Chromatin structure and gene regulation: a dynamic view of enhancer function

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Localized chromatin organization is now recognized as an important determinant of cell identity and developmental pathways. Recent studies have demonstrated that these epigenetic states are unexpectedly dynamic and malleable. In this Extra view we will highlight the transient nature of stimulus-induced enhancer accessibility and its importance for transcription regulation. Using glucocorticoid receptor (GR) as a model system we will discuss spatiotemporal relationships between receptor/chromatin interactions, lifetimes of the DNase I hypersensitivity sites (DHSs), long-range interactions, and gene regulation. We propose that differential temporal activation and utilization of distal regulatory elements plays a role in directing divergent stimulus-induced transcriptional programs.

Introduction

Cells in a multicellular organism are subjected to a plethora of signals, including endocrine and paracrine hormones. These temporal changes in cellular environment impact cell physiology and gene transcription. While some of these changes are stochastic, many hormones are released in a complex temporal fashion.1-4 As an example, GR-stimulating glucocorticoids are released from the adrenal glands in a circadian, as well as hourly (ultradian) manner5-9 (Fig. 1A). As a result, GR-expressing cells experience frequent changes in the level of the activating hormone. Intrinsic cellular processes and differences in physiological states of individual cells may further influence hormone signaling and increase cell-to-cell variability of the transcriptional responses. However, the primary driver of the GR-regulated gene expression is pulsatile, cell extrinsic hormonal signaling.

Considering that endocrine signals govern the function of all tissues and organs in the body, it is surprising how little is known about the genomic effects of the naturally occurring hormone release patterns. The complex and dynamic picture of the GR-mediated transcription regulation10,11 is still not fully appreciated and hormonal fluctuations are frequently dismissed as “noise.” Here we underscore the fact that cells have evolved mechanisms to utilize these naturally occurring transient changes in hormone availability in establishing biologically appropriate patterns of receptor loading, enhancer activation, long-range interactions, and gene activity on the genomic scale.

Dynamic Interactions of TFs with Regulatory Elements

It is becoming apparent that transcription regulation is comprised of many rapid and time-sensitive molecular processes.11-15 To integrate various temporal signals in coherent transcriptional responses, transcription factors (TFs), target chromatin sites, and the transcriptional machinery must detect, adapt, and respond to these changes. It is well established that in vivo the majority of TFs interact with chromatin targets only transiently.12,16-18 Several implications for these dynamic interactions were proposed: (i) regulation of the transcriptional output,19,20 (ii) “sensing” fluctuations in the
levels of activating signals, and (iii) allowing a noncompetitive transient access for binding of secondary regulatory factors. Numerous studies have demonstrated that transient GR association with GR regulatory elements (GREs) in vivo (chromatin cycle) is a key feature of GR signaling. In addition, a much slower cycle involving nuclear chaperone machinery has been implicated. These two superimposed molecular cycles (Fig. 1B and C) ensure that GR interactions with GREs, detectable by microscopic techniques as well as biochemical methods such as chromatin immunoprecipitation (ChIP), occur in a strictly hormone-dependent manner (Fig. 1D).

Figure 1. Ultradian mode of glucocorticoid release and its effects on the GR/chromatin interactions. (A) Schematic representation of the glucocorticoid release pattern: circadian profile consists of discrete ultradian (hourly) pulses. (B) At the peak of an ultradian pulse (red arrow) hormone-activated GR interacts with GREs in a transient manner (chromatin cycle). GR molecules undergo a frequent hormone disassociation-reassociation cycles and the hormone-free receptor must interact with the chaperone machinery to regain hormone binding affinity in the nucleus (chaperone cycle). (C) Hormone disassociation-reassociation cycles allow GR to “sense” a decline in the hormone level (nadir, black arrow), leading to an accumulation of hormone-free receptors incapable of productive interactions with GREs. Unbound receptors will either associate with the chaperone machinery or get degraded by the proteasome. (D) GR loading at GREs strictly follows hormone level fluctuations as demonstrated by chromatin immunoprecipitation against GR followed by high throughput sequencing (ChIP-seq). (E) The GR peaks diminish upon hormone withdrawal, while a subsequent hormone pulse reestablishes GR affinity to GREs. (F) Constant stimulation (reminiscent of the hormone release pattern under stress conditions) increases the level of GR binding at the GREs. Considering that ChIP results are representative of the population average, it is unclear whether under this conditions more GR molecules become engaged at a given GRE or whether the GRE becomes occupied in a bigger number of cells.
Lifetimes of Chromatin Accessibility

All GR binding takes place at “open” chromat sites sensitive to DNase I digestion, either at pre-existing (pre-programmed) sites, or sites actively induced by the receptor. Once considered just a packaging mechanism for DNA, chromatin is now known to be responsible for the functional organization of the genetic material within the nucleus providing a selective access to genomic regulatory elements.

