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A new companion of elongating RNA Polymerase II: TINTIN, an independent sub-module of NuA4/TIP60 for nucleosome transactions

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Multiple factors are involved in the elongation stage of transcription regulation to ensure the passing of RNA polymerases while preserving appropriate nucleosome structure thereafter. The recently reported trimeric sub-module of NuA4 histone acetyltransferase complex involved in this process provides more insight into the sophisticated modulation of transcription elongation.

Keywords: acetylation, chromatin, Eaf3, Eaf5, Eaf7, MRGBP, MRG15, NuA4, transcription elongation, TINTIN

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The cell genome is packaged into chromatin, the basic unit of which is the nucleosome. This packaging of DNA into nucleosomes represents physical barriers for nearly all stages of transcription.[1] The organization of condensed chromatin requires the negotiation of RNA polymerases with nucleosomes for the underlying DNA during transcription. Disrupted transcription leads to gene expression misregulation, which can further result in human diseases and disorders (see review[2]).

While initiation was generally considered as the key step in transcription regulation, recent years of studies have unveiled that transcription elongation is also a crucial regulatory stage which requires complex actions of several players, such as the recruitment of elongation factors, histone chaperones, and post-translational modifications (PTMs) on both histones and RNA Polymerase II (RNAP II) itself (see reviews[3-5]). Highly coordinated regulation is essential for RNAP II moving through nucleosomes while avoiding the inappropriate transcription initiation within ORFs. One good example is the involvement of H3K36me3 during transcription elongation. H3K36me3 is a co-transcriptional histone mark catalyzed by histone methyltransferase Set2 (KMT3).[6,7] Set2 travels along with elongating RNAP II through the interaction with CTD repeats of Rpb1, the largest subunit of RNAP II, specifically phosphorylated on Ser2.[8,9] Interestingly, the deposition of H3K36me3 onto the gene coding regions by Set2 can further recruit a deacetylase complex, Rpd3S, through the chromodomain of its Eaf3 subunit.[10-12] The coding regions are thus maintained at a relatively hypoacetylated nucleosome state to prevent the onset of spurious intragenic transcription.[10] A more recent study has shown that Set2-mediated H3K36me3 also suppresses incorporation of acetylated new histones, which contributes to block spurious transcription.[13]

The detailed chromatin environment required for proper transcription elongation regulation remains to be elucidated. Eaf3 not only resides in Rpd3S, but is also a subunit of another complex with opposing activity, the NuA4 histone acetyltransferase complex. While Rpd3S is associated with repressed chromatin structure of coding regions as mentioned above, NuA4 is generally linked to transcriptional activation by its acetylation activity in "opening" promoter nucleosomes.[14-17] The question is, as both Rpd3S and NuA4 complexes contain Eaf3, why Eaf3 only directs Rpd3S to H3K36me3 over gene bodies, but not NuA4? Subsequent studies have shown that it is the combination of Eaf3 chromo domain and PHD domain in Rpd3S specific subunit Rco1 that endows the specificity,[18] along interaction with phosphorylated RNAP II.[19,20] NuA4 itself had been detected on coding regions of some genes and histone

methylation has been implicated in this binding.[21,22] Nevertheless, this is evidently not the end of the story. Adding to the complexity, recently we provided evidence for the presence of Eaf3 in another small complex consisting of Eaf5/Eaf7/Eaf3.[23] Biochemical data show that Eaf5/7/3 are stable subunits of NuA4 yet can exist as an independent trimeric subcomplex. Compared to NuA4, this trimer exhibits more enrichment over coding regions relative to promoters in genomic mapping, suggesting the involvement in transcription elongation. Additional experiments show that the trimer actually travels with elongating RNAP II over the gene bodies by utilizing a dual interaction surface with both H3K36me3 and phosphorylated RNAP II (Ser2). In contrast to the effect of Rpd3S, this association to coding regions seems to destabilize nucleosomes, as mutants suppress cryptic transcription detected in *Set2* deletion strains. Intriguingly, by interacting with histone chaperons, it also helps recycle/refold nucleosomes after the passage of RNAP II, preventing the incorporation of new histones. These results gave rise to a model in which the trimer stimulates disruption of nucleosomes in front of the polymerase through its association with NuA4 acetyltransferase activity. In parallel it also stabilizes chromatin in the wake of RNAP II by assisting chaperone-mediated recycling/reassembly of the disrupted nucleosomes, a function independent of NuA4. Due to its biochemical characteristics and molecular functions, this newly characterized sub-module is named as the **TINTIN** complex, for **T**rimer **I**ndependent of **N**uA4 involved in **T**ranscription **I**nteractions with **N**ucleosomes (**Fig. 1**).

