

UNDERSTANDING THE ROLE OF THE NUCLEOSOME REMODELING AND
DEACETYLASE COMPLEX IN GLUCOCORTICOID GENE EXPRESSION

By

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Abstract

Creating therapies that minimize off target effects is essential for disease treatment but a comprehensive understanding of the underlying mechanisms are crucial. Lysine deacetylase inhibitors are approved for clinical use in treatment of several diseases but the full effects are not understood. Specific lysine deacetylase inhibitors have been found to have a significant effect on metabolism leading to the discovery of the interplay between glucocorticoid receptor activated genes and lysine deacetylase enzymes. These enzymes are present in multi-protein complexes of which the functions are poorly understood. Furthermore functions of the individual subunits of each of these complexes need to be explored. The Smith lab aims to determine the mechanisms through which lysine deacetylases activate glucocorticoid gene transcription. This project is specifically seeking to identify the role of the lysine deacetylase 1/2 containing nucleosome remodeling complex.

Overview of DNA, RNA, and protein importance

The central dogma of biology is that deoxyribonucleic acid (DNA) is transcribed into ribonucleic acid (RNA) and RNA is translated into proteins. Proteins act as the building blocks and workhorses in biological systems. Genomic DNA is condensed and stored in the form of chromosomes which are made up of chromatin. The basic unit of chromatin is the nucleosome (figure 1). Each nucleosome is made up of DNA wrapped around eight histone proteins, called histone octamers. Along a chromosome, chromatin adopts various levels of compaction, from highly condensed regions to decondensed regions. For gene transcription to take place the chromatin must be in a decondensed form. Complexes that remodel nucleosomes and modify chromatin proteins facilitate this process to make space on the DNA for transcription machinery.

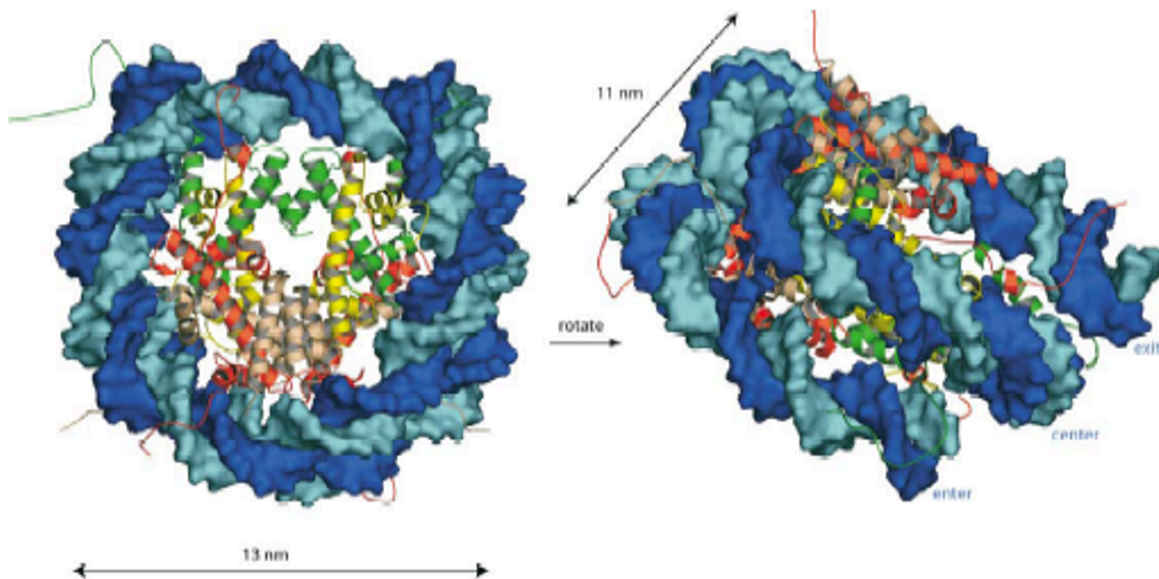


Figure 1: Reproduced from Khorasanizadeh, S. (2004). The Nucleosome. *Cell*. The fundamental unit of chromatin, the nucleosome