Chromatin accessibility is regulated by nucleosome remodeling, utilization of histone variants, DNA methylation, and posttranslational modifications (PTMs). Chromatin remodeling complexes, or enhancer activity upon termination of hypersensitivity state even 40 minutes upon termination of the stimulated hypersensitive state even 40 minutes upon termination of hormone withdrawal. The basis for enhancer activation such as p300 and chromatin remodelers (BRG1), even before hormone induction.

While the hypersensitivity of the transient DHSSs is clearly GR-dependent, the behavior of the persistent sites cannot be explained by a simple mode of hormone dependent GR binding. Transient GR-mediated remodeling was demonstrated to facilitate the access for binding of secondary regulatory factors, which further influence chromatin state, and we envision such factors driving accessibility of the persistent sites even after hormone withdrawal (Fig. 2B). In a limited number of cases we found that AP1 was recruited to persistent DHSSs in a hormone-dependent manner and remained at the site even after hormone withdrawal (unpublished data). In these instances AP1-mediated chromatin remodeling could be responsible for the sustained level of hypersensitivity at these sites. However, the secondary factors required for the sustenance of hypersensitivity are likely to be site specific as this mode of AP1-mediated remodeling was not apparent at other sites (unpublished data).

Thus, the molecular mechanisms driving diverse DHS dynamics are not completely clear, but the abovementioned differences in the initial state of hypersensitivity, existing variations in DHS-associated motifs targeted by various secondary factors, as well as establishment of specific PTMs all could play a role. For example, retention of the H3K4me1 modification at an enhancer upon termination of the activating stimulus was implicated in creation of “memory” of the stimulation as well as in the faster and stronger response upon restimulation. It is unclear whether histone modifications at transient and persistent sites differ and additional studies will be required to fully understand the mechanisms behind epigenomic “memory” of hormone induction as well as its implications for transcription regulation.

Enhancer Dynamics, Long-Range Interactions and Transcription Regulation

We previously demonstrated that ultradian hormone release promotes cyclic GR interaction with regulatory elements, leading to cyclic release of nascent RNA from a number of GR regulated genes. Our genome-wide studies confirmed that gene pulsing is the predominant transcriptional response of the GR-regulated genes. However, we also uncovered transcription profiles which could not be extrapolated from the strictly hormone-dependent GR binding to GREs. Higher order chromatin structure and organization contributes to gene expression regulation. Thus, it is possible that the diverse lifetimes of accessibility and activation of distal regulatory elements could contribute to the observed divergent patterns of gene regulation through combinatorial long-range interactions. This is especially relevant considering that the majority of the GR binding sites are found away from promoters of GR-responsive genes.

It was recently discovered that mammalian genomes are hierarchically organized into megabase-sized topologically associating domains (TADs) within the chromosome territories and that CTCF and cohesins were both implicated in anchoring the loops formed between TAD boundaries. TADs are stably maintained during differentiation and development while the organization within the TADs is cell-type specific. Enhancer-promoter looping within the TADs brings distal TF-bound regulatory sites closer to target genes. Many of the enhancer-promoter loops were found to be conserved between species while others were found to be tissue-specific and dependent on tissue-specific TFs. Interestingly, insertion of artificial zinc fingers to tether a looping factor (or its self-associating domain) to the β-globin promoter resulted in formation of a loop with the LCR as well as activation of the β-globin gene transcription. These data suggest that chromatin looping causally underlies gene regulation and that transcription factors through self-association domains could influence the formation of enhancer-promoter loops.
GR is frequently found at distal enhancer and can self-associate, suggesting that receptor binding could play a role in initiating or strengthening enhancer-promoter contacts, implicating loops in the regulation of glucocorticoid responsive genes. Indeed, the recent findings demonstrate that receptor binding at enhancers and the consequential change in their accessibility correlates with the frequency of interactions of these enhancers with nearby gene targets (Fig 3C-E). It should be noted that nonrandom enhancer-promoter interactions were detectable even before treatment; however receptor loading and alteration of the chromatin structure at enhancers significantly increased the frequency of these contacts.

