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Complex Genomic Interactions in the Dynamic Regulation of Transcription by the Glucocorticoid Receptor

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Abstract

The glucocorticoid receptor regulates transcriptional output through complex interactions with the genome. These events require continuous remodeling of chromatin, interactions of the glucocorticoid receptor with chaperones and other accessory factors, and recycling of the receptor by the proteasome. Therefore, the cohort of factors expressed in a particular cell type can determine the physiological outcome upon treatment with glucocorticoid hormones. In addition, circadian and ultradian cycling of hormones can also affect GR response. Here we will discuss revision of the classical static model of GR binding to response elements to incorporate recent findings from single cell and genome-wide analyses of GR regulation. We will highlight how these studies have changed our views on the dynamics of GR recruitment and its modulation of gene expression.

Keywords

Glucocorticoids; nuclear receptor; glucocorticoid receptor; transcription dynamics; assisted-loading; chromatin structure

1. Introduction

The glucocorticoid receptor (GR) is a ligand-induced transcription factor belonging to the steroid family of nuclear hormone receptors and is involved in the regulation of many physiological processes including glucose, protein, and fat metabolism, bone homeostasis, and anti-inflammatory and immunosuppressive actions (Chrousos and Kino, 2009). GR is activated by glucocorticoids, steroid hormones that bind to the receptor and function as part of a feedback mechanism of the hypothalamic-pituitary-adrenal axis, which regulates many processes including the immune system and reactions to stress. Many diseases including obesity, diabetes, dyslipidaemia, hypertension, depression, and cancer have been linked to malfunctions in the GR pathway (Chrousos and Kino, 2009). In the absence of glucocorticoids, GR resides in the cytoplasmic compartment of the cell sequestered by heat shock proteins. Once bound to its hormone, the receptor dissociates from these proteins and translocates into the nucleus where it binds as a homodimer either to GR response elements (GREs), consensus sequences present on DNA, or to other proteins bound to their respective regulatory sequences through a tethering mechanism. GR can induce a positive outcome on transcription by recruiting components of the basic transcriptional machinery or can

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negatively regulate gene expression. Although GR is constitutively expressed in most tissues, the genes it regulates are tissue specific which dictate the physiological response of each cell-type to glucocorticoids (Evans, 1988). Chromatin structure at specific response elements regulates GR binding in a cell-specific manner in addition to specific accessory factors expressed in different cell types (John et al., 2011; Biddie et al., 2011; Langlais et al., 2012; Rao et al., 2011).

The process of transcriptional activation by the glucocorticoid receptor is highly dynamic, a mechanistic feature which is necessary for the complex regulation of gene expression during development and differentiation. The response elements available for GR in specific cell types determine the transcriptional outcome for that cell upon induction of GR (John et al., 2011). In addition, the release of the glucocorticoid hormone cortisol from the adrenal gland in mammals is highly pulsatile, which has a dramatic impact on transcriptional output (Stavreva et al., 2009). This is controlled by the rapidly transforming binding states of GR and changes in chromatin configuration at these sites (Stavreva et al., 2009; Conway-Campbell et al., 2011a). In this review we will discuss the dynamic processes that regulate GR binding to the genome and the physiological outcomes.

2. Effects of Chromatin Structure on GR Recruitment

DNA is compacted into the nucleus of the cell through interactions with histone proteins to form nucleosomes, which are made up of an octamer of proteins consisting of two each of the following histones: H2A, H2B, H3, and H4. A fifth histone, histone H1, is often found in the linker region between nucleosomes and helps stabilize chromatin. The degree of DNA condensation varies depending on the region of the chromosome, and can affect transcription factor binding to chromatin at any level of DNA compaction. Even a single nucleosome can block a factor from binding to its response element (Beato and Eisfeld, 1997; Pina et al., 1990b; Pina et al., 1990a; Bernstein et al., 2004; Lee et al., 2004; Sekinger et al., 2005). In fact, chromatin structure has proven to be an important factor in determining GR binding to response elements and in hormone-regulated transcription. Further, studies suggest it plays a role in the cell-specific responses observed upon treatment of cells with glucocorticoids (John et al., 2011).

