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TINTIN, at the interface of chromatin, transcription elongation, and mRNA processing

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Recent work including high-resolution genome-wide analysis uncovered a new trimeric complex involved in transcription elongation, both as an integral part of the NuA4 histone acetyltransferase and as an independent functional entity. The complex is conserved in eukaryotes and is named TINTIN, for Trimer Independent of NuA4 for transcription Interactions with Nucleosomes. This point of view covers the current knowledge regarding TINTIN's function in modulating chromatin structure and influencing transcription elongation in eukaryotes. It also points to several physical and functional links to co-transcriptional processes, including interactions with the mRNA splicing machinery and the nuclear exosome.

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

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NuA4/TIP60 for Nucleosome Transactions

Biogenesis of mRNA is vital for translating genetic information into protein. It is a multistep event involving transcription and mRNA processing in the nucleus followed by export of mRNA to the cytoplasm where protein synthesis takes place. All the steps in this process are coupled and tightly regulated to ensure that only properly processed mRNA is efficiently exported to cytoplasm.[1,2] Chromatin structure and dynamics plays a critical role in regulating transcription[3] and also appears to be central to the process of linking transcription to mRNA processing steps.[4]

As with any other DNA related process, the different stages of transcription are profoundly affected by chromatin structure, which is specifically modulated so that transcriptional machinery can access DNA.[3] Specific post-translational modifications of histones within chromatin play critical roles in the process. The majority of chromatin modifying enzymes can only act on chromatin when functioning as part of a multiprotein assembly. One essential and highly conserved chromatin modifying enzyme is the NuA4 (TIP60 in mammals)[5] histone acetyltransferase complex. NuA4 has been implicated in the regulation of gene transcription through local recruitment by DNA bound transcription factors[6-9] or by histone marks and other regulators.[10-13] Notably NuA4 contains subunits, which are also present in other chromatin modifying enzymes. Of particular interest is chromodomain containing subunit Eaf3. It not only resides in NuA4, but is also a subunit of another complex with opposing activity, the Rpd3S histone deacetylase complex. While NuA4 is generally linked to transcriptional activation by its acetylation activity in "opening" promoter nucleosomes,[6,9] Rpd3S is associated with repressed chromatin structure on coding regions. Rpd3S deacetylates the nucleosomes and ensures proper refolding of chromatin and inhibition of spurious transcription from cryptic promoters within the coding region of genes.[14-16] Interestingly the chromodomain of Eaf3 interacts with H3K36me3 and recruits Rpd3S to the gene coding regions.[14-16] It remained puzzling for some time, why Eaf3 directs only Rpd3S to H3K36me3 over gene bodies, but not NuA4? Subsequently it was shown that combined action of PHD domain of Rco1 and

chromodomain of Eaf3 specify recognition of the methyl H3K36 mark by the Rpd3S complex.[17]

Adding to the complexity, we recently provided evidence for the presence of Eaf3 in another small complex consisting of Eaf5/Eaf7/Eaf3.[18] Tandem affinity purification followed by gel filtration clearly demonstrated that the Eaf5/7/3 trimer exists as a native complex outside of NuA4. We further showed that eaf5/7 mutant cells share similar phenotypes, in which some are shared with other components of NuA4 while others are specific to the trimer, further supporting the notion of an independent function. Due to its biochemical characteristics and molecular functions, this newly characterized submodule is named as the **TINTIN** complex, for **T**rimer **I**ndependent of **N**uA4 involved in **T**ranscription **I**nteractions with **N**ucleosomes. The Eaf7 homolog in higher eukaryotes MRGBP interacts directly with MRG15, the human homolog of Eaf3. Gel filtration and a large-scale proteomic study indicate that the MRGBP-MRG15 complex also exists independently of the TIP60 complex.[19] Results in our lab (K. Jacquet and J. Côté; in preparation) also demonstrate the distinct function of MRGBP-MRG15. Since there is no homolog of Eaf5 in human cells, MRGBP-MRG15 dimer is likely the functional homolog of the yeast TINTIN complex.

