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Dynamic Access of the Glucocorticoid Receptor to Response Elements in Chromatin

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Abstract

Transcriptional activation as a rate limiting step of gene expression is often triggered by an environmental stimulus that is transmitted through a signaling cascade to specific transcription factors. Transcription factors must then find appropriate target genes in the context of chromatin. Subsequent modulation of local chromatin domains is now recognized as a major mechanism of gene regulation. The interactions of transcription factors with chromatin structures have recently been observed to be highly dynamic, with residence times measured in seconds. Thus, the concept of static, multi-protein complexes forming at regulatory elements in the genome has been replaced by a new paradigm that envisages rapid and continuous exchange events with the template. These highly dynamic interactions are a property of both DNA-protein and protein-protein interactions and are inherent to every stage of the transcriptional response. In this review we discuss the dynamics of a nuclear receptor, and its transcriptional response in the chromatin context.

INTRODUCTION

DNA encodes the complete blueprint of a living organism. In animal cells, the very large accumulation of DNA is efficiently assembled into a variety of nucleoprotein structures collectively referred to as chromatin. Chromatin serves not only to package DNA into the nucleus but also filters access to the encoded information. Nucleosomes are the primary organizational unit of chromatin. These structures are composed of an octamer of core histone proteins (two copies of H2A, H2B, H3 and H4) encircled by ~146 bp of DNA (Kornberg, 1974; Luger et al., 1997). Unstructured N-terminal tails project from the α -helical protein core of the nucleosome and are sites for the majority of known histone posttranslational modifications (PTMs), though several modifications also appear to reside within the helical secondary structure and loops of folded histones (Cosgrove, 2007). These modifications accompany dramatic variations in gene activity, and have been proposed to form the basis of a “histone code” (Strahl & Allis, 2000). Further diversifying the nucleosome core particle is a set of histone isoforms known as histone variants (Bernstein & Hake, 2006). Structural variability in chromatin can contribute to the accessibility of underlying DNA, ranging from condensed heterochromatin to more accessible euchromatin (Sproul et al., 2005).

Phenotypic traits not encoded in DNA are collectively referred to as epigenetic phenomena and manifest as heritable chromatin states by daughter cells (Goldberg et al., 2007). Early

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formulations of the histone/epigenetic code hypothesis suggested that distinct functional consequences result from histone PTMs, and that a given outcome is encoded in the precise nature and pattern of marks (Jenuwein & Allis, 2001). Recent discussions have also advanced the concept of the ‘nucleosome code’ (Turner, 2007; Ruthenburg et al., 2007). In contrast, others have argued that a specific set of transcription factors must be present in a given cell type to maintain the histone modifications in a given state (Ptashne, 2007).

The spatial and temporal expression of genes required for every biological processes involves a series of precisely orchestrated and regulated steps. Any perturbation which results in dysregulation of gene expression often leads to disease. It has become evident that chromatin is a dynamic and an active participant in regulating transcription of the eukaryotic genome. Thus, the question of how gene expression is regulated in complex eukaryotic genomes has re-focused on the molecular machines that have evolved to navigate through chromatin and mediate transcriptional control (Lemon & Tjian, 2000; Maston et al., 2006). Until recently, alternate states of promoter activity have been associated with the assembly of relatively stable multiprotein complexes on target genes, with transitions in the composition of these complexes occurring on the time scale of minutes or hours. The development of living cell techniques to characterize transcription factor function in real time has led to the discovery that these chromatin interactions are highly dynamic (Hager et al., 2002). It has become very clear that most proteins are highly mobile, and exist in a rapid and dynamic equilibrium with multiple targets within the nuclear compartment (Hager et al., 2002; Voss & Hager, 2008; Hager et al., 2006; Hager et al., 2004).

The potential mechanisms involved in the unexpectedly rapid flux of factor/template interactions have been discussed in the context of a “return-to- template” model for transcription factor function (Hager et al., 2006). The “return -to- template” hypothesis suggests that the interactions active at a given promoter in a given time interval are dynamic and stochastic. The initiating factor, in this model case the glucocorticoid receptor (GR), exists in diverse complexes with coactivators and coregulators. These multi-factorial complexes interact randomly and dynamically with the target regulatory sites. Most of these interactions are nonproductive, because the promoter must exist in the appropriate state for a given coregulator to be effective, either in the catalysis of a particular covalent modification, or in the recruitment of a specific multiprotein complex. Promoter chromatin then evolves through a series of modifications, each state serving as a new substrate for subsequent interaction with alternative coregulator complexes. This dynamic view has now moved to center stage in our understanding of transcriptional regulation (Mellor, 2006; Metivier et al., 2006).

The biological reference to the term “dynamic” in the context of transcriptional regulation usually describes factor/template interactions and the evolution of promoter states in a time frame of 20-30 minutes to hours. We highlight here the concept of dynamic as it applies to both rapid and transient factor interactions, using the extensively characterized glucocorticoid receptor dynamics as a model. The exact mechanics of rapid GR mobility is yet to be precisely characterized, but the unmistakable role of chromatin remodeling is discussed, as well as other contributing factors, including protein refolding (chaperone activity) and ligand exchange. We discuss the widespread implications of rapid factor dynamics for the biological function of regulatory proteins.

BIOLOGY OF THE GLUCOCORTICOID RECEPTOR

The 1950 Nobel Prize in Physiology or Medicine was awarded to Edward Kendall, Tadeus Reichstein, and Philip Hench for their studies on the structure and physiological effects of glucocorticoids. Working independently, Kendall and Reichstein isolated and determined the chemical structure of cortisol. When Hench administered cortisol to patients suffering from

rheumatoid arthritis, glucocorticoids emerged as effective therapeutic agents (Ward et al., 1951). After Elwood Jensen articulated the concept of the hormone receptor in 1958, Allan Munck and colleagues subsequently provided evidence for the glucocorticoid receptor (Munck & Brinck-Johnsen, 1967). Two decades of studies on actions of the glucocorticoids culminated in molecular cloning of GR (Weinberger et al., 1985; Miesfeld et al., 1986), and determination of crystal structures for the DNA binding domain (Hard et al., 1990; Luisi et al., 1991) and ligand binding domain (Bledsoe et al., 2002).

Glucocorticoids (GCs) are key endocrine regulators and are members of the family of steroid hormones. They are cholesterol derivatives and are synthesized and secreted by the adrenal gland. Glucocorticoids play a role in embryonic development, regulation of metabolic homeostasis, central nervous system function and modulation of the immune response. In humans the physiological glucocorticoid is cortisol while the functional equivalent in rodents is corticosterone. Cortisol circulates in blood in three main forms: protein-bound, “free” cortisol or as cortisol conjugates. Only 5% of cortisol is in the “free” form and it is the physiologically active hormone. The remaining 90- 95% cortisol is bound either by the high-affinity, low -capacity cortisol-binding globulin (CBG) or the low-affinity, high capacity albumin (Cole, 2006). The circadian rhythm regulated release of glucocorticoids from the adrenal gland is ultradian and highly pulsatile. Circadian fluctuations are correlated with daily cycles of high and low activity (Lightman, 2006). Ultradian mode of glucocorticoid secretion in rodents is well documented but not completely understood (Windle et al., 1998a; Windle et al., 1998b). Ultradian cortisol secretion in humans is documented but less widely recognized (Young et al., 2004).

Glucocorticoids have binding affinities for two steroid receptors- the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). GR is expressed to varying degrees in all cell types while the expression of MR is more restricted. In the unliganded state, GR forms an inactive cytoplasmic multi-protein complex with heat shock proteins (HSP), such as HSP 90, immunophilins and p23 (Pratt & Toft, 2003). These molecular chaperones function to assist the proper folding of GR into conformation optimal for hormone binding and prevent the nuclear translocation of unbound receptor. Upon encountering glucocorticoid (ligand), GR is activated by the loss of bound heat shock protein. GR then dimerizes and translocates into the nucleus. Once in the nucleus GR has to find its target hormone response element (HRE) in the context of chromatin.