Initial studies on the interaction between chromatin structure and glucocorticoid receptor were performed extensively using the MMTV promoter as a model system. Upon integration into the genome, the MMTV promoter acquires a chromatin conformation consisting of six positioned nucleosomes, with six GR binding sites located within the second and third nucleosome (Fletcher et al., 2002; Payvar et al., 1983; Perlmann and Wrangé, 1988; Scheidereit et al., 1983). Using DNase I accessibility and restriction enzyme analysis, it has been shown that the binding of GR to these response elements results in a localized chromatin transition at these sites and this transition is dependent upon GR forming a complex with the ATP-dependent Swi/Snf complex (Richard-Foy et al., 1987; Richard-Foy and Hager, 1987; Archer et al., 1991). The catalytic subunit of the Swi/Snf complex is from one of four major classes of ATP-dependent chromatin remodelers and promotes chromatin remodeling through sliding nucleosomes along the DNA or by removing nucleosomes or histones completely, making response elements within the genome more accessible to transcription factors (Narlikar et al., 2002; Eberharter and Becker, 2004). Transfection of cells with dominant negative forms of either BRG1 or BRM, interchangeable catalytic ATPase subunits of the Swi/Snf complex, lead to decreases in chromatin decondensation, RNA Polymerase II binding, and transcription at the MMTV construct, in addition to inhibiting transcription activation or repression in a subset of GR responsive genes (Johnson et al., 2008). Cells lacking BRG1 or BRM are weakly transactivated by GR; however, ectopic expression of either BRG1 or BRM can enhance GR response in these cells,

supporting the important role played by the Swi/Snf complex in GR regulation of transcription (Muchardt and Yaniv, 1993). This GR-induced change in chromatin structure results in increases in levels of GR binding at the MMTV promoter, the recruitment of other co-regulatory factors, and transcriptional activation. (Figure 1A) However, although the ATPase subunits of the Swi/Snf complex are interchangeable, switching BRG1 and BRM in the Swi/Snf complex has been shown to define further complexity in the regulation of endogenous remodeling activity mediated by this complex (Engel and Yamamoto, 2011).

Histone H1 is also found bound to the repressed promoter of MMTV and has been shown to be important for glucocorticoid induction at the MMTV promoter. Upon activation of GR, histone H1 is displaced in order to expose regulatory factor binding sites (Bresnick et al., 1992). In addition, H1 phosphorylation status has been shown to be linked to the ability of GR to transactivate the MMTV promoter (Lee and Archer, 1998). Prolonged exposure to glucocorticoids results in global dephosphorylation of histone H1 and it has been suggested that dephosphorylation of H1 affects the ability of GR to transactivate the MMTV promoter. If hormone is removed for more than 24 hours, the promoter can be reactivated and phosphorylation of H1 is re-established. (Deroo and Archer, 2001).

Based on these previous studies, GR was initially identified as a factor that could bind inaccessible chromatin and trigger chromatin remodeling at specific sites priming the chromatin landscape for secondary factors to bind. However, genome-wide studies of GR binding and chromatin accessibility have shown approximately 95% of GR binding sites are at pre-accessible chromatin (John et al., 2011). Therefore, only 5% of GR binding events correspond to classical *de novo* sites described by previous studies. It has been recently shown that pre-existing accessible GR binding sites can undergo further remodeling upon induction of GR (Burd et al., 2012). This suggests that even at these pre-programmed sites activation of GR can lead to induction of further chromatin remodeling at specific sites, which, in turn, could lead to the recruitment of accessory factors.

