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A site to remember: H3K36 methylation a mark for histone deacetylation

Jung-Shin Lee [a,c], Ali Shilatifard [a,b,c,*]

[a] *Edward A. Doisy Department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine, 1402 South Grand Boulevard, St. Louis, MO 63104, USA*
[b] *Saint Louis University Cancer Center, Saint Louis University School of Medicine, St. Louis, MO 63104, USA*
[c] *Stawers Institute for Medical Research, 1000 East South Street, Kansas City, MO 64110, USA*

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Abstract

Chromatin structure exerts vital control over gene expression, DNA replication, recombination, and repair. In addition to altering RNA polymerase II's (Pol II) accessibility to DNA, histones are involved in the recruitment of activator and repressor complex(es) to regulate gene expression. Histone deacetylase Rpd3 exists in two distinct forms, Rpd3S and Rpd3L. Several recent studies demonstrated that the Eaf3 chromodomain, an Rpd3S subunit, recognizes Set2-methylated histone H3K36, initiating Rpd3 deacetylase activity in the wake of transcribing Pol II. Eaf3 and Set2 inhibit internal initiation within mRNA coding regions, similar to the transcription elongation factor and histone chaperone, FACT. Recent studies reviewed here demonstrate that histone deacetylation on the body of a transcribed gene is regulated via Set2-mediated methylation of histone H3-K36. These modifications provide restoration of normal chromatin structure in the wake of elongating Pol II and prevent inappropriate initiation within protein-coding regions masked by chromatin.

Keywords: Transcription elongation; Transcription initiation; RNA polymerase II; Set2

Regulation of transcription by RNA polymerase II occurs during preinitiation, initiation, and elongation, and requires the concerted action of several factors for proper synthesis of messenger RNA (mRNA). Studies of eukaryotic mRNA synthesis during the elongation phase of transcription have historically lag behind studies of mRNA synthesis during preinitiation and initiation stages [1]. However, during the past several years, efforts to define the mechanisms governing the elongation stage of transcription resulted in the discovery of a diverse collection of transcription factors regulating the activity of elongating RNA polymerase II (Pol II) [1,2]. Several years ago, Luse and colleagues showed that transcription elongation proceeding from a naked DNA template varies from transcription proceeding from a nucleosome-containing DNA template [3]. This *in vitro* observation resulted in discovery of a class of elongation factors, including FACT, which are required for proper transcription elongation on nucleosomal templates [1,2,4]. Such studies indicate *in vivo* transcriptional elongation control from a chromatin-containing template is more complicated than expected.

Eukaryotic chromosomal DNA must be compacted into the nucleus as chromatin, while remaining

* Corresponding author at: 1000 East South 50th Street, Kansas City, Missouri 64110, USA. Tel.: +816 926 4465; fax: +816 926 2080.
E-mail address: ash@stowers-institute.org (A. Shilatifard).
URL: www.stowers-institute.org (A. Shilatifard).

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accessible to Pol II and its transcription machinery. *In vitro* chromatin appears as a series of "beads on a string," consisting of individual nucleosome "beads" and linker DNA "string," and each nucleosome consists of eight core histone proteins, two each of H3, H4, H2A, and H2B. In addition to the traditional role of structural regulator and DNA packaging protein, histones play a pivotal role in the regulation of gene expression. X-ray studies suggest that histone amino terminal tails extend outside the core of the nucleosome and are available for interactions with DNA and/or other proteins. Packaged chromatin becomes accessible to transcription factors via either ATP-dependent nucleosome-remodeling complexes or direct posttranslational modification of histones [5]. Posttranslational modifications of histones, including acetylation, phosphorylation, methylation and ubiquitination, occur on the unstructured tail of histones protruding from the core of the nucleosome [6,7]. Changes in chromatin architecture may be regarded as a regulatory principle allowing discrimination of transcriptionally active and inactive regions to facilitate the execution of a gene expression program [8] and to direct establishment of specialized structures such as centromere and telomeres [9]. Given the relevance of chromatin structure during cellular differentiation and development, many laboratories are feverishly working to further characterize epigenetic regulatory mechanisms and the role of chromatin structure/modifications and other factors influencing RNA Pol II activity in the regulation of gene expression.