Glucocorticoids are potent anti-inflammatory and immunosuppressant agents. GCs are frequently prescribed in the treatment of several diseases, to name few- rheumatoid arthritis (Buttgereit et al., 2004), inflammatory bowel diseases (Metge et al., 2001) and obstructive pulmonary disease (Highland, 2004). Unfortunately, GC therapy is sometimes hampered by severe side effects, such as osteoporosis, skin atrophy, cushingoid appearance, diabetes and glaucoma, which place limitations on the use of higher GC dosages and the long-term use of GCs (Song et al., 2005; Schacke et al., 2007). Beneficial anti-inflammatory GC effects are mediated to a major extent via transrepression, while many side effects are due to transactivation. Novel GC receptor ligands, such as selective glucocorticoid receptor agonists (SEGRAs), which show a reduced side-effect profile, while maintaining the anti-inflammatory and immunosuppressive properties of traditional GCs could be answers to this problem (Lowenberg et al., 2007; Schacke et al., 2007). Therefore, a better understanding of the biology and molecular mechanism of GC action is the key to further identification of novel drug targets.

GR AND CHROMATIN INTERACTIONS

When GR enters the nucleus it has the daunting task of targeting itself to the right glucocorticoid response element (GRE) in the context of packaged chromatin. Therefore, we visualize, that

in this scenario GR is constantly sampling the whole population of nucleosomes for a productive interaction. A very recent study reveals that GR binding invariably occurs at DNaseI hypersensitive sites (DHS) and accessibility of the GR binding sites are either constitutive or hormone-inducible. The specificity of GR response is further defined by the choice of the remodeling complex at these hypersensitive sites (John et al., 2008). We propose that GR primarily goes through a “scanning mode” in search for target GREs (Figure 1: *Dynamic scanning of chromatin by GR*). The inherent dynamic property of GR, to interact with chromatin both transiently and rapidly is key to this primary screening stage (Nagaich et al., 2004b).

Current understanding suggests that the purpose of these chromatin-modifying activities is to reorganize the nucleosome architecture of regulated genes to provide greater access for both transcription factors and the basal transcription machinery. The nuclear receptors appear to be members of a restricted group of activators with the ability to bind chromatin and initiate the remodeling process as pioneer proteins (Lemon & Freedman, 1999; Urnov & Wolffe, 2001). The singular role of GR as a “pioneer” factor that can initiate the chromatin remodeling process has currently been revisited. The results from this study show that receptor often binds to pre-existing regions of open chromatin and the authors propose that the chromatin architecture in a given cell type is organized to facilitate receptor template interactions appropriate for that particular cell. Using dominant negative Brg-1 mutants, pre-existing open chromatin detected as regions of hypersensitivity to DNaseI (DHS sites) (Dorschner et al., 2004) can be identified as both Brg-1 dependent and independent sites. Additionally, GR binding events which lead to localized chromatin transitions can be also be distinguished as Brg-1 dependent and independent as a function of these dominant negative mutants (John et al., 2008).

As a class, steroid receptors are believed to bind statically to response elements in the presence of ligand. The DNA-receptor complex serves as a platform for the assembly of coactivators and general transcription factors. These large multifactorial complexes would then perform the wide variety of activities required for local chromatin transitions that eventually lead to transcription initiation. A pre-requisite of this model is that the DNA-receptor complex has a long interaction time in the presence of hormone. While the results of many studies have been interpreted to be in support of this general ordered recruitment model, however, by integrating time into the analysis of transcription, live-cell imaging techniques have revealed the dynamic, cooperative, functionally redundant and cyclical nature of gene expression (Hager et al., 2006; Metivier et al., 2006). A recent review discusses how the current view on steroid hormone receptor (SHR) mediated transcription has progressively shifted from that of a static holoenzyme of transcription factors that steadily build up after the initial binding of receptor to its response element, to a highly dynamic picture where different factors rapidly move in and out to perform temporary and local functions (Griekspoor et al., 2007).

Visualizing factor dynamics

The development of fluorescent proteins has proved to be a powerful tool that allows the researcher to visualize and track the proteins in a live cell scenario. An elegant approach to study a real time view of protein - template dynamics has become possible by marking the protein of interest with green fluorescent protein (GFP) or variations, such as red fluorescent protein (RFP). Since chromosomes do not move rapidly in live cells (Marshall et al., 1997), it is possible, using photobleaching techniques like fluorescence recovery after photobleaching (FRAP), to characterize the interaction of a soluble fluorescent transcription factor with the relatively immobile DNA template. To unambiguously visualize site specific localized molecules against the background of thousands of nuclear, non-bound, molecules, the solution is an amplified set of regulatory elements. The amplification is needed not because of a difficulty in detecting small numbers of protein molecules as it has been reported that even a

single GFP molecule can be observed (Baldini et al., 2005). The localized amplification, through the use of tandem arrays of the gene of interest was first reported for the GR inducible MMTV promoter (McNally et al., 2000). The in vivo real time visualization of template interactions can only be achieved through the use of amplified gene arrays as it allows the discrimination between receptor molecules interacting with DNA against the background of unbound molecules. However, it is very important to establish that the genes within these arrays behave analogously to normal, single copy sequences. The response of the MMTV promoters within the amplified arrays has been rigorously characterized (Fragoso et al., 1998; Kramer et al., 1999) and it was shown that the chromatin reorganization event summed over the individual promoter copies in the array is indistinguishable from the event averaged over many cells with single gene copies. Additionally, it has been established that the kinetics of the receptor induced transcription observed in the array cells is also identical to that originally described in low copy cells (Archer et al., 1994; Smith et al., 1997).

Using an array containing 200 copies of the MMTV promoter, the promoter specific recruitment of GFP-GR in a living cell has been visualized (McNally et al., 2000). This advance permitted the direct measurement of residence times for GR on the amplified MMTV template. The unprecedented results from these experiments was that the receptor was only present on the template for a period in the range of 10-20 seconds, in complete disagreement with the prevailing view of long-term, stable template interactions (McNally et al., 2000). Subsequent studies by other investigators (Agresti et al., 2005), and in other amplified systems (Dundr et al., 2002a), demonstrated correspondingly high mobilities for other transcription factors. Studies on the well-characterized estrogen-regulated promoter pS2 demonstrate that estrogen receptor (ER) recruitment and turnover at the promoter are cyclic events (Reid et al., 2003; Stenoien et al., 2001a; Stenoien et al., 2001b). Interestingly, it has been shown that the iconic transcription factor NF- κ B in living cells is immobilized onto high-affinity binding sites only very transiently and independently of promoter occupancy by other sequence-specific transcription factors. Moreover, changes in the nuclear concentration of NF- κ B are reflected in promoter activity, with a sensitivity in a timescale of seconds. In light of emerging evidence from several groups supporting the transient and dynamic nature of transcription factor and DNA binding site interactions, Bianchi and colleagues propose a revision of the established enhanceosome concept (Bosisio et al., 2006).

Dynamics of GR in vitro

Of the members of the steroid receptor family, GR has led the path to establish the dynamic nature of steroid receptor and chromatin interactions. For over two decades, the mouse mammary tumor virus (MMTV) long terminal repeat (LTR) has been a useful model system to study nuclear receptor transcription regulation. When integrated in cellular chromosomes, the MMTV LTR promoter adopts a specific chromatin organization consisting of six positioned nucleosomes (Fragoso et al., 1995; Richard-Foy & Hager, 1987). Activation of the MMTV promoter by steroid hormones is associated with a region-specific chromatin structural transition detected as an increase in sensitivity to nucleases (Richard-Foy & Hager, 1987; Richard-Foy et al., 1987) and restriction enzymes (Archer et al., 1991). This hormone induced structural remodeling event is implicated, in the secondary binding of transcription factors that are excluded by unremodeled chromatin (Archer et al., 1992; Blomquist et al., 1996; Cordingley et al., 1987; Pina et al., 1990; Truss et al., 1995).

To investigate the nature of GR-induced chromatin interactions in vitro, a 1.8-kb fragment of the MMTV promoter attached to magnetic beads was reconstituted utilizing the *Drosophila* chromatin assembly system into a polynucleosome template followed by direct interaction-pull down assays (Fletcher et al., 2000). This study provided evidence for GR induced chromatin transitions that occurred only in the presence ATP and of HeLa nuclear extracts

using restriction enzyme accessibility assays. In addition, this ATP dependent “open” chromatin coincides with ejection of GR from the template. The first observation of the dynamic behavior of GR *in vitro* is compatible with the “hit-and-run” mechanism for receptor action (Rigaud et al., 1991). According to this model, the receptor interacts transiently with the promoter (“hit”), resulting in a chromatin state more accessible to other factors, and then is dynamically displaced from hormone response elements (“run”). GR dynamics is also consistent with findings from living cell experiments which reveal that the receptor is not statically bound to the MMTV-LTR in the continued presence of ligand, but rather exchanges at a high rate between the chromatin (McNally et al., 2000). It was proposed that this GR induced ATP dependent open chromatin configuration is likely due to recruitment of remodeling factors by GR.