Although initial studies have shown GR activity is dependent upon the Swi/Snf complex, more recent studies have shown that not all GR binding sites are dependent upon this complex, suggesting other remodelers must be involved at other binding sites (John et al., 2008). There are three other major remodeling classes each containing several ATPase proteins, all of which can affect chromatin structure and nucleosomal positioning (Narlikar et al., 2002; Eberharter et al., 2001). Other receptors such as the estrogen receptor have been shown to recruit more than one remodeling complex to response elements upon activation (Okada et al., 2008). In addition, histones can also contain posttranslational modifications, including methylation, acetylation, and phosphorylation, of their amino acid residues, which can affect the chromatin structure and recruitment of transcription factors (Bannister and Kouzarides, 2011; Barski et al., 2007; Roh et al., 2005; Roh et al., 2007; Heintzman and Ren, 2009; Birney et al., 2007). Furthermore, histone variants can be substituted for the canonical histones at specific sites, and both H2A.Z and H3.3 have been shown to be important histones for marking enhancer regions (Barski et al., 2007; Goldberg et al., 2010). In fact, chromatin remodeling is associated with nucleosomes containing H2A.Z at GR response elements, however, there is no published data directly linking H3.3 and GR recruitment (John et al., 2008; He et al., 2010; Jin et al., 2009; Jin and Felsenfeld, 2007). Therefore, chromatin structure, whether it is nucleosome positioning or histone modifications, plays an important role in dictating GR binding.

In addition to local chromatin environment, higher order chromatin structure and nuclear architecture also plays important roles in GR induced transcriptional responses. Chromatin conformation capture (3C) studies and imaging of chromatin structure have shown chromosome territories are spatially arranged in the nucleus allowing for long range inter-

and intra-chromosomal interactions (Cremer and Cremer, 2001; Cremer and Cremer, 2011; Lieberman-Aiden et al., 2009; Hakim et al., 2010). Glucocorticoid receptor response elements have been shown to be located great distances, (up to 50–100kb) from the transcriptional start sites of regulated genes, suggesting chromosomal organization in the cell is important for these elements to be brought into close proximity of the promoters of GR-responsive genes (Hakim et al., 2011; Hakim et al., 2009). Both 3C and 4C analyses have shown this is indeed the case and for GR regulated genes the formation of this loop is hormone-independent, proving these interactions are established prior to induction of GR (Hakim et al., 2011; Hakim et al., 2009). In addition, this loop formation is present only in cells where the two loci are active (Hakim et al., 2009). This suggests higher order chromatin structure is pre-established to allow for rapid transcription response upon activation of GR and is highly important for cell specific transcriptional responses (Figure 1B).

3. Crosstalk between GR and Other Transcription Factors

Recruitment of GR and its effects on transcriptional output of genes is a complex process involving its on-going interactions with other factors. The cohort of proteins expressed in a specific cell can have a direct impact on the cell's response to glucocorticoids and can account for the numerous physiological effects of these hormones. The expression of different cell-type specific GR accessory factors has drastic effects on the recruitment of GR to response elements. In addition, GR can greatly influence the function and recruitment of transcription factors at these elements (Figure 1C). For example, co-activation of GR with Stat3, AP1, or NF- κ B leads to a global rearrangement of these factors when bound in the genome (Langlais et al., 2012; Rao et al., 2011; Biddie et al., 2011).

Different transcription factors affect GR recruitment to binding sites through various mechanisms. For example, GR can bind to secondary factors and be recruited to response elements within the genome that do not contain a composite site consisting of response elements for GR and the accessory factor through a mechanism known as tethering. Tethering occurs through protein-protein interactions between two different proteins. Through tethering, two proteins can form a complex with one another and bind to a region of the genome that contains only one of the tethered proteins recognition sequence. Several transcription factors when expressed or activated have been shown to tether GR to noncomposite binding sites. Recent genome-wide studies have shown GR and AP1 co-occupy numerous binding sites in mouse cells, many of which contain an AP1 binding motif but not a GR response element (Biddie et al., 2011). Double-ChIP experiments looking at direct GR and AP1 interaction at these sites have indicated AP1 and GR are recruited simultaneously at many of these sites (Biddie et al., 2011; Kassel et al., 2004). In addition, co-immunoprecipitation experiments have shown GR is found to be in a complex with AP1 (Yang-Yen et al., 1990; Touray et al., 1991). GR has also been shown to directly interact with the RelA/p65 subunit of NF- κ B (Caldenhoven et al., 1995; Scheinman et al., 1995) and both Stat3 and Stat5 have been shown to tether with GR at noncomposite GR response elements (Stocklin et al., 1996). Tethering can also occur at composite GR response elements where GR interacts directly with GREs but can recruit other factors to this site through tethering. The mode of tethering GR to other factors can have huge changes on transcriptional outcome. GR interactions with AP1, Stat3, and NF- κ B through tethering are thought to inhibit the cellular function of these factors, resulting in transrepression of genes that are normally activated by AP1, Stat3, or NF- κ B (Langlais et al., 2012; Rao et al., 2011; Biddie et al., 2011). However, when Stat3 is tethered to GR there is a synergistic effect on transcription, suggesting different interactions can lead to different outcomes (Langlais et al., 2012).

