Cellular Logistics 1:1, 37-40; January/February 2011; © 2011 Landes Bioscience

mRNA imprinting

Additional level in the regulation of gene expression

Mordechai Choder

Department of Molecular Microbiology; Rappaport Faculty of Medicine; Technion–Israel Institute of Technology; Haifa, Israel

Following its synthesis in the nucleus, mRNA undergoes various stages that are critical for the proper synthesis, localization and possibly functionality of its encoded protein. Recently, we have shown that two RNA polymerase II (Pol II) subunits, Rpb4p and Rpb7p, associate with the nascent transcript co-transcriptionally. This "mRNA imprinting" lasts throughout the mRNA lifetime and is required for proper regulation of all major stages that the mRNA undergoes. Other possible cases of co-transcriptional imprinting are discussed. Since mRNAs can be transported from the synthesizing cell to other cells, we propose that mRNA imprinting can also affect the phenotype of the recipient cells. This can be viewed as "mRNA-based epigenetics."

Key words: mRNA biogenesis, transcription, RNA polymerase II, mRNA export, translation, mRNA decay, mRNA trafficking, exosome, plasmodesmata, yeast

Submitted: 12/14/10

Accepted: 12/15/10

DOI: 10.4161/cl.1.1. 14465

Correspondence to: Mordechai Choder;
Email: choder@technion.ac.il

The yeast RNA polymerase II (Pol II) consists of two substructures: a 10-subunit core enzyme and a heterodimer formed by Rpb4p and Rpb7p.[1,2] The interface between the core and Rpb4/7 is very small and the two substructures can readily be dissociated. In vitro studies, using proteins extracted from human cells, demonstrated that hsRpb7p interacts with the transcript as it emerges from Pol II,[3] raising the possibility that the RNA-binding capacity of Rpb7p is involved in regulating transcription. Recently, we showed that the yeast Rpb4/7 interacts with Pol II transcripts in vivo.[4,5] Despite the excess of Rpb4/7 over Pol II, interaction between Rpb4/7 and mRNA can occur only in the context of Pol II.[4] Significantly, this interaction is critical to the capacity of Rpb4/7 to regulate all major stages that the mRNA undergoes.[4,5] Specifically, Rpb4/7 remains associated with the mRNA throughout its life, as well as regulating processes such as export,[6] translation,[5] movement out of P bodies,[5] 5' to 3' decay[7] and 3' to 5' decay[8] pathways (by Xrn1p and the exosome complex, respectively). Rpb4/7 binds the 3'-untranslated regions of *MFA2* and *RPL30* mRNAs even after most of these mRNAs was exonucleolytically degraded from 5' to 3' in the cytoplasm (Guterman and Choder, unpublished results). After the cytoplasmic mRNA decay is completed, Rpb4/7 returns to the nucleus,[9] probably to stimulate another round of transcription. Thus, in addition to the coding information for protein synthesis, the mRNA carries "imprinted" information. In case of imprinting by Rpb4/7, this information serves to regulate all major post-transcriptional stages. A model is shown in Figure 7 of reference 5.

How can mRNA imprinting regulate so many different mechanisms? A plausible explanation might be related to the capacity of Rpb4/7 to physically interact with components of the translation initiation factor 3 (Nip1p and Hcr1p)[5] and components of P bodies and mRNA decay machinery (Pat1p and Lsm2p).[7,8] It is quite possible that Rpb4/7 interacts with these components temporarily, thereby regulating each stage in due time. Although genetic interaction between Rpb4/7 and a component of the export machinery has been demonstrated,[6] actual physical interactions remain to be determined.

mRNA imprinting might be crucial for proper gene regulation as defects in Rpb4/7 activities result in pleiotropic effects.[5] One remarkable feature is the