Chromatin remodeling and modification

Chromatin remodeling complexes use adenosine triphosphate (ATP) hydrolysis to move the nucleosomes on the chromatin while chromatin modifying complexes covalently alter histones and other chromatin-associated proteins (Mazina & Vorobyeva, 2021). These complexes function to compact or decondense chromatin. For example, remodeling complexes that condense chromatin are able to detect bound transcription factors (TFs) and expel TFs so the nucleosome can be repositioned (Li et al., 2015). It has been found that these remodeling complexes are targeted to specific genes through DNA looping based on the binding of general and specific transcription factors (Yadon et al., 2013). Chromatin modifying complexes play a role in gene transcription through DNA methylation or through direct regulation of post-translational modification of chromatin proteins, either adding these modifications or removing them.

Gene transcription

A gene has a regulatory region made up of promoters and enhancers, a coding region made up of introns and exons, and a termination region. The overall steps of gene transcription include enhancer activation, initiation, elongation, and termination. First transcription factors and coactivators bind to enhancer regions of the DNA. Transcription factors known as pioneer factors bind to nucleosomal DNA to recruit chromatin remodelers and modifiers to the gene (Cramer, 2019). Enhancer-bound transcription factors facilitate transcriptional initiation. During initiation the general transcription factors and RNA polymerase bind to the promoter region of the gene and form a pre-initiation complex. Helicase enzymes unzip the DNA helix and RNA transcription begins. Early on in elongation promoter proximal pausing takes place, allowing for greater regulation of gene expression (Core & Adelman, 2019). At this point the transcript is either prematurely terminated to stop expression of the gene or pausing is released and elongation of the RNA transcript continues (Core & Adelman, 2019). Termination and 3' processing take place simultaneously (Jez, 2021). The RNA transcript is cleaved in the 3' untranslated region and capping enzymes add a poly adenosine tail to the 5' end of the RNA transcript to prevent degradation (Jez, 2021). The RNA polymerase dissociates and the complete RNA transcript is released.

Gene transcription can be activated or repressed through signal transduction, which is initiated through the binding of extracellular molecules to receptors that are located either on the surface of cells or in the cellular cytoplasm. The activation of receptors leads to intracellular signaling cascades that result in the modification and activation of transcription factors (Weidemüller et al., 2021). This leads to the activation or repression of transcription at target genes. There are two ways in which transcription factor activity is regulated for the control of gene expression: post-transcriptional modifications of transcription factors and the accessibility of binding sites (Weidemüller et al., 2021). The Smith lab uses glucocorticoid signaling as a model to study regulation of gene transcription.

Glucocorticoid genes and receptors

Glucocorticoids (GCs) are steroid hormones that are secreted from the adrenal gland (Strehl et al., 2019). These hormones have many functions in the body including but not limited to regulation of glucose, proteins, and lipid metabolism, circadian rhythm, cell growth, differentiation, and apoptosis, inflammation and stress response

(Bereshchenko et al., 2018; Chourpiliadis & Aeddula, 2023; Strehl et al., 2019). Cortisol is the active physiological glucocorticoid in humans and is converted to and from its active and inactive form by enzymes in the liver (Strehl et al., 2019). In the liver GCs can prevent the breakdown of fatty acids through acetyl-CoA dehydrogenase catalyzing beta-oxidation, resulting in the storage of lipids in the liver cells in the form of triglycerides, which can contribute to fatty liver disease (Lettéron et al., 1997). Due to the anti-inflammatory and immunosuppressive effect synthetic glucocorticoids are often prescribed for treatment of inflammatory and autoimmune disorders (Paragliola et al., 2017). Use of these steroids can contribute to metabolic dysfunction-associated steatotic liver disease (MASLD) as well as insulin resistance (Polyzos & Targher, 2024). GCs induce lipolysis in adipose tissue resulting in higher circulating levels of free fatty acids (Xu et al., 2009).

GCs bind to glucocorticoid receptors (GRs) which are intracellular proteins that, when activated through GC binding, act as transcription factors for glucocorticoid responsive genes at specific DNA sequences referred to glucocorticoid response elements (GREs) that occur in enhancers and promoters of target genes (Nicolaidis et al., 2000; Strehl et al., 2019). GCs can also reduce gene expression through binding to negative GREs (Strehl et al., 2019).