Our studies clearly demonstrate that Eaf5/7/3 exists as a separate entity as well as part of the larger NuA4 complex. While we showed that TINTIN tethers NuA4 through Eaf5, preliminary results indicate that the binding of TINTIN to NuA4 is mediated by its interaction with NuA4 scaffold subunit Eaf1 (AL. Steunou, J. Cote, unpublished data). However, it is still not known how this association to NuA4 is regulated. It is possible that the selective association/detachment of TINTIN to NuA4 is regulated through different cell cycle stages or in response to upstream signaling regulated by post-translational modifications (PTMs) on either protein. The identification of the TINTIN complex further highlights the major challenge of shared or common subunits in the study of chromatin modifying complexes. It is thus required to proceed with caution when selecting targets to investigate the specific roles of these multisubunit players.

TINTIN role in transcription elongation

Native chromatin structure is intrinsically incompatible with elongating RNAPII. During transcription elongation, chromatin structure is modulated in order to allow access to elongating RNAPII.[3,20] However, the proper refolding of chromatin after transcription by RNAPII is of utmost importance to the cell. Detrimental consequences that result from its deficiency include spurious transcription from cryptic promoters within the coding region of gene.[21,22] Histone chaperones, chromatin modifying enzymes and ATP dependent chromatin remodelers[23,24] are all key factors necessary for proper chromatin refolding.

Compared to NuA4, TINTIN complex is more highly enriched over coding regions relative to promoters in genomic mapping, suggesting the involvement in transcription elongation.[18] Furthermore, we showed that the trimer travels with elongating Pol II over the gene bodies by utilizing a dual interaction surface with both H3K36me3 and phosphorylated RNAPII (Ser2). Both interactions are required for normal association on the body of transcribed genes, but Ser2-P on Pol II C-terminal domain (CTD) could be more important for targeting while H3K36me3 may be more linked to action on nucleosome dynamics, as is the case for Rpd3S.[25,26] This association to coding regions seems to destabilize nucleosomes, as trimer mutants suppress cryptic transcription detected in set2 deletion strains. Intriguingly, by interacting with histone chaperons, TINTIN prevents the incorporation of new histones and helps recycle/refold nucleosomes after the passage of pol II. Some of the critical players in chromatin refolding and histone exchange include histone chaperones Spt6, FACT, Asf1 and the chromatin remodelers Chd1 and Isw1.[24] It will be interesting to study whether there is crosstalk between TINTIN and these key players of chromatin refolding. If so, the underlying mechanisms would be pursued. Further analysis including mutant effects on cryptic transcription or the global transcriptome by RNA-seq would shed light on this pathway.

TINTIN and mRNA processing

It is well-established that mRNA processing occurs during transcription. Alternative splicing (AS) requires a vast array of proteins that catalyze splicing events. Both optimal RNA polymerase II elongation rate as well as the Pol II CTD play critical role in mRNA processing.[2,27-29] Chromatin structure and epigenetics also play important roles in co-transcriptional splicing.[4,30] One of the key events in the AS process is recognition of the splice site by proteins that either promote or suppress splicing. Polypyrimidine-tract binding protein (PTBP) is a splice junction-binding protein that suppresses splicing resulting in exon exclusion. PTB-dependent genes are enriched in H3K36me3. The high levels of H3K36me3 along the gene attract MRG15, which in turn interacts with PTB, recruiting it to the nascent RNA.[4] Furthermore, the MRGBP-MRG15 dimer has now been linked to the splicing process in mammals linked to pausing of elongating polymerases carrying Ser2-P on their CTD.[31] In yeast, TINTIN is associated with highly expressed genes. Interestingly, most of yeast intron-containing genes are highly expressed ribosomal protein (RP) genes. Moreover, TINTIN shows functional interaction with SWR1-C and Bdf1,[18,32,33] important regulators of alternative splicing.[34] It is likely that the trimer plays a similar role as MRGBP-MRG15 in splicing. Further study on the role of TINTIN in mRNA splicing will shed more light on this critical process in the context of chromatin.

TINTIN and mRNA quality control

Secondary mass-spectrometry hits in Eaf7-TAP purifications also include proteins associated with the exosome[35] (and our unpublished data). An important quality control for mRNA production is the degradation of aberrant transcripts by the exosome. The exosome is a highly conserved multiprotein complex with 3-5' exonuclease activity.[36,37] Depletion of exosome subunits results in an increase in transcription read-through.[38,39]

Interestingly we detected a small but reproducible increase of transcription read-through in *eaf5*/*7* mutants using a reporter plasmid. Importantly, *eaf7* and *eaf5* mutants also show a negative genetic interaction with several components of the nuclear exosome.[40] Since mRNA biogenesis/processing events are tightly coupled, it is not surprising that *eaf5*/*eaf7* also show negative interaction with the THO-TREX mRNA export machinery. Altogether, these studies point to a role for TINTIN, not only in co-transcriptional nucleosome recycling, but also in mRNA processing, splicing, termination and quality control. Further studies are certainly needed to confirm and dissect the function of TINTIN in relation to the processing of the mRNA molecules.

