[RNA Biology 6:2, 145-148; April/May/June 2009]; ©2009 Landes Bioscience

Point of View

Transcription at the proximity of the nuclear pore

A role for the THP1-SAC3-SUS1-CDC31 (THSC) complex

Rosa Luna, Cristina González-Aguilera and Andrés Aguilera*

Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER); Universidad de Sevilla–CSIC; Seville, Spain

Key words: gene expression, transcription elongation, mRNA export, Thp1-Sac3-Sus1-Cdc31, gene tethering

A key aspect of eukaryotic gene expression is the coupling of transcription with RNA processing, polyadenylation and export. The use of new techniques based on tandem affinity purification (TAP) and chromatin immunoprecipitation (ChIP), and of genetic and cell biology approaches has contributed to the beginning of deciphering the network of protein-mRNA interactions accompanying this coupling. Although an extensive amount of work has shed light on this matter, the order of participation and precise role of the different proteins remain to be deciphered. It seems that different and sequential protein interactions must converge to finally promote the anchoring of genes to the nuclear periphery. Here we discuss the new data on the coupling of gene expression and RNA export, with emphasis on the THP1-SAC3-SUS1-CDC31 complex and the possible implications of these results on transcription at the nuclear pore.

Introduction

Formation of a mature and an export-proficient mRNP requires the coupling of transcription with RNA capping, splicing, 3'-end formation, termination and polyadenylation (reviewed in refs. 1 and 2). A key player in this coupling is the *C-t*erminal *d*omain (CTD) of Rbp1, the largest subunit of the RNA polymerase II (RNAPII) that serves as a scaffold for a number of specific factors whose binding to RNAPII often depends on the CTD phosphorylation pattern. Coordination between different steps of transcription, mRNP biogenesis and export, facilitates gene expression, and failures in one step can have a backward or forward effect during the whole process of gene expression. Eukaryotic cells have developed quality control mechanisms to prevent the export of suboptimal mRNPs and synthesis of dysfunctional proteins that retain improperly processed transcripts in the nucleus (reviewed in ref. 3).

A number of recent reports have focused on the understanding of the connection between mRNP biogenesis and export. The use of new techniques based on tandem affinity purification (TAP) and chromatin immunoprecipitation (ChIP), and of genetic and cell biology approaches has contributed to the beginnings of deciphering the network of protein-mRNA interactions accompanying transcription.[4] Proteins which were traditionally thought to act later in mRNP biogenesis, including mRNA export factors, are loaded onto chromatin during transcription to allow RNA export through the nuclear pore complex (NPC).[2] In addition, a functional connection between transcription and RNA export has been provided by the observation that mutations in some of the mRNA export factors lead to transcription defects.[5,6] Although an extensive amount of work has shed light on this matter, the order of participation and precise role of the different proteins remain to be deciphered.

Different reports have revealed that active genes are recruited to the nuclear periphery (reviewed in refs. 7 and 8). These results support the old hypothesis of gene-gating, which postulated that the activation of non-randomly positioned genes in the nucleus was linked to specialized NPCs that in turn facilitate efficient mRNA export.[9] A significant effort has been made to understand the mechanisms underlying this phenomenon, and different models have been proposed. Some studies support the possibility that gene movement to the periphery is RNA independent and promoted by interactions of transcriptional activators with the NPC. Others suggest that transcription activation and/or initiation itself would not to be sufficient for such a relocalization. Instead, proteins that conform to the mRNP could stabilize the anchoring of the genes at the periphery in an RNA-dependent manner.[7,8] It seems that different and sequential protein interactions must converge to finally promote the anchoring of genes to the nuclear periphery. Here we discuss new data about the coupling of gene expression and RNA export, with emphasis on the THSC complex, also termed TREX-2, and we will discuss the possible implications of these results on transcription at the NPC platform.