Protein acetylation

Acetylation is a post translational modification of proteins where an acetyl group is moved from acetyl coenzyme A (acetyl CoA) onto a the α -amino group of the N-terminus of a protein or the ϵ -amino group of a lysine residue of a protein (Drazic et al., 2016). Acetylation of lysine residues is reversible while the acetylation of the N-terminus is not (Drazic et al., 2016). The importance of acetylation is that it affects the protein function through altering the solubility, hydrophobicity, shape, and interactions with substrates and cofactors (Christensen et al., 2019). The traditional model for the role of lysine acetylation in regulation of gene transcription is that acetylation positively regulates transcription via histone acetylation (Grunstein, 1997). The theory is that when lysine residues of nucleosomes are acetylated, the positive charge is neutralized (figure 2). This can have two effects: it causes loss of affinity of the nucleosomal histones for the negatively charged DNA, and it impairs inter-nucleosomal interactions that allow for chromatin folding and compaction (Grunstein, 1997).

Lysine deacetylases

Histone acetyl transferases (HATs) also known as lysine acetyltransferases (KATs) are enzymes responsible for moving the acetyl group from acetyl-CoA to lysine residues in a protein (figure 1). Histone deacetylases (HDACs), also known as lysine deacetylases (KDACs), are the enzymes that remove the acetyl group from lysine (figure 2). There are 2 groups of KDACs: zinc-dependent and NAD⁺-dependent. The latter group is known as sirtuins (Seto & Yoshida, 2014). The zinc-dependent KDACs are further grouped into four classes. Class I KDACs are localized to the nucleus (Haberland et al., 2009) and are subunits of several chromatin remodeling and modifying complexes. Class I KDACs include KDACs 1, 2, 3, and 8 but histones are not the substrates for HDAC8 (Olson et al., 2014)

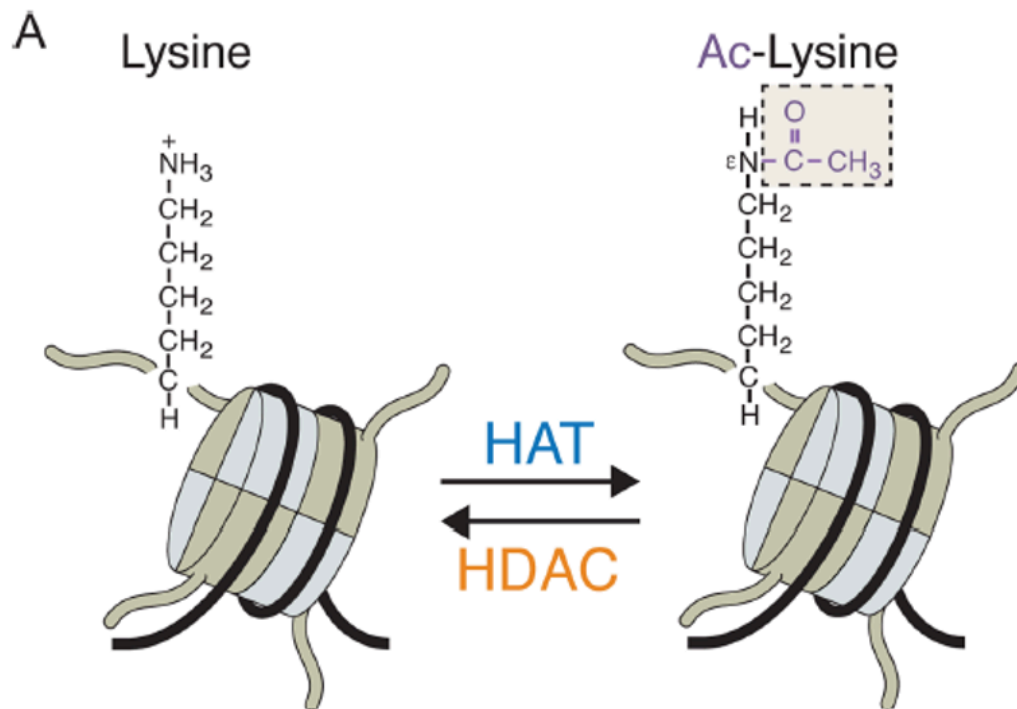


Figure 2: Reproduced from Gong, F., Chiu, L.-Y., & Miller, K. M. (2016). Acetylation Reader Proteins: Linking Acetylation Signaling to Genome Maintenance and Cancer. *PLOS Genetics*.