Disclosure of Potential Conflicts of Interest

J.C. holds a Canada Research Chair.

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References

1. Pandit S, Wang D, Fu X-D. Functional integration of transcriptional and RNA processing machineries. Curr Opin Cell Biol 2008; 20:260-5; PMID:18436438; http://dx.doi.org/10.1016/j.ceb.2008.03.001
2. Hsin J-P, Manley JL. The RNA polymerase II CTD coordinates transcription and RNA processing. Gen Dev 2012; 26:2119-37; PMID:23028141; http://dx.doi.org/10.1101/gad.200303.112
3. Li B, Carey M, Workman JL. The role of chromatin during transcription. Cell 2007; 128:707-19; PMID:17320508; http://dx.doi.org/10.1016/j.cell.2007.01.015
4. Luco RF, Allo M, Schor IE, Kornblihtt AR, Misteli T. Epigenetics in alternative pre-mRNA splicing. Cell 2011; 144:16-26; PMID:21215366; http://dx.doi.org/10.1016/j.cell.2010.11.056
5. Doyon Y, Côté J. The highly conserved and multifunctional NuA4 HAT complex. Curr Opin Genet Dev 2004; 14:147-54; PMID:15196461; http://dx.doi.org/10.1016/j.gde.2004.02.009
6. Utley RT, Ikeda K, Grant PA, Cote J, Steger DJ, Eberharter A, John S, Workman JL. Transcriptional activators direct histone acetyltransferase complexes to nucleosomes. Nature 1998; 394:498-502; PMID:9697775; http://dx.doi.org/10.1038/28886
7. Reid JL, Iyer VR, Brown PO, Struhl K. Coordinate regulation of yeast ribosomal protein genes is associated with targeted recruitment of Esa1 histone acetylase. Mol Cell 2000; 6:1297-307; PMID:11163204; http://dx.doi.org/10.1016/S1097-2765(00)00128-3
8. Brown CE, Howe L, Sousa K, Alley SC, Carrozza MJ, Tan S, Workman JL. Recruitment of HAT complexes by direct activator interactions with the ATM-related Tra1 subunit. Science 2001; 292:2333-7; PMID:11423663; http://dx.doi.org/10.1126/science.1060214
9. Nourani A, Utley RT, Allard S, Cote J. Recruitment of the NuA4 complex poises the PHO5 promoter for chromatin remodeling and activation. EMBO J 2004; 23:2597-607; PMID:15175650; http://dx.doi.org/10.1038/sj.emboj.7600230
10. Morillon A, Karabetsou N, Nair A, Mellor J. Dynamic lysine methylation on histone H3 defines the regulatory phase of gene transcription. Mol Cell 2005; 18:723-34; PMID:15949446; http://dx.doi.org/10.1016/j.molcel.2005.05.009
11. Ginsburg DS, Govind CK, Hinnebusch AG. NuA4 lysine acetyltransferase Esa1 is targeted to coding regions and stimulates transcription elongation with Gcn5. Mol Cell Biol 2009; 29:6473-87; PMID:19822662; http://dx.doi.org/10.1128/MCB.01033-09
12. Ginsburg DS, Anlembom TE, Wang J, Patel SR, Li B, Hinnebusch AG. NuA4 links methylation of histone H3 lysines 4 and 36 to acetylation of histones H4 and H3. J Biol Chem 2014; 289:32656-70; PMID:25301943; http://dx.doi.org/10.1074/jbc.M114.585588
13. Uprety B, Lahudkar S, Malik S, Bhaumik SR. The 19S proteasome subcomplex promotes the targeting of NuA4 HAT to the promoters of ribosomal protein genes to facilitate the recruitment of TFIID for transcriptional initiation in vivo. Nucleic Acids Res 2012; 40:1969-83; PMID:22086954; http://dx.doi.org/10.1093/nar/gkr977
14. Carrozza MJ, Li B, Florens L, Suganuma T, Swanson SK, Lee KK, Shia WJ, Anderson S, Yates J, Washburn MP, et al. Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. Cell 2005; 123:581-92; PMID:16286007; http://dx.doi.org/10.1016/j.cell.2005.10.023
15. Joshi AA, Struhl K. Eaf3 Chromodomain Interaction with Methylated H3-K36 Links Histone Deacetylation to Pol II Elongation. Mol Cell 2005; 20:971-8; PMID:16364921; http://dx.doi.org/10.1016/j.molcel.2005.11.021
16. Keogh MC, Kurdistani SK, Morris SA, Ahn SH, Podolny V, Collins SR, Schuldiner M, Chin K, Punna T, Thompson NJ, et al. Cotranscriptional set2 methylation of histone H3 lysine 36 recruits a repressive Rpd3 complex. Cell 2005; 123:593-605; PMID:16286008; http://dx.doi.org/10.1016/j.cell.2005.10.025
17. Li B, Gogol M, Carey M, Lee D, Seidel C, Workman JL. Combined action of PHD and chromo domains directs the Rpd3S HDAC to transcribed chromatin. Science 2007; 316:1050-4; PMID:17510366; http://dx.doi.org/10.1126/science.1139004