The Thp1-Sac3-Sus1-Cdc31 (THSC) Complex as a Bridge Between Transcription and mRNA Export at the NPC

Thp1-Sac3-Sus1-Cdc31 (THSC) is a conserved multifunctional complex located at the NPC that plays an important role in mRNA export and gene tethering in *Saccharomyces cerevisiae*.[10-15] In this complex, Thp1 and Sac3 are found in association with the centrin Cdc31,[11,12] that interacts with the spindle pole body (SPB) and acts in the duplication of microtubule-organizing centers, and with Sus1, a small protein that interacts with the SAGA complex involved in transcription initiation regulation.[14] Recently, Sem1,

*Correspondence to: Andrés Aguilera; Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER); Universidad de Sevilla-CSIC; Av. Américo Vespucio s/n; Seville 41092 Spain; Tel.: +34.954.468372; Email: aguilo@us.es

Submitted: 09/08/08; Accepted: 01/08/09

Previously published online as an *RNA Biology* E-publication:
http://www.landesbioscience.com/journals/rnabiology/article/7803

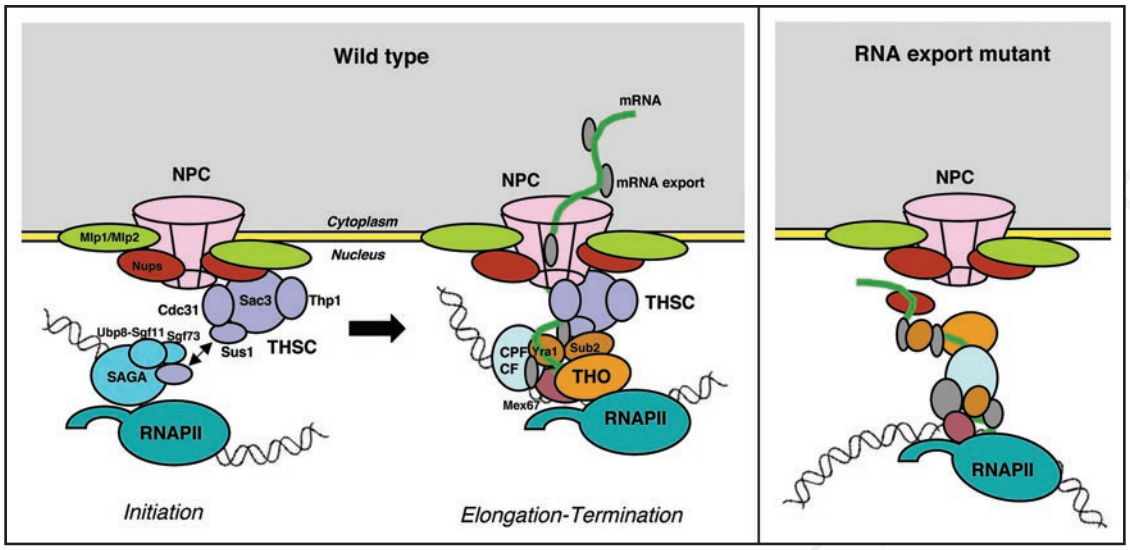

Figure 1. Transcription at the nuclear periphery. mRNA is cotranscriptionally processed and packaged with proteins into messenger ribonucleoproteins (mRNPs) for export to the cytoplasm. Active genes may be recruited to the nuclear periphery via factors involved in transcription and mRNA export. Different phases of this coupling between transcription and mRNA export in *Sacharomyces cerevisiae* are illustrated. First, interactions between promoter and NPC via SAGA and Sus1 may tether the transcribed DNA to the nuclear periphery. Afterwards, during transcription elongation and mRNP processing, other factors would contribute to the gene tethering. In this process, the nuclear-pore associated complex THSC may be key player. In cells lacking mRNA biogenesis export factors, such as THSC, transcription and mRNP export would be disrupted, so that the transcribed DNA would be untangled from the NPC. CPF-CF (Cleavage and polyadenylation complex).