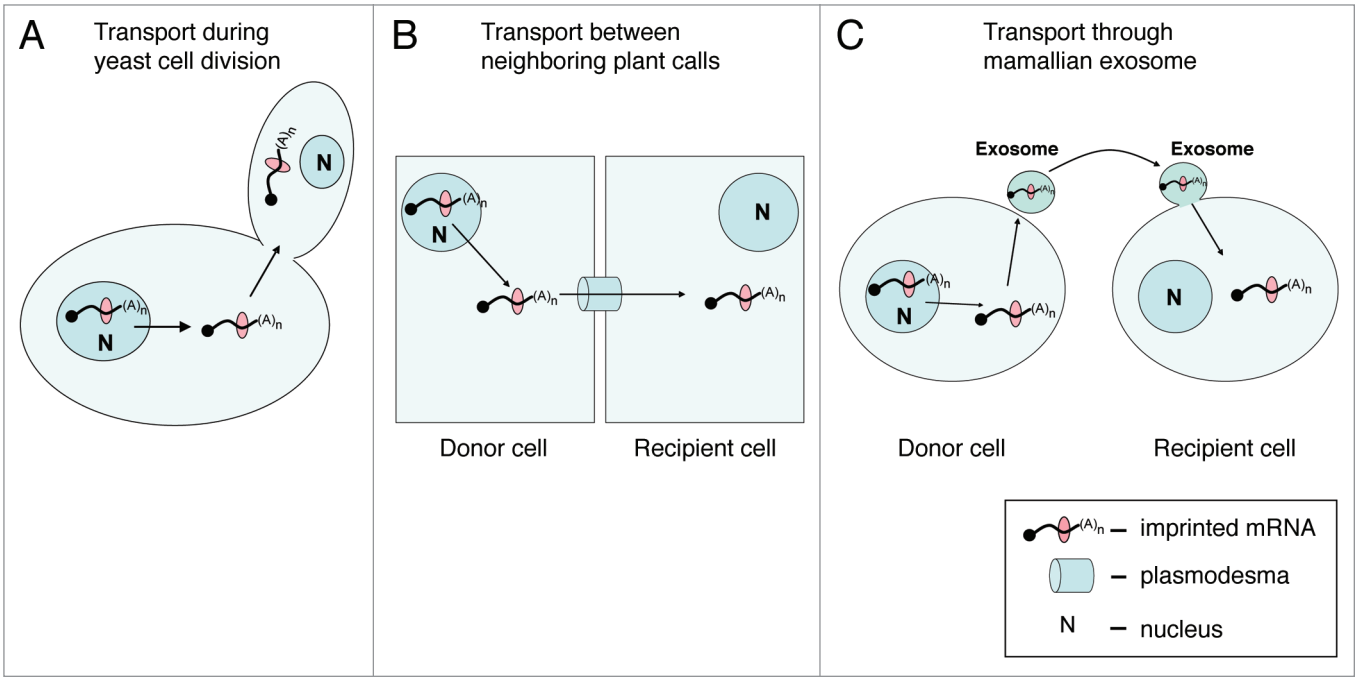

Figure 1. Model for a possible impact of mRNA imprinting on epigentics. Imprinted mRNA can be transported between cells via one of the following ways: (A) Imprinted mRNA can be transferred from mother to daughter cell, thus affecting the phenotype during bud growth and shortly after the daughter cell separates. (B) Imprinted mRNA can be transferred between neighboring cells through specific gates, such as plasmodesmata in plant tissues. (C) Imprinted mRNA can be transferred between distant cells through vesicles, such as exosomes. We propose that, in all these cases, the nature of imprinting can regulate the mRNA transport process itself, as well as the mRNA localization and translatability within the recipient cell. In this way, mRNA imprinting might contribute to epigenetics.

highly variable cell size that characterizes the *rpb4*Δ cell population (proliferating in rich medium at 30°C). It seems that a critical cellular decision regarding if and when to divide[10] requires proper mRNA imprinting by Rpb4/7. It is tempting to speculate that Rpb4/7 coordinates expression of genes required for cell growth (e.g., protein biosynthetic machinery) with those involved in the cell division cycle (CDC). Moreover, similar to DNA imprinting, mRNA imprinting is likely to be transmitted from mother to daughter cell (**Fig. 1A**). Thus, in the case of defective imprinting, CDC regulation becomes chaotic and cell division is not in tune with cell mass. It is also possible that the timing of separation between mother and daughter cell, each carrying improperly imprinted mRNAs, is deregulated.