In continuing experiments using the template pull-down assay, purified GR when present in a chromatin binding reaction with nuclear extract and ATP, it was seen that both GR and the chromatin-remodeling complex, human SWI/SNF, are displaced from the template. This ATP dependent GR displacement was specific to chromatin templates and notably does not occur on a naked DNA template. Although GR dissociates during chromatin remodeling, it was found that its presence in the reaction, along with that of hSWI/SNF, is crucial for NF-1 binding to its site in chromatin. This study further suggested that the dynamic behavior observed for GR may be a general feature of many transcriptional activators, particularly those that are able to interact productively with non-remodeled chromatin.

The development of UV laser technology has overcome the difficulty of poor time resolution in factor-template dynamic studies *in vitro* (Nagaich & Hager, 2004). Laser UV radiation crosslinks sequence-specific DNA-binding proteins to the template much more efficiently than standard UV sources by virtue of a 2-photon effect (Dimitrov & Moss, 2001). The very brief (5 nanosecond) pulses of laser light have sufficient energy to crosslink significant amounts of protein to the template allowing one to follow factor/template interactions as the reaction proceeds (Nagaich & Hager, 2004; Nagaich & Hager, 2004). UV laser crosslinking has irrevocably shown that the binding of GR to MMTV regulatory elements is transient and periodic recurring in five minutes bursts. In addition, this transient binding phenomenon requires the presence of SWI/SNF and ATP, and crosslinking between DNA and the Brg1 subunit of the SWI/SNF complex also varies with a five minute period. Furthermore, crosslinking efficiencies for the H2A and H2B components of the histone octamer core undergo transition with a five minute periodicity, indicating that nucleosome remodeling is intimately associated with receptor recruitment and loss (Nagaich et al., 2004b). This decisive finding suggests that receptor interaction with the chromatin template is a highly dynamic process, in contrast, to the prevailing view of stable, long-term, receptor DNA interactions (Shang et al., 2000; Burakov et al., 2002; Metivier et al., 2003). Nagaich and colleagues (Nagaich et al., 2004a) proposed a specific model for receptor displacement during chromatin remodeling. In this model during early phases of the nucleosome remodeling reaction, receptor binding is assisted, resulting in multiple receptor binding sites becoming available in the “open” chromatin structure. Then, as the remodeling reaction is completed and nucleosomes return to ground state, receptors are actively ejected from the template.

MECHANICS OF GR DYNAMICS

Investigations to identify the possible mechanisms that can account for the high mobility of steroid receptors and other transcription factors on regulatory elements have pointed to the irreplaceable role of ATP. When cells are depleted of ATP stores, protein movement in the nucleus, as well as, site-specific template interactions are strongly retarded (Elbi et al., 2004; Stavreva et al., 2004; Agresti et al., 2005). Two separate, energy dependent mechanisms have recently been implicated in transcription factor mobility, the first involving SWI/SNF

remodeling complexes, the second, the involvement of chaperone molecules. (Figure 2: *Mechanisms of GR mobility*).

Chromatin remodeling -ATP dependent GR dynamics

Chromatin remodeling is a critical step for transcriptional activation on nucleoprotein templates. Although the ability to remodel chromatin is intrinsic to chromatin-remodeling machineries such as SWI/SNF, several lines of evidence from in vitro studies demonstrate that the remodeling activity is targeted to specific promoters by interaction with transcriptional activators. In particular, yeast SWI/SNF can be recruited to chromatin-containing GAL4 sites in vitro by acidic activation domains fused to GAL4 (Yudkovsky et al., 1999). In several model systems, gene activation by steroid receptors has been shown to be dependent on nucleosome remodeling activities such as the SWI/SNF complex (Ichinose et al., 1997; Yoshinaga et al., 1992; Muchardt & Yaniv, 1993; Fryer & Archer, 1998). In vitro-translated GR has been found to interact specifically with the hSWI/SNF subunit BAF 250 (Nie et al., 2000). Evidence of in vivo recruitment of hSWI/SNF by estrogen receptors was demonstrated using chromatin immunoprecipitation assays (DiRenzo et al., 2000). The localized changes in DNA topology were found to require ATP hydrolysis in the remodeling reaction (Fletcher et al., 2002; Gavin et al., 2001; Havas et al., 2000). The chromatin remodeling event at the MMTV promoter by both GR or PR has been recapitulated in vitro with a six nucleosome template and highly purified preparations of receptor and the SWI/SNF remodeling complex (Fletcher et al., 2002; Rayasam et al., 2005). In these experiments, receptor was stably associated with the promoter when presented as free DNA, or with chromatin in the absence of remodeling activity, but was specifically lost from the template during the remodeling reaction thus implying a role for chromatin remodeling in factor dynamics.

While displacement of GR occurs only on chromatin, the ATP-dependent release of SWI/SNF is observed on both chromatin and DNA templates (Fletcher et al., 2002). Like GR, ATP-dependent changes in NF-1 binding are exclusive to chromatin. However, an increase in NF-1 binding which requires the presence of GR during chromatin remodeling by SWI/SNF is observed (Fletcher et al., 2002). PR also induces MMTV transcription and recruits NF-1 to the promoter. This PR action is facilitated by remodeling complexes; and it has been proposed that direct protein-protein interactions contribute to a stable PR/NF-1/template complex (Di Croce et al., 1999).

A recent global study by John and colleagues (John et al., 2008) characterizes GR binding events and chromatin structural transitions across a wide spectrum of both GR induced and repressed genes. DHS sites were shown to require the Brg1 containing SWI/SNF complex, but others are Brg1 independent. This observation that there is widespread requirement for chromatin remodeling at both GR inducible and repressed genes supports the view that the rapid exchange of receptor proteins occurs during nucleosome reorganization.

Chaperone- ATP dependent GR dynamics

Steroid/nuclear receptors are assembled in the cytoplasm into large dynamic super-complexes that contain a consortium of accessory proteins, including hsp90, hsp70, hsp40, Hop, p23 and immunophilins (Pratt & Toft, 2003; Pratt & Toft, 1997). It has been reported (Freeman & Yamamoto, 2002) that molecular chaperones, p23 and Hsp90 are selectively recruited to genomic response elements in a hormone dependent manner. p23, and to a lesser extent Hsp90 respond to signaling changes by promoting the disruption of receptor-mediated transcriptional activation in vivo and in vitro by targeting receptor interactions with both response elements and coactivators. Unliganded PR and GR normally reside in a large molecular weight complex with several chaperones (Kosano et al., 1998; Smith & Toft, 1993). Addition of hormone releases the chaperones, producing an activated receptor that will bind hormone response

elements and induce gene expression. The folding activity of the chaperones is thought to be required to generate an open conformation of the hydrophobic steroid binding cleft that can be assessed by the non-polar steroid. If ligand is lost from the activated receptor, the ligand-free protein cannot rebinding hormone without reformation of the chaperone/receptor complex (Pratt et al., 1996). This process is referred to as the “chaperone cycle” for steroid receptors.

In an attempt to understand the mechanism of GR dynamics, cells are permeabilized with digitonin to release much of the free cytoplasmic protein (Greber & Gerace, 1995; Elbi et al., 2004). The GFP-labeled receptors that remain in the nucleus were found to be completely immobile, showing no detectable recovery after laser bleaching. Surprisingly, addition of a cocktail of seven chaperone proteins, including hsp70, Ydj-1, Hop, hsp90, p23, FKBP51 and CHIP, led to the recovery of a major fraction of glucocorticoid receptor mobility. Recovery of mobility was completely dependent on ATP (Elbi et al., 2004). The chaperone cocktail was also effective in the recovery of progesterone receptor, but failed to elicit recovery of mobility for the HP1 heterochromatin binding protein, which is reported to be highly mobile in living cells (Cheutin et al., 2003). These findings suggest a second, ATP-dependent component of receptor mobility and argue that chaperone function is required not only for ligand assimilation, but also for the movement of receptor from target to target in the nucleus. This observation is particularly interesting in light of the findings from Thompson and colleagues (Kumar et al., 1999) that the unstructured AF1 domain of the glucocorticoid receptor acquires structure upon binding to DNA. These investigators have also reported induced structural transitions upon interaction with receptor coactivators (Kumar et al., 2001; Kumar & Thompson, 2003). It is thus possible to argue that major structural reorganizations occur in receptor domains upon interaction with both DNA and coregulators, and these transitions could be assisted by the chaperone refolding activities. In this view, receptor refolding would be required for receptor movement from target to target, and chaperones would serve as a “molecular grease” for this mobility.