a component of the 19S proteosome, has also been shown to be physically and functionally related to THSC complex.[16] Thp1 and Sac3 mediate docking of the mRNP at the NPC by its interaction with nucleoporins and the export receptor Mex67-Mtr2.[10] In addition to its effect on mRNA export, mutations in *THP1* and *SAC3* showed low levels of mRNA, suggesting a possible role for Thp1 and Sac3 in transcription.[12,17]

An additional link of THSC and transcription is provided by the interaction of SAGA with the Sus1 subunit. SAGA is involved in transcription regulation facilitating the access of general transcription factors to chromatin, mediating the assembly of a pre-initiation complex at the promoter of some genes. Sus1, in yeast, and its human and Drosophila orthologs physically interact with the subunits Ubp8 and Sgf11 of the deubiquitylation module of SAGA, and participate in the remodeling of histones necessary for gene expression.[18,19] Thus, it seems that THSC could influence transcription via Sus1 by direct interaction with SAGA during transcription initiation (Fig. 1). Nevertheless, THSC also participates in transcription elongation. THSC mutants show low levels of mRNAs of long and GC-rich ORFs and reduced RNAPII-occupancy at the 3' end of the genes.[20,21] Taken into account all data, an emerging question asks if the transcription effect of THSC mutants is only a consequence of its interaction of THSC with SAGA or if it relies on the downstream function in mRNP biogenesis and export.

Recently, it has been shown that the Sgf73 subunit of SAGA stabilizes the interaction between Sus1-Sgf11 and the Ubp8 subunit required for activation of the deubiquitylation module, and that Sgf73 also seems to be necessary for the stable association of Thp1 and Sac3 with Sus1 and Cdc31. The synthetic growth defects of *sgf73* mutant carrying THSC mutations is indeed consistent with a functional relationship between these factors.[22] Sus1 has recently been shown to bind to the ORF of the SAGA-dependent gene *ARG4*.[21] In another study it was shown that Sus1 only binds to the promoter of *GAL1*, a gene whose transcription is also regulated by SAGA.[14] As Sus1 recruitment has been shown to be dependent on Sgf73 and Sac3, it is possible that Sus1 functions during transcription elongation with SAGA and THSC.

There is evidence supporting the idea that THSC plays a role in transcription elongation that is linked to its function in mRNP biogenesis and export. In vitro experiments using whole cell extracts of THSC mutants do not show a significant elongation defect, in contrast to THO-complex mutants, which also affect mRNP biogenesis and export, suggesting that the effect of THSC mutations in transcription elongation can only be observed when it is physically coupled to RNA export at the NPC.[20] This is in agreement with the observed physical and functional interaction of THSC with factors involved in mRNP export such as Sub2, Yra1 and Mex67, whereas this relationship has not been observed with the SAGA complex.[10,20,21] Indeed, THSC seems to act during transcription elongation in a RNA dependent manner, as the transcription defects of the mutants can be suppressed by the action of a ribozyme that degrades the mRNA nascent molecule.[20] THSC has been shown to be necessary not only for the anchoring of an active gene to the periphery, but for posttranscriptional retention.[23] These data are in agreement with a view in which THSC connects transcription elongation with mRNA export by a RNA dependent process (Fig. 1).

It is possible that the stable association of genes with the NPC is achieved by different means. A transient contact could be established by physical interaction between the NPC and the promoter of the gene via transcription factors.[7,8,24] This could explain confinement of the *GAL1* gene to the nuclear periphery mediated by the Nup1 nucleoporin plus Sac3, Sus1 and different subunits of SAGA such as the Ada2 subunit necessary for its integrity,[15] and the Sgf73 and Ubp8 deubiquytilation subunits.[22] Alternatively, the THSC

complex via its putative ability to interact with the nascent mRNP could either stabilize or promote an alternative mechanism of association, accordingly with the relevance of the mRNP in the gene anchoring[23,25] (Fig.1).