Clinical relevance of lysine deacetylase inhibitors

KDAC inhibitors (KDACi) are a class of drugs that induce hyperacetylation of histones as well as non-histone proteins, such as transcription factors (Bondarev et al., 2021). These are approved for the treatment of seizure disorders, migraines, and cancer (Bondarev et al., 2021; Luo & Li, 2020). However, scientists are evaluating their potential use in other diseases (Bondarev et al., 2021; Luo & Li, 2020). KDACi have been found to induce cell cycle arrest through upregulation of cyclin dependent kinase inhibitors and enhancing p53 activity through acetylation at specific p53 lysine residues (Shanmugam et al., 2022). A characteristic of many cancers is the loss of acetylation on histone 4, which makes KDACi a potential for targeted anti-cancer therapy (Fraga et al., 2005; Jenke et al., 2021). Though class I KDACs would be the ideal targets, most current KDACi use the mechanism of binding to the active site in zinc dependent KDACs making them act on multiple classes of KDAC proteins (Luo & Li, 2020; Shanmugam et al., 2022). General side effects associated with KDACi use include mild issues such as headaches, rashes, nausea, vomiting, fatigue, and cough but specific KDCAi drugs have more severe effects (Shah, 2019). There have been cases of cardiac toxicity, insulin resistance, kidney failure, acute liver failure, myelosuppression, and gastrointestinal issues (G. Luef et al., 2009; Shah, 2019).

The Smith lab found that the class I specific KDACi valproic acid (VPA) significantly impairs GR-activated gene transcription (Kadiyala et al., 2013). This result supports the concept that KDACs play a role in the induction of GR mediated transcription. Through small interfering RNA (siRNA) knockdowns of each of the class I

KDACs it was determined that only KDAC1 is required for GR activated gene expression (Kadiyala et al., 2013).

Lysine deacetylases 1 and 2

KDAC1/2 are very similar in structure, have been found to be partially redundant in specific cell types (Hess et al., 2022). KDACs 1 and 2 exist in cells associated with five distinct multi-protein complexes of which the functions are currently unknown (Yang & Seto, 2008). The Smith lab found only a single GR activated gene that needed both KDAC1/2 depleted to be repressed, suggesting the two may function together for specific genes (Kadiyala et al., 2013).

Nucleosome remodeling complex

The nucleosome-remodeling and deacetylase (NuRD) complex is one of the five HDAC1/2 containing complexes. This complex is composed of multiple paralogs of six subunits. Variations of the paralogs in this complex differ by cell type (Reid et al., 2023). It has also been found that specific paralogs of the complex are required for fetal hemoglobin (HbF) gene repression while the other paralogs have no effect (Sher et al., 2019). This suggests the possibility of gene specific roles of each paralog in the complex. NuRD complex has been found to directly modulate pluripotency related genes of undifferentiated embryonic stem cells (ESC) at the promoter and gene body regions both during ESC renewal and when the cells commit to differentiation (Reynolds et al., 2012), showing that the complex is involved in both positive and negative gene regulation.

NuRD is composed of a helicase module and a deacetylase module (Jason et al., 2016). The helicase module is made up of one CHD (chromodomain helicase DNA binding protein) protein, which can be CHD3, 4, or 5, and two GATAD2 proteins, p66 α/β , (Figure 3). The deacetylase module consists of two histone deacetylases, KDACs 1 and/or 2, two metastases tumor antigen paralogs, MTA1/2/3, and four of the two histone chaperones RBBP4/7 (Figure 3). Two methyl-CpG binding domain proteins, MBD2 and/or MBD3, hold the two modules together (figure 3), (Jason et al., 2016).

