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Review

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 Thus, the concept of static, multi-protein complexes forming at regulatory elements in the genome has been replaced by a new paradigm that envisages rapid and continuous exchange events with the template.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.

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Abbreviations: 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.

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1. 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 and Allis, 2000). Further diversifying the nucleosome core particle is a set of histone isoforms known as histone variants (Bernstein and 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 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 and 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 process involves a series of precisely orchestrated and regulated steps. Any perturbation which results in dis-regulation 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 refocused on the molecular machines that have evolved to navigate through chromatin and mediate transcriptional control (Lemon and Tjian, 2000; Maston et al., 2006). Until recently, alternate states of promoter activity have been associated with the assembly of relatively stable multi-protein 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, 2004, 2006; Voss and Hager, 2008).

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 multifactorial 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 multi-protein 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 min 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.

2. 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, Munck and Brinck-Johnsen (1967) subsequently provided evidence for the glucocorticoid receptor. Two decades of studies on actions of the glucocorticoids culminated in the 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,b). 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 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 hsp90, immunophilins and