The biological relevance of the coupling between transcription and RNA export at the NPC is provided by other factors, such as Mlp1 and Mex67, which have been shown to contribute to gene tethering.[26] Mlp1 is a myosine-like protein that interacts with mRNA export factors and it serves as a docking site at the NPC for hnRNP proteins,[27] and is associated with transcribing genes in an RNA-dependent manner.[28] Interestingly, Mex67 is also recruited to active chromatin.[29] The observations that a reporter-gene lacking the coding region is able to recruit Mex67 upon transcriptional activation and to relocate to the nuclear periphery, and that the Mex67 recruitment to active chromatin is insensitive to RNase have led to the proposal that Mex67 participates in gene-tethering, probably, in an RNA-independent manner.[26,29] However, the physical interaction of Mex67 with other mRNP biogenesis and export factors, such as THSC,[10] abrogate for a putative role of the mRNP in tethering.

Altogether, the data suggest a scenario in which proteins that are associated with the NPC and with a putative function in later steps of mRNP biogenesis and export, also affect earlier steps of transcription. Indeed THSC mutations, in addition to RNA export impairment, lead to transcription elongation defects and transcription-dependent hyper-recombination that are dependent on the nascent mRNA in yeast,[20] presumably with the involvement of R-loop formation as was previously shown for yeast THO-Sub2 mutants.[2] THO is a 4-subunit complex recruited to active chromatin that participates in transcription elongation and interacts physically and functionally with the export factors Sub2 and Yra1 forming a bigger complex termed TREX.[2] The interaction of THSC with chromatin is not yet defined, and even though THSC would act later than THO/TREX during mRNP biogenesis and export, THSC depletion results in a feedback effect on transcription elongation in vivo (Fig. 1).[20]

A number of nuclear proteins have been found with either an impact on transcription elongation or in association with the ORF during elongation but without a clear function in elongation. The latter can be seen in some SAGA subunits,[30,31] including Sus1 when acting as a subunit of the SAGA complex. SAGA is involved in nucleosome eviction and histone deubiquitylation and some subunits could have an indirect effect on transcription elongation via the impact of these chromatin modifications or on the access of some other factors to the RNAPII itself. Instead, the action of Sus1 during transcription elongation may be played as part of THSC. The observation that Sus1 is not recruited to the coding region of SAGA-dependent genes as *GAL1* but to its promoter[14] or that it shares the features of Thp1 and Sac3,[20,21] opens the possibility that Sus1 relationship with elongation is a consequence of it being a subunit of THSC.

Transcription and mRNP Biogenesis at the Nuclear Periphery

Increasing evidence, mainly in yeast, suggests that a number of transcription-coupled RNA processing steps converge at the nuclear pore. Different nucleoporins have been shown to interact with active genes.[7,8] Also, a specific interaction between Nup2 and the promoter of numerous genes have been shown by in vivo mapping of the cleavage sites of Nup2 fused to micrococcal nuclease.[24] The stable association of a gene with the nuclear periphery is mediated via interactions of the NPC with the promoter or with the 3'UTR.[25] The involvement of promoters and termination regions of a gene in localization to the nuclear periphery is particularly intriguing on the basis of the new gene-looping model for transcription.[32] In this model, transcription and termination are physically interconnected, implying the formation of an actively transcribed loop in which a number of factors, such as the Ssu72 Ser5-phosphatase or TFIIB participate during transcription at both promoter and termination regions. Physical association between promoters and poly(A) sites suggests that active genes exist in a looped conformation that could promote transcription reinitiation and facilitate the recycling of RNAPII from the terminator to the promoter.[32,33] In this sense it is particularly noticeable that Mlp1, a factor involved in gene anchoring at the nuclear periphery, maps preferentially at the 3' end of the genes[28] and has recently been shown to interact with SAGA and to bind to the *GAL1* promoter.[34] This opens the possibility that some genes form loops in the proximity of the NPC.