Ligand dependent factor dynamics

Steroid receptors, such as the glucocorticoid (GR), progesterone (PR), and estrogen receptor (ER), require ligand to bind DNA and are believed to assist in the formation of large, multifactor complexes that reside on the DNA template in the continued presence of hormone (Xu et al., 1999). This cycling behavior of ER has shown to be modulated by agonists, antagonists and proteasome inhibitors (Stenoien et al., 2001a; Sharp et al., 2006). The kinetics of ligand bound GR mobility has been attributed to the binding affinity of the ligand. Notably, for the agonists, the binding affinity of dexamethasone is greater than corticosterone and consequently, GR mobility is lower when bound to dexamethasone (Schaaf & Cidlowski, 2003). The exchange rate for GR at the MMTV array is also reflective of the bound ligand. Antagonist liganded receptors show a higher rate of exchange in comparison to receptors bound with agonists such as dexamethasone or corticosterone. Longer GR residence times are associated with productive transcription. It was also observed that the synthetic hormone dexamethasone is a more efficient agonist than the natural hormone corticosterone (Stavreva et al., 2004). Unfortunately, the high efficiency of an agonist in vitro will induce debilitating side effects in vivo. Efforts to identify “dissociated” GR ligands- SEGRA’s - are now a major component of drug discovery in the pharmaceutical industry. Rapid ligand dynamics may emerge as a key factor in identifying new compounds with advantageous therapeutical activity.

While characterizing the dynamic interactions of progesterone receptor with the MMTV template. Rayasam et al. (Rayasam et al., 2005) demonstrated that like GR, PR is also lost from the template during chromatin remodeling. Additionally, it was demonstrated that the type of ligand associated with the receptor can have a dramatic impact on the interaction of the receptor with the chromatin remodeling apparatus. A distinguishing characteristic of agonist R5020

bound PR is its rapid exchange with the MMTV promoter, in contrast, to the slow exchange of antagonist RU486-PR complex. Significantly, the recruitment of SWI/SNF and consequently the ATP-dependent displacement of PR was a property determined by the ligand, wherein the agonist R5020 or antagonist RU486 - PR complex can recruit SWI/SNF, while PR activated with antagonist ZK98299 cannot do so. These observations allowed the authors to propose that ligand specific interactions with remodeling complexes strongly influence receptor nuclear dynamics and rates of exchange with chromatin in living cells.

In corroborating studies, in the case of the androgen receptor (AR), the mobility of unliganded and antagonist-bound AR was found to be different to that of agonist-bound AR (Farla et al., 2005). In a more recent study (Klokk et al., 2007), show that the residence time of AR on the template is significantly higher in the presence of agonists, than when it is bound to antagonists. Another significant observation was the ligand-specific recruitment of the chromatin-remodeling complex SWI/SNF by AR to the MMTV promoter. Agonists and partial antagonist CPA induced the recruitment of Brm to the MMTV array, while no specific recruitment was observed for RU486 and the pure antagonists OHF and bicalutamide, strongly suggesting that agonist/AR induced chromatin remodeling, leading to longer receptor residence time on the template. Compared to PR (Rayasam et al., 2005) and GR (McNally et al., 2000), AR had displayed a slower recovery kinetics, suggesting that there are differences in the individual mechanism of receptor- promoter interactions.

CLASSIC vs DYNAMIC KINETICS

Two contrasting views still exist for the sequential development of factor complexes and chromatin modifications on regulated promoters. The classic model suggests that large, relatively stable, multiprotein complexes are assembled on target DNA response elements initiated by sequence specific DNA-binding proteins. These complexes would engage the template for relatively long periods, measured in minutes or even hours, which would lead to the eventual recruitment of the RNA polymerase II machinery. In some cases, factor exchange could occur between template bound proteins and alternative activating and repressing activities, as has been argued in the nuclear receptor coactivator/ corepressor model (Perissi et al., 2004; Zhang & Lazar, 2000). The concepts of rapid dynamic movement and template exchange have emerged only recently through the development of single cell biochemistry (Tsien & Miyawaki, 1998), and the application of these real time methodologies to the study of genome/ factor interactions (Hager, 2002; Nagaich et al., 2004a). As a result of these transient interactions, promoters move through many states during activation and repression (Figure 3 *Models contrasting classic & dynamic view*).

Two general concepts emerge from current literature. First, various transcription factors appear to follow “ordered recruitment” to promoters on a time scale of minutes to hours in response to a stimulus. Secondly, during this response the proteins that interact with chromatin may cycle on and off the promoter multiple times. During these ordered recruitment cycles, the individual molecules that form functional complexes often exchange rapidly on a time scale of seconds. This rapid exchange of molecules within a formed complex occurs independently of long-term cycling on chromatin. Rapid nuclear dynamics, can be attributed to chromatin remodeling complexes, molecular chaperones, the proteasome degradation machinery and ligands.

Many secondary implications further arise from the dynamic view of receptor behavior. As an example, the receptors would be immediately and continually available for interaction with other signaling pathways. Coordination of the receptor response with other regulatory pathways can be integrated much more effectively under the dynamic model. Biological significance of the ultradian hormone secretion for GR function and GR-regulated gene

transcription is unknown and is being studied for the first time in our lab. Unpublished data (Stavreva et al., unpublished) shows that when corticosterone is applied in an ultradian manner (consecutive hormone additions and withdrawals) cyclic GR loading at the MMTV array promptly reflects the hormone cycles. This fast GR recycling is of central importance for the appropriate GR activation in response to changing hormone levels. In striking contrast, when the addition of synthetic hormone dexamethasone is used to mimic the ultradian glucocorticoid secretion cycle, GR interaction with the array loses its cyclic pattern, suggesting a much stronger and longer GR/dexamethasone binding. The much stronger GR-dexamethasone interactions in vivo have been previously reported (Munck & Foley, 1976; Stavreva et al., 2004). These physiologically relevant results emphasize that GR interactions are highly dynamic and that this dynamic behavior is crucial for proper GR responses to the constantly fluctuating extracellular hormone levels.

The term dynamics is frequently used in the description of factor/ template interactions and the evolution of promoter states. When referring to dynamics in the context of transcriptional activation, transcription factors are believed to bind/unbind over time intervals of 20 to 30 minutes, or even longer. For example, ER cycle times of 40 to 60 minutes was initially reported to be (Shang et al., 2000), and later explored in detail (Metivier et al., 2003). Factor exchange is envisioned as a slow process, and the key nucleating protein (a nuclear receptor) remains constantly and stably bound to the template for extended periods of time. Evidence supporting this model has been obtained primarily from experiments based on the chromatin immunoprecipitation technology which is an end point reaction and thus shows a given transcription complex associated with a target promoter for long periods. In contrast, experiments that examine the real time interaction of fluorescently tagged GR with response elements in living cells are less than one minute (Rayasam et al., 2005; Hager et al., 2004; Becker et al., 2002; McNally et al., 2000). Evidence consistent with rapid exchange is now emerging from other systems suggesting that the dynamic interaction of regulatory proteins with the template may be a common mechanism for many transcription factors - PolII transcription apparatus (Dundr et al., 2002a) and NF- κ B (Bosisio et al., 2006). These observations are incompatible with the classic view of stable complexes, and show regulatory proteins moving rapidly on and off the template. Proteins involved in transcriptional activation, chromatin modification, DNA repair, RNA splicing and processing have all been shown to exhibit dynamic behavior (Misteli, 2001).

It has been demonstrated (Dundr et al., 2002b) that while GR exchanges rapidly with the MMTV array, the number of GR molecules associated with the promoter is consistent with the number of binding sites. It is likely that most of these binding events, though specific, are stochastic and nonproductive. The rate-limiting step in transcription might reflect the time necessary to form productive and functional, transcriptionally competent complexes. Consistent with this concept, it has been demonstrated that RNA Pol I subunits enter their target region as distinct subunits and not as an assembled holocomplex (Dundr et al., 2002a; Dundr et al., 2002b). Surprisingly, proteins such as H1 that have classically been thought to be stable components of chromatin have been shown to exchange very rapidly (Misteli et al., 2000; Cheutin et al., 2003). This rapid exchange has been observed in both euchromatin and heterochromatin. In contrast, core histones appear to be more stably associated with chromatin (Kimura & Cook, 2001). In well-characterized systems, such as the IFN-beta promoter and the HO promoter in yeast, it has been proposed that the long-lived interactions of proteins with chromatin result in the biochemically stable enhanceosome complex (Agalioti et al., 2000; Cosma et al., 1999). A recent report that counters the prevailing view that slow cycling initiates transcription demonstrates that fast and slow cycling can occur simultaneously on an endogenous yeast promoter. It is proposed that fast cycling initiates transcription and that slow cycling regulates the quantity of mRNA produced (Karpova et al., 2008).