18. Rossetto D, Cramet M, Wang AY, Steunou AL, Lacoste N, Schulze JM, Côté V, Monnet-Saksouk J, Piquet S, Nourani A, et al. Eaf5/7/3 form a functionally independent NuA4 submodule linked to RNA polymerase II-coupled nucleosome recycling. EMBO J 2014; 33:1397-415; PMID:24843044
19. Kirkwood KJ, Ahmad Y, Larance M, Lamond AI. Characterization of Native Protein Complexes and Protein Isoform Variation Using Size-fractionation-based Quantitative Proteomics. Mol Cell Proteomics 2013; 12:3851-73; PMID:24043423; http://dx.doi.org/10.1074/mcp.M113.032367
20. Selth LA, Sigurdsson S, Svejstrup JQ. Transcript elongation by RNA polymerase II. Ann Rev Biochem 2010; 79:271-93; PMID:20367031; http://dx.doi.org/10.1146/annurev.biochem.78.062807.091425
21. Cheung V, Chua G, Batada NN, Landry CR, Michnick SW, Hughes TR, Winston F. Chromatin-and transcription-related factors repress transcription from within coding regions throughout the Saccharomyces cerevisiae genome. PLoS Biol 2008; 6:e277; PMID:18998772; http://dx.doi.org/10.1371/journal.pbio.0060277
22. Smolle M, Workman JL. Transcription-associated histone modifications and cryptic transcription. Biochim Biophys Acta 2013; 1829:84-97; PMID:22982198; http://dx.doi.org/10.1016/j.bbagrm.2012.08.008
23. Avvakumov N, Nourani A, Cote J. Histone chaperones: modulators of chromatin marks. Mol Cell 2011; 41:502-14; PMID:21362547; http://dx.doi.org/10.1016/j.molcel.2011.02.013
24. Smolle M, Workman JL, Venkatesh S. reSETting chromatin during transcription elongation. Epigenetics 2013; 8:10-5; PMID:23257840; http://dx.doi.org/10.4161/epi.23333
25. Drouin S, Laramee L, Jacques PE, Forest A, Bergeron M, Robert F. DSIF and RNA polymerase II CTD phosphorylation coordinate the recruitment of Rpd3S to actively transcribed genes. PLoS Genet 2010; 6:e1001173; PMID:21060864; http://dx.doi.org/10.1371/journal.pgen.1001173
26. Govind CK, Qiu H, Ginsburg DS, Ruan C, Hofmeyer K, Hu C, Swaminathan V, Workman JL, Li B, Hinnebusch AG. Phosphorylated Pol II CTD recruits multiple HDACs, including Rpd3C(S), for methylation-dependent deacetylation of ORF nucleosomes. Mol Cell 2010; 39:234-46; PMID:20670892; http://dx.doi.org/10.1016/j.molcel.2010.07.003
27. Fong N, Kim H, Zhou Y, Ji X, Qiu J, Saldi T, Diener K, Jones K, Fu XD, Bentley DL. Pre-mRNA splicing is facilitated by an optimal RNA polymerase II elongation rate. Gen Dev 2014; 28:2663-76; PMID:25452276; http://dx.doi.org/10.1101/gad.252106.114
28. Dujardin G, Lafaille C, de la Mata M, Marasco LE, Muñoz MJ, Le Jossic-Corcos C, Corcos L, Kornblihtt AR. How Slow RNA Polymerase II Elongation Favors Alternative Exon Skipping. Mol Cell 2014; 54:683-90; PMID:24793692; http://dx.doi.org/10.1016/j.molcel.2014.03.044
29. Bentley DL. Coupling mRNA processing with transcription in time and space. Nat Rev Genet 2014; 15(3):163-75; PMID:24514444; http://dx.doi.org/10.1038/nrg3662
30. Brown SJ, Stoilov P, Xing Y. Chromatin and epigenetic regulation of pre-mRNA processing. Hum Mol Genet 2012: 21(R1):R90-6 dds353; PMID: 22936691
31. Gowher H, Brick K, Camerini-Otero RD, Felsenfeld G. Vezf1 protein binding sites genome-wide are associated with pausing of elongating RNA polymerase II. Proc Natl Acad Sci U S A 2012; 109:2370-5; PMID:22308494; http://dx.doi.org/10.1073/pnas.1121538109
32. Krogan NJ, Baetz K, Keogh MC, Datta N, Sawa C, Kwok TC, Thompson NJ, Davey MG, Pootoolal J, Hughes TR, et al. Regulation of chromosome stability by the histone H2A variant Htz1, the Swr1 chromatin remodeling complex, and the histone acetyltransferase NuA4. Proc Natl Acad Sci U S A 2004; 101:13513-8; PMID:15353583; http://dx.doi.org/10.1073/pnas.0405753101
33. Lin YY, Qi Y, Lu JY, Pan X, Yuan DS, Zhao Y, Bader JS, Boeke JD. A comprehensive synthetic genetic interaction network governing yeast histone acetylation and deacetylation. Gen Dev 2008; 22:2062-74; PMID:18676811; http://dx.doi.org/10.1101/gad.1679508
34. Albulescu LO, Sabet N, Gudipati M, Stepankiw N, Bergman ZJ, Huffaker TC, Pleiss JA. A quantitative, high-throughput reverse genetic screen reveals novel connections between Pre-mRNA splicing and 5' and 3' end transcript determinants. PLoS Genet 2012; 8:

e1002530; PMID:22479188; http://dx.doi.org/10.1371/journal.pgen.1002530
35. Synowsky SA, van Wijk M, Raijmakers R, Heck AJ. Comparative multiplexed mass spectrometric analyses of endogenously expressed yeast nuclear and cytoplasmic exosomes. J Mol Biol 2009; 385:1300-13; PMID:19046973; http://dx.doi.org/10.1016/j.jmb.2008.11.011
36. Lykke-Andersen S, Brodersen DE, Jensen TH. Origins and activities of the eukaryotic exosome. J Cell Sci 2009; 122:1487-94; PMID:19420235; http://dx.doi.org/10.1242/jcs.047399
37. Parker R. RNA degradation in Saccharomyces cerevisae. Genetics 2012; 191:671-702; PMID:22785621; http://dx.doi.org/10.1534/genetics.111.137265
38. Torchet C, Bousquet-Antonelli C, Milligan L, Thompson E, Kufel J, Tollervey D. Processing of 3′-extended read-through transcripts by the exosome can generate functional mRNAs. Mol Cell 2002; 9:1285-96; PMID:12086625; http://dx.doi.org/10.1016/S1097-2765(02)00544-0
39. Lemay J-F, Larochelle M, Marguerat S, Atkinson S, Bähler J, Bachand F. The RNA exosome promotes transcription termination of backtracked RNA polymerase II. Nat Struct Mol Biol 2014; 21:919-26; PMID:25240800; http://dx.doi.org/10.1038/nsmb.2893
40. Wilmes GM, Bergkessel M, Bandyopadhyay S, Shales M, Braberg H, Cagney G, Collins SR, Whitworth GB, Kress TL, Weissman JS. A genetic interaction map of RNA-processing factors reveals links between Sem1/Dss1-containing complexes and mRNA export and splicing. Mol Cell 2008; 32:735-46; PMID:19061648; http://dx.doi.org/10.1016/j.molcel.2008.11.012