A fundamental problem in long range enhancer contacts remains unresolved. As depicted in Fig 3F, many of the factors acting at these elements, including GR, have residence times in the range of 5-10 sec. Domain factors such as CTCF and cohesion are often assumed to have very long residence times, providing potential stability to these interactions. However there is as yet no direct evidence to support this concept. How rapidly exchanging proteins such as GR function within the actual molecular biochemistry at the long-range contacts is a major challenge for the field. Recent advances in live cell imaging, particularly single molecule tracking, hold some promise to address these difficult issues.

In addition to TFs and the well-established architectural proteins CTCF and cohesion, other factors such as mediator, coactivators (p300 and CBP), and eRNAs are also implicated in establishing promoter-enhancer loops. In a number of cases we noted hormone-dependent recruitment of Pol II at enhancers and confirmed the release of eRNA in a stimulus-dependent manner (unpublished data). Enhancer transcription could be just a reflection of opportunistic Pol II loading at accessible chromatin. However, previous studies reporting a correlation between the eRNA levels and the expression of nearby genes suggests that eRNA might be important for enhancer function. More systematic studies will be required to determine whether transient release of eRNA in a treatment-specific manner could influence the activity of enhancers and their participation in long-range interactions. Genome editing by CRISPR-Cas9 technology or targeting various factors to specific genomic sites using a variation of the CRISPR-Cas9 system will further add to our understanding of the role of the chromatin state of enhancers as well as their function(s).

Overall, our findings suggest that changes in chromatin accessibility may influence the frequency of the enhancer-promoter interactions. However, assuming that transient DHSs are always in the vicinity of transiently induced genes or that persistent DHSs are always in a close proximity to persistently active genes would be an over-simplification. Multiple enhancers likely contribute to the regulation of a gene in a combinatorial manner; furthermore, it is not always apparent which enhancers are functionally involved. Thus, additional studies addressing the role of chromatin structure and accessibility in gene regulation will be required. Individual genes and enhancers should be considered and a combination of high-resolution chromosome conformation

Figure 2. A model for hypersensitivity regulation at transient and persistent DHSs. (A) GR loading at transient sites recruits chromatin remodelers (not shown) leading to an increases accessibility at these sites, as well as Pol II recruitment and release of enhancer RNA (eRNA). Upon hormone withdrawal GR dissociation from GREs leads to dissociation of the remodelers and consequently to a loss of accessibility at these sites. (B) At the persistent DHSs, the GR-created accessibility allows binding of secondary TFs. These factors remain at the sites even after hormone withdrawal and continue to remodel it. This carry-over hypersensitivity may over time return to its initial state or lead to a permanent open state at the site.
capture-based methods as well as super-resolution microscopy methods applied to further elucidate this matter.

In summary, the recent study shows that hormone-driven interactions of GR with GREs lead to a complex variety of changes in chromatin accessibility at distal sites. In a number of cases changes in chromatin structure and long-range contacts correlated with gene activity, suggesting that accessibility-driven long-range interactions may have regulatory functions. Under this scenario, differential utilization of distal enhancers could be achieved by temporal alteration of their chromatin structure in a stimulus-dependent manner which may represent a novel principle for gene transcription regulation from a distance.

**Relevance to Physiological Hormone Action**

Recent advancements in our understanding of the interplay between lifetimes of chromatin states and transcription regulation reinforce the view that complex dynamics on multiple timescales are crucial for the execution of appropriate transcriptional programs. Thus it is not surprising that the pulsed and constant hormone stimulations are associated with divergent transcriptional patterns which may provide the molecular basis for the differential physiological outcomes associated with these different modes of hormone exposure.

These findings may have significant implications for glucocorticoid function in vivo and for steroid therapies. Changes in glucocorticoid levels in plasma are paralleled by similar changes in tissues where gene activity is regulated in a dynamic manner. It is plausible that
the disruptions of glucocorticoid release patterns, either as a result of a systemic disease, continuous stress, or treatment with synthetic glucocorticoids, may lead to aberrant patterns of activation of distal regulatory elements and aberrant transcriptional response in the glucocorticoid-target organs that have significant repercussions at the level of their normal physiology. Conversely, restoring the proper hormone delivery pattern may benefit glucocorticoid therapies in various clinical settings.

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No potential conflicts of interest were disclosed.

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