A recent work using a modified chromatin immunoprecipitation protocol has revealed the existence of a heavy chromatin complex containing 3'-end processing factors and nucleoporin components, suggesting that the NPC itself associates with the 3' ends of the genes during transcription.[35] This large particle is observed in THO-Sub2 mutants consistent with a participation of THO from transcription elongation to the final release of the mRNP at the nuclear pore, which is in agreement with the pattern of crosslinking of THO to active chromatin[36] and its functional interaction with mRNA 3'-end processing proteins.[37,38] In this sense is also worth noticing the recent observation that the mRNA export factor Yra1 is initially recruited to the transcription elongation complex through a direct interaction with Pcf11, a 3' end processing factor that associates with the poll CTD[39] (Fig. 1).

That a large molecule intermediate formed at the NPC in THSC and THO mutants would be an intriguing possibility that could be related to a general impact on transcription elongation. In light of this and the increasing experimental evidence for the gene-looping model of transcription, it may not be surprising that an increasing number of nuclear proteins might affect transcription elongation. It would certainly be important to establish the physiological relevance of this nucleoporins-containing particle in the nuclear structure, considering that THSC and other RNA export mutations have been shown to disentangle the transcribed DNA from the nuclear periphery (Fig. 1).

Concluding Remarks and Future Perspectives

Gene gating has been described for some genes that are highly transcribed under stress-induced conditions or in special developmental processes. It is unlikely that all transcriptional activity takes place at the nuclear periphery, but this may be the case for highly expressed genes to favor a rapid and coordinated export to the cytoplasm. In light of the gene looping model of transcription, the uncovering of large nucleoprotein complexes containing transcription, mRNA export and nucleoporins, and the known functional impact that proteins found at the nuclear periphery, such as the THSC complex, has on transcription, it is possible that transcription initiation and termination sites are brought together with the nuclear pore in activated and highly expressed genes. The contribution of the

mRNP biogenesis process during transcription elongation may be critical for the tethering, but reciprocally it may cause transcription initiation and elongation to be affected by feedback mechanisms that sense further steps of transcription and mRNP biogenesis and export. Our full understanding of nuclear gene expression certainly requires a better understanding of the cellular context in which reactions take place, but at the same time introduces us to a new body of knowledge that opens new and intriguing questions about the physical connection of transcription with the nuclear periphery and its impact on other nuclear processes, including genome dynamics.

Acknowledgements

We would like to thank Diane Haun for style supervision. Research in A.A.'s lab is funded by grants from the Spanish Ministry of Science and Education and the Junta de Andalucía. We apologize for relevant findings that were not cited due to space limitations.

