodimerization did not always co-occur with loss of trans-repression potential [82].

The application of single-base resolution TF ChIP technology, attained by inclusion of a lambda exonuclease digestion step in the ChIP protocol (ChIP-exo), was used to reveal that

many GR-bound half-sites (GRACA) coincide with recognition sequences of unrelated TFs at composite elements [83, 84]. For instance, Lim et al. revealed co-localization with liver-specific TF-binding sites, explaining part of GR's liver cell-specific binding profiles [84]. The molecular mode of regulation at composite sites still remains to be elucidated, although it was hypothesized to fit a model in which only the co-association of the involved TFs results in productive DNA binding, as seen for classical heterodimeric TFs [85, 86].

Next to its ability to bind half-sites, monomeric GR has been reported to counter the effects of other TFs through protein-protein tethering which would result in trans-repression [87, 88]. One such proposed GR-tethering partner is the activator protein 1 (AP-1) heterodimer made-up of heterodimers of bZIP TF family members. A second major proposed GR tethering partner is NF-κB, a TF that consists of heterodimers of RELA and RELB with NFKB1 and NFKB2 subunits [89–92].

For long, AP-1 and NF-κB tethering of GR to DNA were considered the dominant mechanism for GR-mediated trans-repression of transcription, through "on-DNA" repression of the GR tethering TF's transcription activation potential, as reviewed by Glass and Saijo [93]. Genomic studies showed a significant reduction of GR association upon AP-1 loss, but a majority of regulatory scenarios could neither be disentangled nor rationalized through genome-wide ChIP analyses [94]. Indeed, recent experiments indicate that the mode of GR "trans-repression" is still not fully understood. For instance, Oh et al., showed that activation of GR after LPS treatment caused similar gene repression as activation of GR before LPS treatment, and that DNA occupancy by GR was not predictive of gene expression repression, contradicting the "trans-repression by tethering" model. Rather, GR activation was found to directly induce the expression of inhibitors of NF-κB (and AP-1) and this was proposed to cause genome-wide blockade of NF-κB interaction with chromatin [95]. This suggests that protein tethering leading to DNA-bound monomeric GR trans-repression can only account for a minority of repressive events [96]. Indeed, single-molecule imaging suggests that tethering can account for only ~3% of DNA recruitment events [35].

In yet another twist of the GR tethering saga, Weikum et al. showed that GR associates with a GRE half-site that is located within an AP-1 recognition element, even in the absence of AP-1 [97]. Since AP-1 occupancy was not directly required for GR-mediated trans-repression, Weikum et al. proposed that AP-1 establishes an accessible chromatin state for subsequent GR binding to the half-sites which results in transcription repression [34]. Whether AP-1 trans-repression by GCs relies on co-repressor recruitment [98, 92] or rather on exclusion of other TFs and their co-activators is an unresolved issue at this point in time.

## 5. Non-genomic mechanisms of gene regulation by glucocorticoids

The classical model for GR action involves ligand-dependent release from a repressive HSP90 complex followed by genomic DNA binding and consequent transcription modulation [12, 75, 99–101]. However, over the years, non-genomic physiologically relevant GR responses have been proposed, as reviewed by Boldizsar et al. [102]. These include direct membrane

binding effects of (synthetic) glucocorticoids, a putative non-GR membrane-associated receptor [103], functional interactions of GR with proteins involved in signal transduction such as kinases and phosphatases [104, 105], and mitochondrial GR translocation as a mechanism leading to T-cell apoptosis [106]. An advantage of non-genomic regulation over genomic regulation of gene expression is that non-genomic regulation can take place much faster than the transcription-translation process, which often take >20 minutes to begin to change a cell's molecular composition [107–109].

Over the past decades, it has become apparent that non-genomic mechanisms may also play vital roles in GC action, particularly in the context of immune cell regulation [108]. The mechanism we will review below concerns the apparent capacity of GR to bind to RNA.

There are reports that the growth arrest-specific 5 transcript (GAS5), which is a non-coding RNA, can sequester GR, as well as progesterone and androgen receptors, away from their genomic sites of action by acting as a "GRE decoy" [110–112]. This GAS5-dependent GC inhibitory pathway appears to also be active in some immune cells [113, 114]. Although there are no crystal structures of GR bound to RNA, such structures have been modeled [110].