Insights as to how chromatin is remodeled to reprogram gene expression come from careful comparisons of biochemical properties of "active" versus "inactive" chromatin [10–12]. The active state of chromatin is associated with one or more of the following: depletion and/or phosphorylation of H1-type linker histones; increased core histone acetylation; increased content of specific high-mobility group (HMG) proteins; and increased incorporation of specific histone variants.

Histone methylation on lysines 4 and 36 of histone H3 on the body of actively transcribed genes is associated with the elongating form of Pol II [12]. The MLL-like, Set1 containing complex, complex of proteins associated with Set1 (COMPASS) was the first H3K4 methylase to be identified [13–15]. COMPASS associates with the early elongating form of Pol II via its interaction with the elongation factor, the Paf1 complex [16,17]. The histone methyltransferase Set2 associates with serine 2-phosphorylated elongating Pol II and can methylate lysine 36 of H3 in the body of an actively transcribed gene [18–20]. Deletion of approximately 10 heptapeptide repeats of the C-terminal domain of Pol II results in a significant global loss of histone H3 lysine 36 methylation. Additionally, the enzymatic activity of the CTK kinase required for Pol II CTD phosphorylation, is also required for Set2-dependent lysine 36 methylation of histone H3. In this review, we focus on recent studies indicating histone methylation by Set2 is associated with recruitment of histone modifying machineries to the body of actively transcribed genes to suppress spurious intragenic transcription initiation.

Acetylation and deacetylation of lysine residues of histone are respectively linked to transcriptional activation or repression [5]. Many enzymes responsible for acetylation of histones at different residues were first characterized as transcriptional co-activators and later as enzymes [5]. Generally, histone acetylation and transcriptional activity are correlated, but there are exceptions at some residues [21]. Rpd3 is a histone deacetylase in *S. cerevisiae* that functions as a transcriptional corepressor at many promoters via interaction to the DNA binding factor Ume6 [22–25]. Rpd3 is known to function as a part of protein complex that contains Ume1, Sin3, Sap30, Sds3, Pho23, and Cti6/Rxt1 and several other factors [26–30]. Also, Rpd3 is required to regulate Sir2-mediated repression at telomeres, ribosomal loci, and HMR [31]. Carozza et al. and Keogh et al. initially sought to purify and analyze subunits of the histone deacetylase Rpd3 with the ultimate goal of determining its functions and mechanisms of recruitment beyond targeted promoters [32,33]. At the *INO*1 promoter, DNA binding transcription repressor Ume6 recruits Rpd3 to corepressor Sin3, deacetylating the histones around the Ume6 binding site region [22,24]. Rpd3 recruitment and subsequent intergenic deacetylation were thought to occur via a repressor/corepressor-independent mechanism, though the molecular components of this signal pathway were less understood [34]. In *S. cerevisiae*, there are two known Rpd3 complexes, Rpd3L (1.2 MDa), and RpdS (0.6 MDa). Keogh et al. and Carozza et al. isolated and characterized these two Rpd3 histone deacetylase complexes by tandem affinity purification (TAP) and found Rpd3L and Rpd3S to vary in composition and function. Carozza et al. used a modified TAP to resolve the Rpd3 complexes on a MonoQ ion exchange column and purify fraction pools of the MonoQ peaks on calmodulin (CaM)-sepharose. Silver-stained SDS/PAGE and MudPIT analyses were used to analyze the composition of the complex(es). While the subunits of Rpd3L were consistent with the promoter-recruited corepressor model described above, Rpd3S revealed two unique subunits, Rco1 and Eaf3 [32,33]. Keogh et al. confirmed by synthetic genetic array analysis (SGA) that when a miniarray of 384 transcription-related deletion strains