In addition to tethering as a mechanism by which GR is recruited to specific sites, genome-wide sequencing and analysis of global changes in chromatin structure have shown GR binding can be determined by an “assisted loading” mechanism (Voss et al., 2011)(Voss et al., 2011). Under this mechanism, a given factor with chromatin access can recruit chromatin remodeling complexes to specific sites in the genome. This leads to rapid reprogramming of chromatin structure and a transient window by which secondary factors with response elements in the remodeled region can bind. Remodeling events at these sites allow GR access to these recognition elements that were previously unavailable. This was demonstrated for AP1 and GR in which expression of a dominant negative form of AP1 inhibits changes in chromatin structure and GR binding at assisted loading sites, providing evidence for this mechanism (Biddie et al., 2011). In addition, Stat3 has recently been shown to potentially recruit GR through a similar mechanism (Langlais et al., 2012) suggesting this mechanism may be used by numerous factors to regulate GR binding. The expression of lineage specific factors during the differentiation process has also been shown to be important for determining the GR binding landscape. Assisted-loading of GR by CEBPB has been shown to be important during the differentiation of adipocytes (Siersbaek et al., 2011). Similar mechanisms have been shown for FoxA1 and ER binding (Hurtado et al., 2010).

Since induction of GR also leads to reprogramming of chromatin structure at *de novo* sites, GR can also be responsible for the assisted loading of other factors. It has been shown that GR can assist the binding of both AP1 and Stat3, suggesting this mechanism can act in either direction depending on the chromatin context at the response element (Biddie et al., 2011). In addition, studies on the binding dynamics of a mutant estrogen receptor (ER pbox) that binds to GR response elements instead of ER response elements, revealed the ER pbox mutant could only bind at certain sites when GR was first activated even though both receptors recognized the same response element (Voss et al., 2011). These sites also had an increase in sensitivity to DNase I digestion upon activation of GR, suggesting GR initially recruits chromatin modifiers to these sites before the binding of the ER mutant (Voss et al., 2011). These results suggest assisted loading may be a general mechanism used by transcription factors, and the determination as to which factor takes part is dependent on the context of its binding element at specific sites. We therefore hypothesize that “pioneer factors,” factors that are expressed first in the cellular differentiation process and prime the landscape for transcription factor binding, may not exist in the classical sense. As the binding patterns of transcription factors genome-wide are being determined, it is becoming apparent that any transcription factor may have the potential to bind to a “closed” chromatin conformation depending on the site specific context and induce remodeling and recruitment of other factors. There is not a subset of “pioneer factors” solely responsible for this function. The time of induction and expression of transcription factors during the differentiation process determines the binding landscape of other factors; however, every expressed factor can potentially affect the binding landscape of every other factor.

GR can also interact cooperatively with other transcription factors and these cooperative interactions have been shown to have an enhanced impact on gene regulatory programs. For example, GR and Oct1 can synergistically activate the mouse mammary tumor virus (MMTV) promoter through cooperative DNA binding of GR and Oct1 to adjacent GRE and Oct1 sequences, respectively, found in this promoter (Bruggemeier et al., 1991). Oct1 binding at the MMTV promoter is dependent on GR binding and is necessary for transcriptional induction by GR (Bruggemeier et al., 1991). In a similar fashion, GR and the cAMP response element (CRE) binding protein (CREB) can cause cooperative binding of each other to GRE and CRE response elements (Richardson et al., 1999).

