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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.

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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 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

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element (Beato and Eisfeld, 1997; Pina et al., 1990a,b; 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 Wrange, 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 (Fig. 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 been 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 Hh1 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 h, 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, 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 confirmation 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, 2011; Lieberman-Aiden et al., 2009; Hakim et al., 2010). Glucocorticoid receptor response elements have been shown to be located great distances (up to 50–100 kb), 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., 2009, 2011). 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 (Fig. 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 (Fig. 1C).