EPIGENETICS AND DYNAMICS

Epigenetic marks have been demonstrated to exhibit unique gene expression patterns which are stably inherited over generations. Several inheritable diseases are attributed to epigenetics and genome imprinting (Reik, 2007). Thus, the potential role of “epigenetic therapy” is being actively embraced. Histone modifications and their regulation have dominated the role of chromatin in epigenetic processes, but both histone modifications and chromatin remodeling are required for all chromatin based mechanisms. Recently other chromatin-based processes have been proposed to play a role in epigenetic regulation, including the deposition of histone variants and nucleosome remodeling (Li et al., 2007; Turner, 2007). Henikoff (Henikoff, 2008) discusses dynamic chromatin regulation, with nucleosome remodelers providing the driving forces that disrupt chromatin and result in diverse patterns of histone modifications and variants. The cell cycle dependent analysis of Brg1 and hBrm has revealed that both proteins undergo phosphorylation on entry into mitosis, and although the level of Brg1 remains constant, hBrm appears to be degraded during mitosis (Muchardt et al., 1996; Reyes et al., 1997). It was proposed that this transitional inactivation and reactivation of hSWI/SNF is required for the formation of repressed chromatin structure during mitosis and reformation of active chromatin structure as cells leave mitosis (Sif et al., 1998).

In an analysis of the GR response element (GRE), it was reported that activation of GR led to the recruitment of hepatocyte nuclear factor 5 wherein it appears that GR initiates the nucleosome transition but is immediately ejected from the template during chromatin remodeling, leaving behind a region of reorganized chromatin (Rigaud et al., 1991). The reorganization event has been suggested to be responsible for secondary transcription factor recruitment (Archer et al., 1990; Archer et al., 1991). Contrary to the view of steroid receptors as pioneer proteins that can interact globally with non-remodeled chromatin, a major finding of a recent study is that the glucocorticoid receptor often binds to “pre-existing,” or constitutive hypersensitive transitions. The authors propose that access to a given receptor binding site is likely to be governed by the nucleoprotein organizational state of a given HRE and that the DHS profile is highly cell specific, implicating cell selective organization of the chromatin landscape as a critical determinant of tissue-selective receptor function (John et al., 2008). In other words the epigenetic marks associated with chromatin are the signposts for transcriptional activation or repression and are not only cell type specific but also inheritable. Many groups are now investigating the genome-wide location of histone variants and epigenetic marks (Mikkelsen et al., 2007; Barski et al., 2007; Roh et al., 2004). In the case of GR, it has been documented that the histone variant H2A.Z is not only enriched at both constitutive and hormone inducible DNase I hypersensitive sites but it is also lost in response to hormone (John et al., 2008). In recent reports, two groups make an unprecedented observation that the epigenetically preserved DNA methylation at CpG dinucleotides is not a stable modification but rather both transient and cyclic in response to transcriptional activation (Metivier et al., 2008; Kangaspeska et al., 2008). Therefore the dynamic nature of regulatory complexes is not limited to transcription factors but interestingly also to an epigenetic mark like methylation. The dynamic nature inherent to transcription regulation has clearly been understated and as more reports emerge of its prevalence we certainly cannot ignore its significance. Thus, the molecular understanding of dynamics in detail is essential to our understanding of transcriptional regulation in the context of inheritable epigenetic patterns and a dynamic chromatin environment. As it is becoming evident that epigenetics is more than histone modifications the role of chromatin remodelers as potential drug targets in epigenetic therapy should be an intensive area of research.

References

- Agalioti T, Lomvardas S, Parekh B, Yie J, Maniatis T, Thanos D. Ordered recruitment of chromatin modifying and general transcription factors to the IFN-beta promoter. *Cell* 2000;103:667–678. [PubMed: 11106736]
- Agresti A, Scaffidi P, Riva A, Caiolfa VR, Bianchi ME. GR and HMGB1 interact only within chromatin and influence each other's residence time. *Mol Cell* 2005;18:109–121. [PubMed: 15808513]
- Archer TK, Cordingley MG, Wolford RG, Hager GL. Transcription factor access is mediated by accurately positioned nucleosomes on the mouse mammary tumor virus promoter. *Mol Cell Biol* 1991;11:688–698. [PubMed: 1846670]
- Archer TK, Hager GL, Omichinski JG. Sequence specific DNA binding by glucocorticoid receptor "finger peptides". *Proc Natl Acad Sci USA* 1990;87:7560–7564. [PubMed: 2120703]
- Archer TK, Lee HL, Cordingley MG, Mymryk JS, Fragoso G, Berard DS, et al. Differential steroid hormone induction of transcription from the mouse mammary tumor virus promoter. *Mol Endocrinol* 1994;8(5):568–576. [PubMed: 8058066]
- Archer TK, Lefebvre P, Wolford RG, Hager GL. Transcription factor loading on the MMTV promoter: A bimodal mechanism for promoter activation. *Science* 1992;255:1573–1576. [PubMed: 1347958]
- Baldini G, Cannone F, Chirico G. Pre-unfolding resonant oscillations of single green fluorescent protein molecules. *Science* 2005;309:1096–1100. [PubMed: 16099991]
- Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, et al. High-resolution profiling of histone methylations in the human genome. *Cell* 2007;129:823–837. [PubMed: 17512414]
- Becker M, Baumann CT, John S, Walker D, Vigneron M, McNally JG, et al. Dynamic behavior of transcription factors on a natural promoter in living cells. *EMBO Rep* 2002;3:1188–1194. [PubMed: 12446572]
- Bernstein E, Hake SB. The nucleosome: a little variation goes a long way. *Biochem Cell Biol* 2006;84:505–517. [PubMed: 16936823]
- Bledsoe RK, Montana VG, Stanley TB, Delves CJ, Apolito CJ, McKee DD, et al. Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition. *Cell* 2002;110:93–105. [PubMed: 12151000]
- Blomquist P, Li Q, Wrangé O. The affinity of nuclear factor 1 for its DNA site is drastically reduced by nucleosome organization irrespective of its rotational or translational position. *Journal of Biological Chemistry* 1996;271:153–159. [PubMed: 8550551]
- Bosisio D, Marazzi I, Agresti A, Shimizu N, Bianchi ME, Natoli G. A hyper-dynamic equilibrium between promoter-bound and nucleoplasmic dimers controls NF-kB-dependent gene activity. *EMBO J* 2006;25:798–810. [PubMed: 16467852]
- Burakov D, Crofts LA, Chang CP, Freedman LP. Reciprocal recruitment of DRIP/mediator and p160 coactivator complexes in vivo by estrogen receptor. *Journal of Biological Chemistry* 2002;277:14359–14362. [PubMed: 11893728]
- Buttgereit F, Straub RH, Wehling M, Burmester GR. Glucocorticoids in the treatment of rheumatic diseases: an update on the mechanisms of action. *Arthritis Rheum* 2004;50:3408–3417. [PubMed: 15529366]
- Cheutin T, McNairn AJ, Jenuwein T, Gilbert DM, Singh PB, Misteli T. Maintenance of stable heterochromatin domains by dynamic HP1 binding. *Science* 2003;299:721–725. [PubMed: 12560555]
- Cole TJ. Glucocorticoid action and the development of selective glucocorticoid receptor ligands. *Biotechnol Annu Rev* 2006;12:269–300. [PubMed: 17045197]
- Cordingley MG, Riegel AT, Hager GL. Steroid-dependent interaction of transcription factors with the inducible promoter of mouse mammary tumor virus in vivo. *Cell* 1987;48:261–270. [PubMed: 3026639]
- Cosgrove MS. Histone proteomics and the epigenetic regulation of nucleosome mobility. *Expert Rev Proteomics* 2007;4:465–478. [PubMed: 17705705]
- Cosma MP, Tanaka T, Nasmyth K. Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle- and developmentally regulated promoter. *Cell* 1999;97:299–311. [PubMed: 10319811]