Taken together, these studies support the idea that GR can have various interactions with a vast number of other proteins within the cell. These interactions dictate the binding of GR and/or secondary factors to response elements and regulate transcriptional outputs upon stimulation of cells with glucocorticoids, controlling the cellular response to treatments. Therefore, the presence of other factors within a specific cell type can control the cellular outcome following GR induction.

4. Dynamics of GR Recruitment

Our understanding of the binding kinetics of GR to response elements has been largely based on studies performed with a few well-characterized model systems. Although the classical model of GR binding suggests a static interaction of GR with DNA where GR's interactions with its response elements are stable and persists for hours (Perlmann et al., 1990), recent advances in technology have uncovered that GR is actually rapidly exchanged at binding sites. Initial evidence suggesting GR binding at response elements was a dynamic process was initially observed using *in vivo* DNA footprinting analysis. These studies indicated that GR transiently cooperates with the hepatocyte nuclear factor 5 (HNF5) to promote binding at the rat tyrosine aminotransferase gene (Rigaud et al., 1991). Over a decade later, UV crosslinking of GR to chromatinized DNA *in vitro* showed GR could rapidly bind to its response element, but then was actively displaced, supporting the earlier study (Nagaich et al., 2004; Nagaich and Hager, 2004). However, the occupancy rate of GR at a response element *in vivo* could not be determined until recently.

The stochastic of transcription factor binding in live cells has been made possible by the emergence of fluorescence protein-tagging and monitoring by microscopy (Htun et al., 1996). Studies involving real time kinetics of fluorescent proteins in live cells have shown, in general, transcription factors rapidly diffuse through the nucleus and frequently interact with non-specific binding sites in the genome with retention times on the order of hundreds of milliseconds. Occasionally, a transcription factor will interact with an accessible binding site increasing its residence time (Hager, 2009). Using this genome scanning mechanism, factors are able to locate and interact with their target sequences within seconds of activation.

The development of the 3617 cell line, which was derived from the mouse mammary carcinoma cell line 3134, has enabled high-resolution analysis of GR binding at a response element (Kramer et al., 1999; Walker et al., 1999). The 3617 cell line contains a construct for a green fluorescent protein-tagged version of GR (GFP-GR) that is stably integrated into the genome, in addition to containing a large tandem array of the MMTV/v-HaRas reporter fusion (200 copies) integrated on chromosome 4 resulting in 800 to 1200 GR binding sites (Kramer et al., 1999; Walker et al., 1999). Photobleaching techniques, fluorescence recovery after photobleaching (FRAP), and fluorescence loss in photobleaching (FLIP) has allowed for the direct measurement of GFP-GR mobility in this cell line. Studies using these methods have shown that upon addition of hormone, GR rapidly and transiently binds at the MMTV array with rates registering on the order of 10–20 seconds (Figure 2A) (McNally et al., 2000). Other lines of evidence result from the expression of an ER mutant that recognizes GR response elements in the 3617 cell line (Voss et al., 2011). These studies have shown the binding of GR at the GRE does not reduce the steady-state binding of the ER mutant to the same GRE, suggesting these receptors have short residency times at this binding site and there is a relatively long time interval between DNA binding events (Voss et al., 2011). In addition, mathematical simulations using these concepts are able to reproduce this noncompetitive state (Voss et al., 2011).