References

1. Bentley DL. Rules of engagement: co-transcriptional recruitment of pre-mRNA processing factors. Curr Opin Cell Biol 2005; 17:251-6.
2. Luna R, Gaillard H, Gonzalez-Aguilera C, Aguilera A. Biogenesis of mRNPs: integrating different processes in the eukaryotic nucleus. Chromosoma 2008; 117:319-31.
3. Schmid M, Jensen TH. Quality control of mRNP in the nucleus. Chromosoma 2008; 117:419-29.
4. Farny NG, Hurt JA, Silver PA. Definition of global and transcript-specific mRNA export pathways in metazoans. Genes Dev 2008; 22:66-78.
5. Jimeno S, Rondon AG, Luna R, Aguilera A. The yeast THO complex and mRNA export factors link RNA metabolism with transcription and genome instability. EMBO J 2002; 21:3526-35.
6. Jensen TH, Patricio K, McCarthy T, Rosbash M. A block to mRNA nuclear export in *S. cerevisiae* leads to hyperadenylation of transcripts that accumulate at the site of transcription. Mol Cell 2001; 7:887-98.
7. Akhtar A, Gasser SM. The nuclear envelope and transcriptional control. Nat Rev Genet 2007; 8:507-17.
8. Brown CR, Silver PA. Transcriptional regulation at the nuclear pore complex. Curr Opin in Genet Dev 2007; 17:100-6.
9. Blobel G. Gene gating: a hypothesis. Proc Natl Acad Sci USA1985; 82:8527-9.
10. Fischer T, Strasser K, Racz A, Rodriguez-Navarro S, Oppizzi M, Ihrig P, et al. The mRNA export machinery requires the novel Sac3p-Thp1p complex to dock at the nucleoplasmic entrance of the nuclear pores. EMBO J 2002; 21:5843-52.
11. Fischer T, Rodriguez-Navarro S, Pereira G, Racz A, Schiebel E, Hurt E. Yeast centrin Cdc31 is linked to the nuclear mRNA export machinery. Nat Cell Biol 2004; 6:840-8.
12. Gallardo M, Luna R, Erdjument-Bromage H, Tempst P, Aguilera A. Nab2p and the Thp1p-Sac3p complex functionally interact at the interface between transcription and mRNA metabolism. J Biol Chem 2003; 278:24225-32.
13. Kurshakova MM, Krasnov AN, Kopytova DV, Shidlovskii YV, Nikolenko JV, Nabirochkina EN, et al. SAGA and a novel Drosophila export complex anchor efficient transcription and mRNA export to NPC. EMBO J 2007; 26:4956-65.
14. Rodriguez-Navarro S, Fischer T, Luo MJ, Antunez O, Brettschneider S, Lechner J, et al. Sus1, a functional component of the SAGA histone acetylase complex and the nuclear pore-associated mRNA export machinery. Cell 2004; 116:75-86.
15. Cabal GG, Genovesio A, Rodriguez-Navarro S, Zimmer C, Gadal O, Lesne A, et al. SAGA interacting factors confine sub-diffusion of transcribed genes to the nuclear envelope. Nature 2006; 441:770-3.
16. Wilmes GM, Bergkessel M, Bandyopadhyay S, Shales M, Braberg H, Cagney G, et al. 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.
17. Gallardo M, Aguilera A. A new hyperrecombination mutation identifies a novel yeast gene, THP1, connecting transcription elongation with mitotic recombination. Genetics 2001; 157:79-89.
18. Kohler A, Pascual-Garcia P, Llopis A, Zapater M, Posas F, Hurt E, et al. The mRNA export factor Sus1 is involved in Spt/Ada/Gcn5 acetyltransferase-mediated H2B deubiquitinylation through its interaction with Ubp8 and Sgf11. Mol Biol Cell 2006; 17:4228-36.
19. Zhao Y, Lang G, Ito S, Bonnet J, Metzger E, Sawatsubashi S, et al. A TFTC/STAGA module mediates histone H2A and H2B deubiquitination, coactivates nuclear receptors, and counteracts heterochromatin silencing. Mol Cell 2008; 29:92-101.
20. Gonzalez-Aguilera C, Tous C, Gomez-Gonzalez B, Huertas P, Luna R, Aguilera A. The THP1-SAC3-SUS1-CDC31 Complex Works in Transcription Elongation-mRNA Export Preventing RNA-Mediated Genome Instability. Mol Biol Cell 2008.