- Di Croce L, Koop R, Venditti P, Westphal HM, Nightingale KP, Corona DF, et al. Two-step synergism between the progesterone receptor and the DNA-binding domain of nuclear factor 1 on MMTV minichromosomes. *Mol Cell* 1999;4:45–54. [PubMed: 10445026]
- Dimitrov S, Moss T. UV laser-induced protein-DNA crosslinking. *Methods Mol Biol* 2001;148:395–402. [PubMed: 11357601]
- DiRenzo J, Shang Y, Phelan M, Sif S, Myers M, Kingston RE, et al. BRG-1 is recruited to estrogen-responsive promoters and cooperates with factors involved in histone acetylation. *Mol Cell Biol* 2000;20:7541–7549. [PubMed: 11003650]
- Dorschner MO, Hawrylycz M, Humbert R, Wallace JC, Shafer A, Kawamoto J, et al. High-throughput localization of functional elements by quantitative chromatin profiling. *Nat Methods* 2004;1:219–225. [PubMed: 15782197]
- Dundr M, Hoffmann-Rohrer U, Hu Q, Grummt I, Rothblum LI, Phair RD, et al. A kinetic framework for a mammalian RNA polymerase in vivo. *Science* 2002a;298:1623–1626. [PubMed: 12446911]
- Dundr M, McNally JG, Cohen J, Misteli T. Quantitation of GFP-fusion proteins in single living cells. *J Struct Biol* 2002b;140:92–99. [PubMed: 12490157]
- Elbi C, Walker DA, Romero G, Sullivan WP, Toft DO, Hager GL, et al. Molecular chaperones function as steroid receptor nuclear mobility factors. *Proc Natl Acad Sci USA* 2004;101:2876–2881. [PubMed: 14978266]
- Farla P, Hersmus R, Trapman J, Houtsmuller AB. Antiandrogens prevent stable DNA-binding of the androgen receptor. *J Cell Sci* 2005;118:4187–4198. [PubMed: 16141232]
- Fletcher TM, Ryu B-W, Baumann CT, Warren BS, Fragoso G, John S, et al. Structure and dynamic properties of the glucocorticoid receptor-induced chromatin transition at the MMTV promoter. *Mol Cell Biol* 2000;20:6466–6475. [PubMed: 10938123]
- Fletcher TM, Xiao N, Mautino G, Baumann CT, Wolford RG, Warren BS, et al. ATP-dependent mobilization of the glucocorticoid receptor during chromatin remodeling. *Mol Cell Biol* 2002;22:3255–3263. [PubMed: 11971959]
- Fragoso G, John S, Roberts MS, Hager GL. Nucleosome positioning on the MMTV LTR results from the frequency-biased occupancy of multiple frames. *Genes Dev* 1995;9:1933–1947. [PubMed: 7649476]
- Fragoso G, Pennie WD, John S, Hager GL. The position and length of the steroid-dependent hypersensitive region in the mouse mammary tumor virus long terminal repeat are invariant despite multiple nucleosome B frames. *Mol Cell Biol* 1998;18:3633–3644. [PubMed: 9584204]
- Freeman BC, Yamamoto KR. Disassembly of transcriptional regulatory complexes by molecular chaperones. *Science* 2002;296:2232–2235. [PubMed: 12077419]
- Fryer CJ, Archer TK. Chromatin remodeling by the glucocorticoid receptor requires the BRG1 complex. *Nature* 1998;393:88–91. [PubMed: 9590696]
- Gavin IM, Horn PJ, Peterson CL. SWI/SNF Chromatin Remodeling Requires Changes in DNA Topology. *Mol Cell* 2001;7:97–104. [PubMed: 11172715]
- Goldberg AD, Allis CD, Bernstein E. Epigenetics: a landscape takes shape. *Cell* 2007;128:635–638. [PubMed: 17320500]
- Greber UF, Gerace L. Depletion of calcium from the lumen of endoplasmic reticulum reversibly inhibits passive diffusion and signal-mediated transport into the nucleus. *J Cell Biol* 1995;128:5–14. [PubMed: 7822421]
- Griekspoor A, Zwart W, Neeffjes J, Michalides R. Visualizing the action of steroid hormone receptors in living cells. *Nucl Recept Signal* 2007;5:e003. [PubMed: 17464358]
- Hager, GL. Recent Advances in Glucocorticoid Receptor Action. Berlin: Springer-Verlag; 2002. The dynamics of intranuclear movement and chromatin remodeling by the glucocorticoid receptor; p. 111-129.
- Hager GL, Elbi C, Johnson TA, Voss TC, Nagaich AK, Schiltz RL, et al. Chromatin dynamics and the evolution of alternate promoter states. *Chromosome Res* 2006;14:107–116. [PubMed: 16506100]
- Hager GL, Elbi CC, Becker M. Protein dynamics in the nuclear compartment. *Curr Opin Genet Dev* 2002;12:137–141. [PubMed: 11893485]
- Hager GL, Nagaich AK, Johnson TA, Walker DA, John S. Dynamics of nuclear receptor movement and transcription. *Biochim Biophys Acta* 2004;1677:46–51. [PubMed: 15020044]

- Hard T, Kellenbach E, Boelens R, Maler BA, Dahlman K, Freedman LP, et al. Solution structure of the glucocorticoid receptor DNA-binding domain. *Science* 1990;249:157–160. [PubMed: 2115209]
- Havas K, Flaus A, Phelan M, Kingston RE, Wade PA, Lilley DM, et al. Generation of Superhelical Torsion by ATP-Dependent Chromatin Remodeling Activities. *Cell* 2000;103:1133–1142. [PubMed: 11163188]
- Henikoff S. Nucleosome destabilization in the epigenetic regulation of gene expression. *Nat Rev Genet* 2008;9:15–26. [PubMed: 18059368]
- Highland KB. Inhaled corticosteroids in chronic obstructive pulmonary disease: is there a long-term benefit? *Curr Opin Pulm Med* 2004;10:113–119. [PubMed: 15021180]
- Ichinose H, Garnier JM, Chambon P, Losson R. Ligand-dependent interaction between the estrogen receptor and the human homologues of SWI2/SNF2. *Gene* 1997;188:95–100. [PubMed: 9099865]
- Jenuwein T, Allis CD. Translating the histone code. *Science* 2001;293:1074–1080. [PubMed: 11498575]
- John S, Sabo PJ, Johnson TA, Sung MH, Biddie SC, Lightman SL, et al. Interaction of the glucocorticoid receptor with the global chromatin landscape. *Mol Cell* 2008;29:611–624. [PubMed: 18342607]
- Kangaspeska S, Stride B, Metivier R, Polycarpou-Schwarz M, Ibberson D, Carmouche RP, et al. Transient cyclical methylation of promoter DNA. *Nature* 2008;452:112–115. [PubMed: 18322535]
- Karpova TS, Kim MJ, Spriet C, Nalley K, Stasevich TJ, Kherrouche Z, et al. Concurrent fast and slow cycling of a transcriptional activator at an endogenous promoter. *Science* 2008;319:466–469. [PubMed: 18218898]
- Kimura H, Cook PR. Kinetics of core histones in living human cells: little exchange of H3 and H4 and some rapid exchange of H2B. *J Cell Biol* 2001;153:1341–1353. [PubMed: 11425866]
- Klokk TI, Kurys P, Elbi C, Nagaich AK, Hendarwanto A, Slagsvold T, et al. Ligand-specific dynamics of the androgen receptor at its response element in living cells. *Mol Cell Biol* 2007;27:1823–1843. [PubMed: 17189428]
- Kornberg RD. Chromatin structure: a repeating unit of histones and DNA. *Science* 1974;184:868–871. [PubMed: 4825889]
- Kosano H, Stensgard B, Charlesworth MC, McMahon N, Toft D. The assembly of progesterone receptor-hsp90 complexes using purified proteins. *Journal of Biological Chemistry* 1998;273:32973–32979. [PubMed: 9830049]
- Kramer P, Fragoso G, Pennie WD, Htun H, Hager GL, Sinden RR. Transcriptional state of the mouse mammary tumor virus promoter can effect topological domain size in vivo. *Journal of Biological Chemistry* 1999;274:28590–28597. [PubMed: 10497225]
- Kumar R, Baskakov IV, Srinivasan G, Bolen DW, Lee JC, Thompson EB. Interdomain signaling in a two-domain fragment of the human glucocorticoid receptor. *Journal of Biological Chemistry* 1999;274:24737–24741. [PubMed: 10455143]
- Kumar R, Lee JC, Bolen DW, Thompson EB. The conformation of the glucocorticoid receptor af1/tau1 domain induced by osmolyte binds co-regulatory proteins. *Journal of Biological Chemistry* 2001;276:18146–18152. [PubMed: 11279138]
- Kumar R, Thompson EB. Transactivation functions of the N-terminal domains of nuclear hormone receptors: protein folding and coactivator interactions. *Mol Endocrinol* 2003;17:1–10. [PubMed: 12511601]
- Lemon B, Tjian R. Orchestrated response: a symphony of transcription factors for gene control. *Genes Dev* 2000;14:2551–2569. [PubMed: 11040209]
- Lemon BD, Freedman LP. Nuclear receptor cofactors as chromatin remodelers. *Curr Opin Genet Dev* 1999;9:499–504. [PubMed: 10508693]
- Li B, Carey M, Workman JL. The role of chromatin during transcription. *Cell* 2007;128:707–719. [PubMed: 17320508]
- Lightman SL. Patterns of exposure to glucocorticoid receptor ligand. *Biochem Soc Trans* 2006;34:1117–1118. [PubMed: 17073764]
- Lowenberg M, Stahn C, Hommes DW, Buttgeriet F. Novel insights into mechanisms of glucocorticoid action and the development of new glucocorticoid receptor ligands. *Steroids*. 2007
- Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* 1997;389:251–260. [PubMed: 9305837]see comments