The mechanisms controlling rapid GR displacement is poorly understood and it is hypothesized that many other interacting factors may be involved in this process. It has been shown that transient exchange of GR at response elements is regulated in part by chromatin remodelers. *In vitro* studies using chromatin assembled MMTV DNA has shown that GR is actively displaced from the chromatin template by remodeling of chromatin by the Swi/Snf complex (Fletcher et al., 2002). In fact, both GR and Swi/Snf undergo periodic binding and displacement during chromatin remodeling (Nagaich et al., 2004; Nagaich and Hager, 2004). *In vivo*, the mobility of GR and the induction of transcription have been shown to depend on ATPase activity (Stavreva et al., 2004; Elbi et al., 2004; Agresti et al., 2005). FRAP studies at the MMTV array demonstrate that the recovery of GFP-GR is significantly depreciated when ATP levels are reduced with sodium azide and deoxyglucose (Stavreva et al., 2004). These studies suggest chromatin remodelers may play a major role in GR binding dynamics. These observations are consistent with the previously described hit-and-run model of receptor binding where GR binds to the chromatin and recruits a chromatin remodeling activity, which facilitates transcription factor binding as GR is simultaneously lost from the binding site (Fletcher et al., 2002).

In addition to chromatin remodeling, the residence times of GR at the MMTV array has been shown to be dependent on proteasome activity and chaperone functions *in vivo*, and both of these processes are also ATP-dependent (Stavreva et al., 2004) (Figure 2B). Proteasomes can regulate steroid receptor function through proteolysis of the receptor or possibly by interfering with its mobility (Molinari et al., 1999; Reid et al., 2003; Collins and Tansey, 2006; Baker and Grant, 2005; Lipford and Deshaies, 2003). Inhibition of the proteasome complex with MG132 reduced the mobility of GR when measured by FRAP (Stavreva et al., 2004; Elbi et al., 2004). Chaperones are known to interact with unliganded GR in either the cytoplasm or nucleus (Liu and DeFranco, 1999). The heat shock protein 90 (hsp90) and the p23 chaperones are both recruited to GREs upon induction of GR and may play a role in the disassembly of transcriptional regulatory complexes at these sites (Freeman and Yamamoto, 2002). It has been shown that disruption of either the proteasome pathway or the chaperone Hsp90 have opposing effects on the exchange rate of GR suggesting that a balance between both mechanisms needs to be in place for proper GR cycling (Stavreva et al., 2004).

The dynamic binding of GR is also affected by ultradian cycling of hormones (Stavreva et al., 2009). In mammals, hormones are released from the adrenal gland in a circadian cycle and this secretion is highly pulsatile with a cycling period of 1 hour (Lightman et al., 2002; Lightman, 2006; Droste et al., 2008; Young et al., 2004). Ultradian hormone stimulation induces cyclic transcriptional regulation, which is controlled by the rapid exchange of GR at binding elements and chaperone-mediated GR recycling (Figure 2D) (Stavreva et al., 2009). GR exchange and recycling allows for the reactivation of the receptor during the pulsatile hormone cycles (Stavreva et al., 2009). The ultradian cycling of glucocorticoids has also been shown to induce the cyclical activity of CBP, acetylation of histone H4 and Polymerase II recruitment at GR responsive genes, resulting in gene pulsing (Conway-Campbell et al., 2011b). The progressive cycling of GR binding and recruitment of other accessory factors allows for the coupling of the physiological fluctuations of hormones to transcriptional output. The dynamic availability of ligand-hormone is responsive to the demands of the organism and pulses of glucocorticoids are related to a physiological hit and run mechanism.

5. Downstream Effects: Gene Expression and Physiological Outcomes

Induction of GR leads to changes in the expression levels of a large number of genes; however, the transcriptional output in a cell as a result of GR induction is not binary. Studies conducted on the hippocampus of rats have shown that upon GR stimulation different cycles

of transcription occur with transrepression followed by a later period of transactivation (Morsink et al., 2006). Transcriptional induction at the MMTV reporter upon glucocorticoid treatment has also been shown to be rapid, but transient suggesting a complex kinetic cycling of transcription (Archer et al., 1994). In addition, expression patterns in cell lines reveal glucocorticoid dependent transcription occurs with gene specific alternate phases of activation and repression suggesting the overall effect of glucocorticoids on cellular responses was implicated by a highly complex and tightly regulated process (Figure 2C) (John et al., 2009). The role of other factors or resonance times of GR at specific promoters in determining the kinetic output of a specific gene is not yet known.