This identification of TINTIN, the "dually-functional" new companion of RNAP II, opens many interesting questions. Firstly, how is TINTIN physically associated with NuA4? While it is shown that TINTIN tethers NuA4 through Eaf5, it is of interest to know which part of NuA4 serves as the contact point for the trimer. Our lab recently found that the binding of TINTIN to NuA4 is mediated by the interaction with NuA4 scaffold subunit Eaf1 (Steunou and Côté, unpublished data). However, it remains unknown how this association to NuA4 is regulated. While TINTIN is more abundant than NuA4 in the cell, it is possible that PTMs on either one during different cell cycle stages or response to upstream signaling regulate the selective association/detachment of TINTIN with NuA4. Secondly, it will be necessary to discern whether there is crosstalk between TINTIN and other important players such as elongation-coupled histone chaperones, Spt6, FACT and Asf1, or chromatin remodelers that are implicated in histone exchange, Chd1 and Isw1?[24] If so, what are the underlying mechanisms? Further analysis including mutant effects on cryptic transcription or the global transcriptome by RNA-seq would shed light on this pathway. Last, but not the least, what is the possible function of "TINTIN" in mammalian cells? Eaf3 and Eaf7 are conserved from yeast to human, and the mammalian homologs are called MRG15 and MRGBP, respectively.[25] Data from a large-scale proteomic study suggest the existence of a MRG15-MRGBP submodule independent of the mammalian version of NuA4, the TIP60 complex.[26] Consistent with TINTIN's function in yeast, a study from the Felsenfeld lab detected the interaction of MRG15-MRGBP with transcriptional regulatory factor Vezf1 and its involvement in transcription elongation and alternative splicing.[27] It is of great interest to further characterize the role of the mammalian TINTIN counterpart in transcription.

Whether TINTIN also functions beyond transcription? Several lines of evidence suggesting the involvement of Eaf3/MRG15 in DNA damage response favor this possibility. Large-scale proteomic studies have found that Eaf3 is phosphorylated at putative Mec1/Tel1 (ATR/ATM)-target sites upon DNA damage in yeast.[28-31] Furthermore, MRG15, the mammalian homolog of Eaf3, has been shown to directly interact with PALB2, a member of BRCA2 complex, to mediate its DNA damage response functions.[32,33] Future examination is required to study the potential involvement of TINTIN in DNA damage response pathways in both yeast and higher eukaryotes.

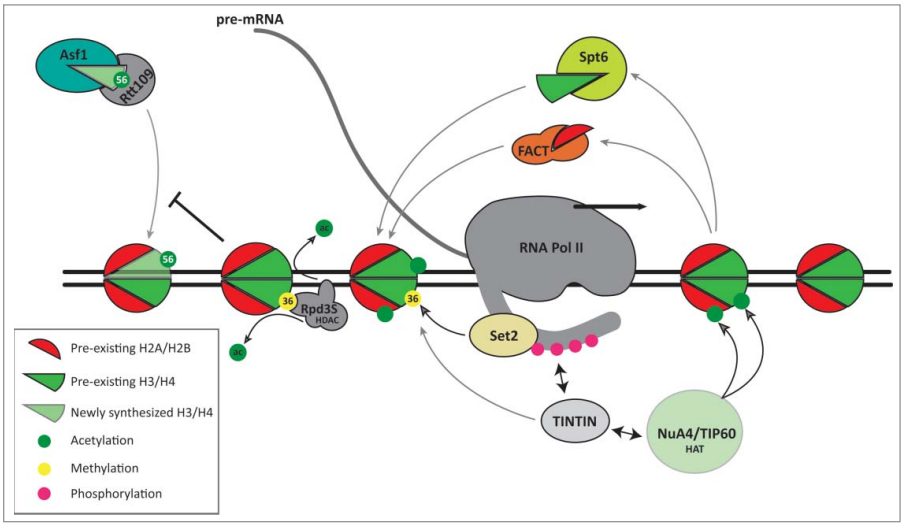

Figure 1. Model for the involvement of TINTIN in nucleosome transactions during transcription elongation. TINTIN represents a trimeric module which exists both within and outside of the NuA4/TIP60 histone acetyltransferase (HAT) complex. It interacts with phosphorylated CTD domain of RNAP II and Set2-mediated H3K36me3-containing nucleosomes during transcription elongation. In front of elongating RNAP II, TINTIN-containing NuA4 acetylates nucleosomes to assist nucleosome disruption. Behind the polymerase, independent TINTIN assists recycling/refolding of nucleosomes in coordination with FACT and Spt6 histone chaperones. This, together with the deacetylation of reassembled nucleosomes by Rpd3S histone deacetylase (HDAC) complex, contributes to the suppression of new histone incorporation (by Asf1/Rtt109).

The challenge remains regarding the study of chromatin modifying complexes as they often possess/share common subunits. It is thus required to proceed with caution when selecting targets to study the role of specific complexes. In the case of NuA4, this report along with previous findings[34-36] renders Eaf1 as the sole subunit that can exclusively represent the NuA4 complex.[35]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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