- Luisi BF, Xu WX, Otwinowski Z, Freedman LP, Yamamoto KR, Sigler PB. Crystallographic analysis of the interaction of the glucocorticoid receptor with DNA. *Nature* 1991;352:497–505. [PubMed: 1865905]see comments
- Marshall WF, Straight A, Marko JF, Swedlow J, Dernburg A, Belmont A, et al. Interphase chromosomes undergo constrained diffusional motion in living cells. *Curr Biol* 1997;7:930–939. [PubMed: 9382846]
- Maston GA, Evans SK, Green MR. Transcriptional regulatory elements in the human genome. *Annu Rev Genomics Hum Genet* 2006;7:29–59. [PubMed: 16719718]
- McNally JG, Mueller WG, Walker D, Wolford RG, Hager GL. The glucocorticoid receptor: Rapid exchange with regulatory sites in living cells. *Science* 2000;287:1262–1265. [PubMed: 10678832]
- Mellor J. Dynamic nucleosomes and gene transcription. *Trends Genet* 2006;22:320–329. [PubMed: 16631276]
- Metge CJ, Blanchard JF, Peterson S, Bernstein CN. Use of pharmaceuticals by inflammatory bowel disease patients: a population-based study. *Am J Gastroenterol* 2001;96:3348–3355. [PubMed: 11774948]
- Metivier R, Gallais R, Tiffocche C, Le PC, Jurkowska RZ, Carmouche RP, et al. Cyclical DNA methylation of a transcriptionally active promoter. *Nature* 2008;452:45–50. [PubMed: 18322525]
- Metivier R, Penot G, Hubner MR, Reid G, Brand H, Kos M, et al. Estrogen receptor- α directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter. *Cell* 2003;115:751–763. [PubMed: 14675539]
- Metivier R, Reid G, Gannon F. Transcription in four dimensions: nuclear receptor-directed initiation of gene expression. *EMBO Rep* 2006;7:161–167. [PubMed: 16452926]
- Miesfeld R, Rusconi S, Godowski PJ, Maler BA, Okret S, Wikström AC, et al. Genetic complementation of a glucocorticoid receptor deficiency by expression of cloned receptor cDNA. *Cell* 1986;46:389–399. [PubMed: 3755378]
- Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, et al. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* 2007;448:553–560. [PubMed: 17603471]
- Misteli T. Protein dynamics: implications for nuclear architecture and gene expression. *Science* 2001;291:843–847. [PubMed: 11225636]
- Misteli T, Gunjan A, Hock R, Bustin M, Brown DT. Dynamic binding of histone H1 to chromatin in living cells. *Nature* 2000;408:877–881. [PubMed: 11130729]
- Muchardt C, Reyes JC, Bourachot B, Leguoy E, Yaniv M. The hbrm and BRG-1 proteins, components of the human SNF/SWI complex, are phosphorylated and excluded from the condensed chromosomes during mitosis. *EMBO J* 1996;15:3394–3402. [PubMed: 8670841]
- Muchardt C, Yaniv M. A human homologue of *Saccharomyces cerevisiae* SNF2/SWI2 and *Drosophila* brm genes potentiates transcriptional activation by the glucocorticoid receptor. *EMBO J* 1993;12:4279–4290. [PubMed: 8223438]
- Munck A, Brinck-Johnsen T. Specific metabolic and physicochemical interactions of glucocorticoids in vivo and in vitro with rat adipose tissue and thymus cells. *Excerpta Med Int Cong Ser* 1967;132:472–481.
- Munck A, Foley R. Kinetics of glucocorticoid-receptor complexes in rat thymus cells. *J Steroid Biochem* 1976;7:1117–1122. [PubMed: 1025357]
- Nagaich AK, Hager GL. UV laser cross-linking: A real-time assay to study dynamic protein/DNA interactions during chromatin remodeling. *Sci STKE* 2004;256:PL13. [PubMed: 15507594]
- Nagaich AK, Rayasam GV, Martinez ED, Johnson TA, Elbi C, John S, et al. Subnuclear trafficking and gene targeting by nuclear receptors. *Ann N Y Acad Sci* 2004a;1024:213–220.
- Nagaich AK, Walker DA, Wolford RG, Hager GL. Rapid periodic binding and displacement of the glucocorticoid receptor during chromatin remodeling. *Mol Cell* 2004b;14:163–174. [PubMed: 15099516]
- Nie Z, Xue Y, Yang D, Zhou S, Deroo BJ, Archer TK, et al. A specificity and targeting subunit of a human SWI/SNF family-related chromatin-remodeling complex. *Mol Cell Biol* 2000;20:8879–8888. [PubMed: 11073988]

- Perissi V, Aggarwal A, Glass CK, Rose DW, Rosenfeld MG. A corepressor/coactivator exchange complex required for transcriptional activation by nuclear receptors and other regulated transcription factors. *Cell* 2004;116:511–526. [PubMed: 14980219]
- Pina B, Brüggemeier U, Beato M. Nucleosome positioning modulates accessibility of regulatory proteins to the mouse mammary tumor virus promoter. *Cell* 1990;60:719–731. [PubMed: 2155706]
- Pratt WB, Gehring U, Toft DO. Molecular chaperoning of steroid hormone receptors. *EXS* 1996;77:79–95. [PubMed: 8856970]
- Pratt WB, Toft DO. Steroid receptor interactions with heat shock protein and immunophilin chaperones. *Endocr Rev* 1997;18:306–360. [PubMed: 9183567]
- Pratt WB, Toft DO. Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. *Exp Biol Med* (Maywood) 2003;228:111–133. [PubMed: 12563018]
- Ptashne M. On the use of the word ‘epigenetic’. *Curr Biol* 2007;17:R233–R236. [PubMed: 17407749]
- Rayasam GV, Elbi C, Walker DA, Wolford RG, Fletcher TM, Edwards DP, et al. Ligand specific dynamics of the progesterone receptor in living cells and during chromatin remodeling in vitro. *Mol Cell Biol* 2005;25:2406–2418. [PubMed: 15743833]
- Reid G, Hubner MR, Metivier R, Brand H, Denger S, Manu D, et al. Cyclic, proteasome-mediated turnover of unliganded and liganded ERalpha on responsive promoters is an integral feature of estrogen signaling. *Mol Cell* 2003;11:695–707. [PubMed: 12667452]
- Reik W. Stability and flexibility of epigenetic gene regulation in mammalian development. *Nature* 2007;447:425–432. [PubMed: 17522676]
- Reyes JC, Muchardt C, Yaniv M. Components of the human SWI/SNF complex are enriched in active chromatin and are associated with the nuclear matrix. *J Cell Biol* 1997;137:263–274. [PubMed: 9128241]
- Richard-Foy H, Hager GL. Sequence specific positioning of nucleosomes over the steroid-inducible MMTV promoter. *EMBO J* 1987;6:2321–2328. [PubMed: 2822386]
- Richard-Foy H, Sistare FD, Riegel AT, Simons SS Jr, Hager GL. Mechanism of dexamethasone 21-mesylate antiglucocorticoid action: II. Receptor-antiglucocorticoid complexes are unable to interact productively with MMTV LTR chromatin in vivo. *Mol Endocrinol* 1987;1:659–665. [PubMed: 2856414]
- Rigaud G, Roux J, Pictet R, Grange T. In vivo footprinting of rat TAT gene: dynamic interplay between the glucocorticoid receptor and a liver-specific factor. *Cell* 1991;67:977–986. [PubMed: 1683601]
- Roh TY, Ngau WC, Cui K, Landsman D, Zhao K. High-resolution genome-wide mapping of histone modifications. *Nat Biotechnol* 2004;22:1013–1016. [PubMed: 15235610]
- Ruthenburg AJ, Li H, Patel DJ, Allis CD. Multivalent engagement of chromatin modifications by linked binding modules. *Nat Rev Mol Cell Biol* 2007;8:983–994. [PubMed: 18037899]
- Schaaf MJ, Cidlowski JA. Molecular determinants of glucocorticoid receptor mobility in living cells: the importance of ligand affinity. *Mol Cell Biol* 2003;23:1922–1934. [PubMed: 12612067]
- Schacke H, Berger M, Rehwinkel H, Asadullah K. Selective glucocorticoid receptor agonists (SEGRAs): novel ligands with an improved therapeutic index. *Mol Cell Endocrinol* 2007;275:109–117. [PubMed: 17630119]
- Shang Y, Hu X, DiRenzo J, Lazar MA, Brown M. Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. *Cell* 2000;103:843–852. [PubMed: 11136970]
- Sharp ZD, Mancini MG, Hinojos CA, Dai F, Berno V, Szafran AT, et al. Estrogen-receptor-alpha exchange and chromatin dynamics are ligand- and domain-dependent. *J Cell Sci* 2006;119:4101–4116. [PubMed: 16968748]
- Sif S, Stukenberg PT, Kirschner MW, Kingston RE. Mitotic inactivation of a human SWI/SNF chromatin remodeling complex. *Genes Dev* 1998;12:2842–2851. [PubMed: 9744861]
- Smith CL, Htun H, Wolford RG, Hager GL. Differential activity of progesterone and glucocorticoid receptors on mouse mammary tumor virus templates differing in chromatin structure. *Journal of Biological Chemistry* 1997;272:14227–14235. [PubMed: 9162055]
- Smith DF, Toft DO. Steroid receptors and their associated proteins. *Mol Endocrinol* 1993;7:4–11. [PubMed: 8446107]