The natural physiological pulses of glucocorticoids are an integral part of normal mammalian physiology and can also have a significant effect on transcriptional output. Microarray analysis of the transcriptome response to ultradian stimulation with glucocorticoids identified a set of genes that respond differently to pulsatile versus continuous treatments (McMaster et al., 2011). In addition, pulsatile delivery caused a reduction in cell survival due to increases in apoptosis (McMaster et al., 2011). Ultradian hormone stimulation also induces gene pulsing in both cultured cells and animal models allowing for precise control of glucocorticoid responsive genes (Stavreva et al., 2009).

Crosstalk of GR with other receptors can also have a great effect on the transcriptome response to glucocorticoids. Co-activation of both GR and ER has been shown to regulate a subset of pro-inflammatory genes in USO2 cells (Cvoro et al., 2011). In addition, glucocorticoids can reverse the effects of estradiol on a small subset of genes in human leiomyoma (Whirledge et al., 2012). It has also been shown that crosstalk between either GR and Stat3 or GR and NF- κ B can function to regulate gene networks (Langlais et al., 2012; Rao et al., 2011). GR and Stat3 crosstalk results in a 10-fold increase in the number of affected genes compared to activation of Stat3 alone with the vast majority of the genes being involved in different cellular defense mechanisms (Langlais et al., 2012) whereas co-activation of GR and NF- κ B modifies signaling pathways that are modulated by each factor separately by altering the repertoire of regulated genes (Rao et al., 2011). In addition, GR can also repress functionally related inflammatory response genes through the disruption of p65/interferon regulatory complexes required for toll-like receptors dependent-transcription (Ogawa et al., 2005). Therefore, other factors that are activated along with GR can have crucial effects on the transcriptional response.

The dynamic nature of GR enables it to adapt to changes in glucocorticoid levels or changes in the cellular or chromatin environment, thus allowing for significant physiological outcomes. GR has a vast role in sensing changes in physiological ligand concentrations and adjusting transcriptional output in order to control cellular processes during the circadian and ultradian cycles (Stavreva et al., 2009). In addition, glucocorticoids have been shown to synchronize circadian clocks in peripheral tissues, and in a jet lag mouse model the adrenal glucocorticoids have been shown to play a key role in circadian resynchronization (Balsalobre et al., 2000; Kiessling et al., 2010). It has also been shown that the two circadian co-regulators, cryptochromes 1 and 2, can interact with GR and alter the transcriptional response of GR regulated genes in mouse embryonic fibroblasts (Lamia et al., 2011). If cells are deficient in cryptochromes, the number of GR-induced genes doubles, suggesting these co-regulators oppose GR function and favor gene repression (Lamia et al., 2011). Taken together, these studies demonstrate the important role GR plays in establishment and transduction of circadian signals and physiological regulation.

6. Conclusion and Perspectives

For decades, GR was thought to be a pioneer factor, which upon activation would statically bind to response elements and recruits the activity of chromatin remodelers allowing for other regulatory factors to bind. However, the advancement of technologies involving single cell analysis and genome-wide studies has led to findings that dictate major revisions of this model. We now know GR dynamically interacts with chromatin with residence times on the order of seconds. In addition, GR primarily binds to pre-accessible chromatin, with very few sites bound by the traditional *de novo* mode. Recruitment of GR to response elements occurs by multiple mechanisms, including tethering to other factors, assisted-loading, direct interactions with DNA, and cooperative binding. Therefore the cell-specific expression of collaborating factors determines to a great extent the GR response elements that are active in a given tissue.

Although there has been much progress made in understanding the cellular reaction to glucocorticoids, we have learned GR recruitment and regulation of gene expression are complex processes, and there are still many avenues we do not clearly understand. Comprehensive spatial enhancer/promoter maps in multiple tissues are required so individual GR binding events can be directly mapped to specific transcriptional outcomes. In addition, we need a better understanding of why a binding site may be a pre-accessible or a *de novo* site in one cell type, but not another. It is likely other chromatin modifiers, transcription factors, or accessory factors are involved in marking these sites in a cell-type specific fashion. Therefore, characterization of these collaborating factors and discovering how they modulate the cellular response to glucocorticoids can result in improved treatments for diseases involving the glucocorticoid receptor.