On the other hand, evidence was published that GCs affect the turnover of specific mRNAs. Regulation of mRNA stability is an intricate process controlled by a complex set of interaction between phosphorylation-mediated signaling pathways like the phosphorylation of UPF1 or SMG-2, which together with *cis*-regulatory RNA elements accelerate an mRNA's decay rate [107, 115–118]. RNA *cis*-acting elements that regulate mRNA stability are usually found in their 5′ and 3′ untranslated regions (UTRs) [119, 120]. The most widely found sequences in the 3′ UTRs of unstable mRNAs belong to the adenylate-uridylate-rich elements, consisting of AUUUA ribonucleotide sequences [119]. It has been proposed that GCs can accelerate mRNA decay by inducing the transcription of genes that code for protein factors implicated in mRNA decay. One such example being the gene that codes for tristetraprolin (*TTP*, also known as *ZFP36*), which is inducible by GCs under some circumstances [121, 122]. Pro-inflammatory factor mRNAs indeed display differential half-lives through such an indirect GC-induced mechanism, an example of which is TNF$\alpha$ [122, 123].

Strikingly, in addition to upregulating the expression of mRNA decay factors, it would appear that GR can act directly as a ligand-dependent activator of mRNA decay. In 1999, a 5′ UTR RNA element was reported to be of particular importance for GC regulation of the expression of the MCP-1/CCL2 inflammatory chemokine [124]. In 2007, it was first reported that GR binds specifically to *CCL2* mRNA, to cause its decay [125]. In 2011, an RNA immunoprecipitation protocol was employed to define an RNA motif that recruits GR and the 5′ UTRs of *CCL2* and *CCL7* mRNAs [126]. The mechanism of GR binding to an mRNA to mediate its decay was termed "GR-mediated mRNA decay" (GMD) by Park et al. in 2015 [127]. This research group investigated how GMD occurs. They reported that GMD is a distinct mRNA decay pathway that shares factors with other forms of RNA decay [128, 129]. GMD depends on a number of proteins that have to be recruited to the mRNA. These include GR, PNRC2, UPF1, DCP1A, HRSP12, and YBX1 which then instigate rapid mRNA degradation (**Figure 3**). PNRC2 and UPF1 are known to bind to each other to bring RNA helicase activity into the complex. Another pair of factors that are known for their ability to degrade mRNA