21. Pascual-Garcia P, Govind CK, Queralt E, Cuenca-Bono B, Llopis A, Chavez S, et al. Sus1 is recruited to coding regions and functions during transcription elongation in association with SAGA and TREX2. Genes Dev 2008; 22:2811-22.
22. Kohler A, Schneider M, Cabal GG, Nehrbass U, Hurt E. Yeast Ataxin-7 links histone deubiquitination with gene gating and mRNA export. Nat Cell Biol 2008; 10:707-15.
23. Chekanova JA, Abruzzi KC, Rosbash M, Belostotsky DA. Sus1, Sac3 and Thp1 mediate post-transcriptional tethering of active genes to the nuclear rim as well as to non-nascent mRNP. Rna 2008; 14:66-77.
24. Schmid M, Arib G, Laemmli C, Nishikawa J, Durussel T, Laemmli UK. Nup-PI: the nucleopore-promoter interaction of genes in yeast. Mol Cell 2006; 21:379-91.
25. Abruzzi KC, Belostotsky DA, Chekanova JA, Dower K, Rosbash M. 3'-end formation signals modulate the association of genes with the nuclear periphery as well as mRNP dot formation. EMBO J 2006; 25:4253-62.
26. Dieppois G, Iglesias N, Stutz F. Cotranscriptional recruitment to the mRNA export receptor Mex67p contributes to nuclear pore anchoring of activated genes. Mol Cell Biol 2006; 26:7858-70.
27. Green DM, Johnson CP, Hagan H, Corbett AH. The C-terminal domain of myosin-like protein 1 (Mlp1p) is a docking site for heterogeneous nuclear ribonucleoproteins that are required for mRNA export. Proc Natl Acad Sci USA2003; 100:1010-5.
28. Casolari JM, Brown CR, Drubin DA, Rando OJ, Silver PA. Developmentally induced changes in transcriptional program alter spatial organization across chromosomes. Genes Dev 2005; 19:1188-98.
29. Gwizdek C, Iglesias N, Rodriguez MS, Ossareh-Nazari B, Hobeika M, Divita G, et al. Ubiquitin-associated domain of Mex67 synchronizes recruitment of the mRNA export machinery with transcription. Proc Natl Acad Sci USA 2006; 103:16376-81.
30. Govind CK, Zhang F, Qiu H, Hofmeyer K, Hinnebusch AG. Gcn5 promotes acetylation, eviction and methylation of nucleosomes in transcribed coding regions. Mol Cell 2007; 25:31-42.
31. Wyce A, Xiao T, Whelan KA, Kosman C, Walter W, Eick D, et al. H2B ubiquitylation acts as a barrier to Ctk1 nucleosomal recruitment prior to removal by Ubp8 within a SAGA-related complex. Mol Cell 2007; 27:275-88.
32. O'Sullivan JM, Tan-Wong SM, Morillon A, Lee B, Coles J, Mellor J, et al. Gene loops juxtapose promoters and terminators in yeast. Nat Genet 2004; 36:1014-8.
33. Ansari A, Hampsey M. A role for the CPF 3'-end processing machinery in RNAP II-dependent gene looping. Genes Dev 2005; 19:2969-78.
34. Luthra R, Kerr SC, Harreman MT, Apponi LH, Fasken MB, Ramineni S, et al. Actively transcribed GAL genes can be physically linked to the nuclear pore by the SAGA chromatin modifying complex. J Biol Chem 2007; 282:3042-9.
35. Rougemaille M, Dieppois G, Kisseleva-Romanova E, Gudipati RK, Lemoine S, Blugeon C, et al. THO/Sub2p functions to coordinate 3'-end processing with gene-nuclear pore association. Cell 2008; 135:308-21.
36. Kim M, Ahn SH, Krogan NJ, Greenblatt JF, Buratowski S. Transitions in RNA polymerase II elongation complexes at the 3' ends of genes. EMBO J 2004; 23:354-64.
37. Luna R, Jimeno S, Marin M, Huertas P, Garcia-Rubio M, Aguilera A. Interdependence between Transcription and mRNP Processing and Export, and Its Impact on Genetic Stability. Mol Cell 2005; 18:711-22.
38. Saguez C, Schmid M, Olesen JR, Ghazy MA, Qu X, Poulsen MB, et al. Nuclear mRNA surveillance in THO/sub2 mutants is triggered by inefficient polyadenylation. Mol Cell 2008; 31:91-103.
39. Johnson SA, Cubberley G, Bentley DL. Cotranscriptional Recruitment of the mRNA Export Factor Yra1 by Direct Interaction with the 3' End Processing Factor Pcf11. Mol Cell 2008.