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Highlights

- GR is recruited to response elements through complex interactions with the genome.
- GR predominantly binds pre-programmed sites, but can be recruited to *de novo* sites.
- GR binding at response elements is highly dynamic.
- Ultradian fluctuations of glucocorticoids induce cyclic gene expression cycles.

Figure 1. Recruitment of GR to response elements and activation of gene expression

A. GR recruitment at the MMTV promoter. A schematic representation of GR-mediated transcriptional activation. Upon activation, GR is recruited to the MMTV LTR, which contains six nucleosomes labeled A–F. GR binds to nucleosome B and recruits the Swi/Snf chromatin remodeling complex, and removes the nucleosome at this position. Nucleosome removal creates a permissive chromatin environment for the binding of other regulatory factors and the RNA Polymerase II (PolII) complex leading to transcriptional activation of the downstream Ras reporter gene.

B. Spatial arrangement and long-range interactions between GR response elements and promoters. Depiction of chromosome territories spatially arranged in the nucleus. These arrangements contain transcription hubs, which harbor steroid receptor binding sites, allowing for long range inter- and intra-chromosomal interactions. Within these hubs are

bound transcription factors (yellow boxes), which help maintain spatial orientation of the chromosomes as well as accessibility at GR binding sites. This pre-established configuration allows for GR to rapidly control the expression of genes found up to 50–100 kb away from the GR response element.

C. Mechanisms that recruit GR to specific sites in the genome. Similar to the mechanism described in Figure 1A, depicted are GR binding events at *de novo* sites in the genome and recruitment of chromatin remodelers. Chromatin modifiers remodel chromatin at these sites allowing for other transcription factors to bind in a mechanism known as an assisted loading. In contrast, GR can bind to pre-accessible sites and, at many of these sites, the accessibility has been shown to be dependent upon other regulatory factors being activated or expressed in a cell. GR can also co-bind to sites with other factors through tethering, in addition to cooperative binding at specific sites.

Figure 2. Dynamics of GR function and transcriptional regulation

A. Dynamic binding of GR at the MMTV array. Analysis of GFP-GR binding at the MMTV array (shown by yellow arrow), which contains 200 consecutive copies of the MMTV construct. Fluorescence recovery after photobleaching (FRAP), fluorescence loss in photobleaching (FLIP), and competition studies demonstrate GR dynamically interacts with the array with an on/off rates on the order of seconds.

B. The dynamic interaction of GR with the MMTV array is dependent upon the Swi/Snf complex, protein chaperones, and the proteasome. Schematic representation of GR activation and degradation. In the absence of hormone, GR is found in the cytoplasm bound by heat shock proteins. Upon activation, GR dissociates from the heat shock proteins and translocates into the nucleus. Once bound to the MMTV array, GR recruits the Swi/Snf complex which causes eviction of GR from the array. Once evicted, GR can either bind to chaperone proteins found in the nucleus or cytoplasm, or can be degraded by the proteasome.

C. Complex kinetics of transcriptional regulation by GR. Transcription kinetics of GR-regulated genes, which are clustered based on their expression patterns (based on data from (John et al., 2009)). Some genes are rapidly activated and then repressed (orange line), whereas other genes may be gradually activated (light blue line) or rapidly activated and then plateau, however, at different rates (gray and black lines). In addition, some genes are rapidly repressed and then gradually increase (yellow and red lines) or are repressed followed by a plateau in the expression level (light blue and light brown lines).

D. Ultradian fluctuations within the circadian cycle induce cyclic gene expression.

Graph of ultradian and circadian release of glucocorticoids by adrenal glands. The ultradian release of glucocorticoids result in a cycling of GR regulated gene expressions.