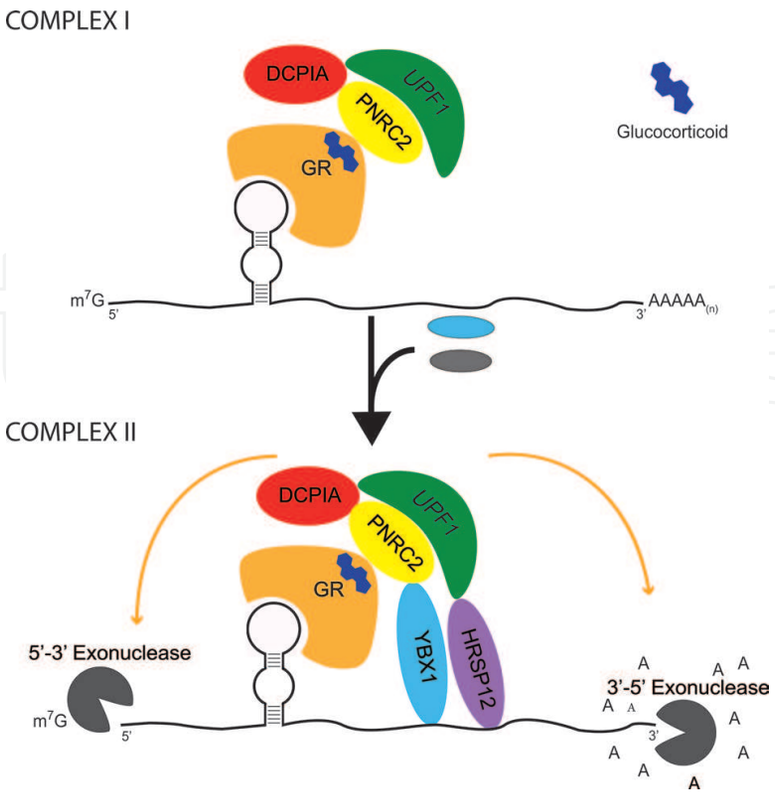


**Figure 3.** Model of the assembly and composition of the glucocorticoid mediated mRNA decay pathway as described by Park et al. [129]. GR with bound GC recruits PNRC2 and DCP1A together with UPF1 to the 5′ UTR of a target mRNA to form Complex I. HRSP12 and YBX1 are then recruited to form Complex II and mRNA decay is performed by exonucleases.

are DCP1A, which promotes mRNA decapping by DCP1 activity, and HRSP12, an endoribonuclease that can attack mRNA [129]. Although exciting, GMD still needs to be confirmed by unbiased approaches such as genome-wide transcriptomic comparisons of nascent RNA and steady-state RNA which have the capacity to simultaneously report mRNA transcription and decay rates [130].

## 6. Conclusion

Over the past decade, GR action has been studied at the molecular level in model systems using DNA accessibility assays, GR ChIP, epigenetic profiling of histone-borne epigenetic marks, transcriptome profiling, and RNA immunoprecipitation. Furthermore, chromosome conformation capture assays have been deployed to investigate the impact of GC signaling on chromosome domain topology.

In the cases where it was studied, GR was found to bind for less than a minute to its genomic targets. GR does not appear to affect the configuration of the topologically associated domains to which it binds. It therefore appears that GR binds to loci where enhancers and promoters are dynamically pre-configured in three-dimensional space. The observation that GR complies with chromosome conformation rather than influencing it offers the exciting perspective of

being able to map intergenic GRE's, which are often located very far away from their target promoters, to TADs. The genes encompassed by these TADs can then be earmarked as potential GR target genes, a hypothesis that can be confirmed by monitoring their expression upon GC exposure.

Results obtained by many laboratories suggest that GR is dependent on other pioneer transcription factors to access its response elements in chromosomal DNA. Co-pioneer factor combinations appear to be cell-type-specific lineage determining TFs, largely explaining the tissue-specific responses elicited by GCs. Furthermore, much evidence indicates that GR is not only restricted to the classical inverted repeat steroid response element, but can also bind to a variety of DNA sequences that only encompass one half site. Furthermore, the concept that GR is tethered indirectly to DNA via other TFs, whose activity it would then repress "on DNA," is no longer the only model to explain trans-repression in the field. Indeed, other genomic and non-genomic interactions may explain the repression of NFKB and AP-1 target genes observed upon GC exposure.

Interestingly, GR itself appears to be subject to miRNA-mediated regulation, as recently reviewed [131].

Excitingly, following on early reports of RNA binding, it was reported multiple times that GR is also an mRNA-binding protein that induces mRNA decay. A particular target for this pathway are CCL chemokine family mRNAs that have long been known to undergo a dramatic down-regulation upon GC exposure.

Altogether, we conclude that although much effort has been invested in glucocorticoid research since the discovery in the 1940s that glucocorticoids are anti-inflammatory wonder drugs, much remains to be discovered about the molecular mechanisms of action of glucocorticoids.

## Abbreviations

| | |
|---|---|
| 3C | Chromosome Conformation Capture |
| 4C | Circularized Chromatin Conformation Capture |
| ChIP | Chromatin immunoprecipitation |
| DHS | DNaseI hypersensitive site |
| GC | glucocorticoid |
| GMD | GR-mediated mRNA decay |
| GR | Glucocorticoid receptor |
| GRE | GR response elements |
| Hi-C | Chromosome conformation capture with high-throughput sequencing |
| LPS | Lipopolysaccharides |

| | |
|---|---|
| NGS | Next-generation sequencing |
| TAD | Topologically associating domain |
| TF | Transcription factor |
| TNF$\alpha$ | Tumor necrosis factor |
| TSS | Transcription start site |
| UTR | mRNA untranslated region |

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