- Song IH, Gold R, Straub RH, Burmester GR, Buttgereit F. New glucocorticoids on the horizon: repress, don't activate! *J Rheumatol* 2005;32:1199–1207.
- Sproul D, Gilbert N, Bickmore WA. The role of chromatin structure in regulating the expression of clustered genes. *Nat Rev Genet* 2005;6:775–781. [PubMed: 16160692]
- Stavreva DA, Muller WG, Hager GL, Smith CL, McNally JG. Rapid glucocorticoid receptor exchange at a promoter is coupled to transcription and regulated by chaperones and proteasomes. *Mol Cell Biol* 2004;24:2682–2697. [PubMed: 15024059]
- Stenoien DL, Nye AC, Mancini MG, Patel K, Dutertre M, O'Malley BW, et al. Ligand-mediated assembly and real-time cellular dynamics of estrogen receptor alpha-coactivator complexes in living cells. *Mol Cell Biol* 2001a;21:4404–4412. [PubMed: 11390668]
- Stenoien DL, Patel K, Mancini MG, Dutertre M, Smith CL, O'Malley BW, et al. FRAP reveals that mobility of oestrogen receptor-alpha is ligand- and proteasome-dependent. *Nat Cell Biol* 2001b;3:15–23. [PubMed: 11146621]
- Strahl BD, Allis CD. The language of covalent histone modifications. *Nature* 2000;403:41–45. [PubMed: 10638745]
- Truss M, Bartsch J, Schelbert A, Hache RJ, Beato M. Hormone induces binding of receptors and transcription factors to a rearranged nucleosome on the MMTV promoter in vivo. *EMBO J* 1995;14:1737–1751. [PubMed: 7737125]
- Tsien RY, Miyawaki A. Seeing the machinery of live cells. *Science* 1998;280:1954–1955. [PubMed: 9669950]
- Turner BM. Defining an epigenetic code. *Nat Cell Biol* 2007;9:2–6. [PubMed: 17199124]
- Urnov FD, Wolffe AP. A necessary good: nuclear hormone receptors and their chromatin templates. *Mol Endocrinol* 2001;15:1–16. [PubMed: 11145735]
- Voss TC, Hager GL. Visualizing chromatin dynamics in intact cells. *Biochim Biophys Acta Mol Cell Res.* 2008in press
- Ward E, Slocumb CH, Polley HF, Kendall EC, Hench PS. Clinical effects of cortisone administered orally to 100 patients with rheumatoid arthritis. *Ann Rheum Dis* 1951;10:477–484. [PubMed: 14895098]
- Weinberger C, Hollenberg SM, Ong ES, Harmon JM, Brower ST, Cidlowski JA, et al. Identification of human glucocorticoid receptor complementary DNA clones by epitope selection. *Science* 1985;228:740–742. [PubMed: 2581314]
- Windle RJ, Wood SA, Lightman SL, Ingram CD. The pulsatile characteristics of hypothalamo-pituitary-adrenal activity in female Lewis and Fischer 344 rats and its relationship to differential stress responses. *Endocrinology* 1998a;139:4044–4052. [PubMed: 9751481]
- Windle RJ, Wood SA, Shanks N, Lightman SL, Ingram CD. Ultradian rhythm of basal corticosterone release in the female rat: dynamic interaction with the response to acute stress. *Endocrinology* 1998b;139:443–450. [PubMed: 9449609]
- Xu L, Glass CK, Rosenfeld MG. Coactivator and corepressor complexes in nuclear receptor function. *Curr Opin Genet Dev* 1999;9:140–147. [PubMed: 10322133]
- Yoshinaga SK, Peterson CL, Herskowitz I, Yamamoto KR. Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. *Science* 1992;258:1598–1604. [PubMed: 1360703]
- Young EA, Abelson J, Lightman SL. Cortisol pulsatility and its role in stress regulation and health. *Front Neuroendocrinol* 2004;25:69–76. [PubMed: 15571755]
- Yudkovsky N, Logie C, Hahn S, Peterson CL. Recruitment of the SWI/SNF chromatin remodeling complex by transcriptional activators. *Genes Dev* 1999;13:2369–2374. [PubMed: 10500094]
- Zhang J, Lazar MA. The mechanism of action of thyroid hormones. *Annu Rev Physiol* 2000;62:439–466. [PubMed: 10845098]

Glossary

AR

Androgen receptor

DBD

	DNA binding domain
DHS	DNase I hypersensitive site
ER	Estrogen receptor
FRAP	Fluorescence recovery after photobleaching
GFP	Green fluorescent protein
GR	Glucocorticoid receptor
HREs	Hormone response elements
HSP	Heat shock proteins
LBD	Ligand binding domain
MMTV	Mouse mammary tumor virus
NF-1	Nuclear factor- 1
PR	Progesterone receptor
PTMs	Posttranslational modifications
SEGRAs	Selective glucocorticoid receptor agonists
SHR	Steroid hormone receptor
SWI/SNF	Switching/sucrose nonfermenting remodeling complex

Fig. 1. Dynamic scanning of chromatin by GR

(A) Transcription factors must sample a large number of potential binding sites throughout the genome, prior to successful interaction at a hormone response element. (B) Productive interaction of the receptor at a response element invariably involves a local chromatin remodeling event (John et al., 2008), catalyzed by Swi/Snf (or other) remodeling systems. Laser crosslinking experiments (C)(Nagaich et al., 2004) indicate that the receptor is actively ejected from the template (time scale of sec.). This process is continuously and rapidly repeated.

Fig. 2. Mechanisms of GR mobility

(1) Receptor cycles continuously on and off chromatin (McNally et al., 2000). (2) The released receptor may either retain ligand, and return to the template, or lose ligand, and enter the chaperone cycle for rebinding of hormone. (3) During these return cycles, receptor may complex with many alternative cofactors, introducing a highly stochastic element to the activation process.

Fig. 3. Models contrasting classic and dynamic view of receptor chromatin interactions

(A) Receptor occupancy of response elements has been classically modeled as long term interactions with life times measured in minutes or hours. Thus ChIP data (B) has been interpreted as reflecting the direct occupancy status of the receptor (C). The dynamic view (D) argues that receptors return continuously to binding sites (Figure 2), giving rise to transient binding events (E). Averaging of these transient events over the cell population leads to the integrated ChIP signal (B, C).