The deacetylase module is the focus of the NuRD complex as it contains KDAC1/2. The MTA Proteins act as the scaffolding for the NuRD complex, as well as directly bind to the HDACs and RBPs (Fig. 3). Domains within the MTA1/2/3 proteins are associated with nucleosome recognition (Low et al.). MTA1/2 have two binding domains for RBBP while MTA3 only has one (Reid et al.; Schmidberger et al.), this further supports the hypothesis that the specific paralogs present in the complex may hold specific roles in gene regulation through the NuRD complex. The RBBP proteins can bind to MTAs or histone H4, and histone H3, as well as multiple transcriptional coregulators (Reid et al.; Schmidberger et al.). It is suggested that RBBP4 can only bind to histone 4 when not found in the NuRD complex because that same binding site is used to bind to MTA1 (Alqarni et al.). Through two arginine fingers, MBD2 binds to methylated regions of DNA called CpG sites that are cytosine followed by guanine in the 5' to 3' direction (Liu et al.). This leads to the association of MBD2 with repressed genes (Günther et al.). While MBD3 can bind to both methylated and unmethylated CpG sites, it is more highly associated with activated genes (Günther et al.; Liu et al.).

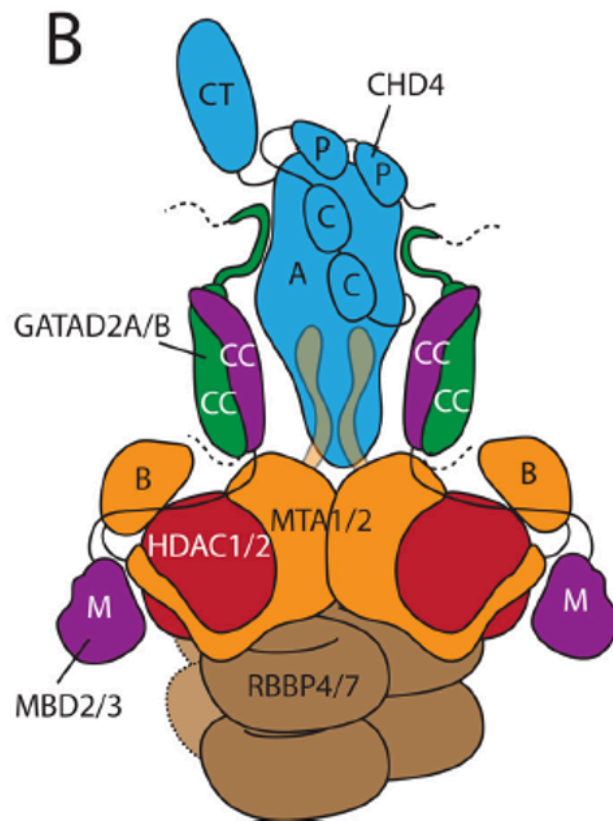


Figure 3: Reproduced from Jason, Webb, S. R., Ana, Saathoff, H., Ryan, D. P., Torrado, M., Mattias Brofelth, Parker, B. L., Shepherd, N. E., & Mackay, J. P. (2016). CHD4 Is a Peripheral Component of the Nucleosome Remodeling and Deacetylase Complex. *Journal of Biological Chemistry*

Project findings and future directions

Because of its observed role in regulating gene expression, we in the Smith lab are aiming to determine whether the NuRD complex plays a role in GR-activated transcription using murine hepatoma cells (Hepa-1c1c7). If so, this complex may mediate the positive effect of deacetylase activity on GR-activated transcription. The approach is to specifically deplete the NuRD complex in these cells by targeting the scaffold proteins that hold the complex together. Thus, siRNA targeted to the MTA proteins, MTA1/2/3, is used to deplete their expression to inhibit complex formation. Because an individual NuRD complex contains two MTA proteins which can be the same or different, the siRNAs targeting individual MTA proteins are used to deplete their expression individually or in combination. A non-targeting siRNA is included as a negative control. Successful and specific MTA knockdowns are confirmed with western blotting. Once the desired depletions are achieved, GR-activated gene transcription is measured by quantification of nascent RNA transcripts generated from specific GR target genes by RT-qPCR. Currently all the results have shown that MTA depletions have no effect on GR-activated transcription, suggesting that the NuRD complex does not mediate the positive effects of KDAC activity at these genes. However, this project

is ongoing and does need more replicates of the paralog combinations to make any statistically sound conclusions.

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