Is mRNA imprinting regulated? To answer this question we need to understand what controls the dissociation of Rpb4/7 from Pol II and its association with the mRNA. Dissociation of Rpb4/7 from Pol II can be regulated by ubiquitination of two Pol II subunits, Rpb1p and Rpb2p.[11] Whether this ubiquitin-stimulated dissociation of Rpb4/7 is required for its binding with Pol II transcripts remains to be determined. Another possibility for regulation is post-translational modification of Rpb4/7. This kind of modulation can also occur post-transcriptionally, at any location the mRNA is visiting. Of note, both Rpb4p and Rpb7p are highly modified post-translationally (Duek and Choder, unpublished observation). At least one of the modifications is phosphorylation.[12]

Is the function of Rpb4/7 in higher eukaryotes similar to that of the yeast heterodimer? Rpb7p is a highly conserved protein.[13] The human Rpb7p can functionally replace the yeast Rpb7p.[14] However, Rpb4p is less conserved. Most notable is the presence of an unstructured domain in the yeast Rpb4p, which is absent in higher eukaryotes. Nevertheless, hRpb4 can partially complement the temperature sensitivity and proliferation defects of *rpb4*Δ yeast cells.[15] It is therefore possible that the human Rpb4/7 functions similarly to that of the yeast homolog. Indeed, the human Rpb7p can interact with Pol II transcript co-transcriptionally,[3] like we propose for yeast Rpb4/7. On the other hand, the absence of the unstructured domain in the human Rpb4p, a domain that might be responsible for the capacity of the yeast Rpb4/7 to interact with different partners,[16] raises the possibility that Rpb4/7-mediated regulation in higher eukaryotes is more complex and might involve additional factors that provide the function(s) of the missing domain.

Discovering the Rpb4/7-mediated mRNA imprinting raises the question whether similar versions of RNA imprinting exist in yeast and other organisms. She2p is associated with some specific Pol II transcripts co-transcriptionally. This interaction is required to promote the transport of these mRNAs from the nucleus of yeast mother cell to the tip of the daughter cell.[17] A number of additional proteins have been proposed to associate with Pol II transcripts during transcription. Examples are: ZBP1,[18] THO/TREX complex,[19] Mext67,[19] CPEB,[20] Npl3p,[21] Sus1p[22] and Sro9p.[23] Notably, all these

factors regulate post-transcriptional stages. ZBP1 has been implicated in regulation of localization and translatability of several different RNAs.[24] TREX and Mex67 mediate mRNA export.[19] CPEB affects alternative splicing as well as cytoplasmic polyadenylation and translation regulation,[20] Npl3p affects both mRNA export and translation,[25] and Sus1p regulates mRNA decay.[22] Sro9p associates with polysomes and might regulate translation.[26] Interestingly, overexpression of *SRO9* partially suppresses the temperature sensitivity of *rpb4*Δ cells.[36] Like Rpb4/7,[9] Sro9p is a shuttling protein whose export is dependent on transcription.[23] It is possible that there is some functional link between the two factors. The exon junction complex (EJC) is deposited on the mRNA following splicing in the nucleus and also regulates translation and nonsense mediated decay.[27,28] Finally, the kinase Ctk1p phosphorylates the Pol II C-terminal domain[29] as well as the ribosomal protein Rps2p, thereby regulating translation.[30] Ctk1p interacts with TREX complex and with polysomes and probably associates with mRNAs both in the nucleus and in the cytoplasm.[30] Hence, Ctk1p has the potential capacity to regulate mRNA fate by phosphorylating its associated proteins temporarily (in tune with the environment?). However, it is important to emphasize that involvement of a given factor in more than one process does not necessarily signify that its various functions are mechanistically linked. It is possible that, during evolution, this factor has acquired more than one unrelated function, and that each function is carried out by a separate pool of this factor. In our recent papers, we demonstrated that recruitment of Rpb4/7 to Pol II is a prerequisite for its subsequent functions in all major post-transcriptional stages.[4,5] Thus, the Rpb4p and Rpb7p molecules that interact with the mRNA co-transcriptionally are the ones regulating mRNA export, translation and decay. Likewise, co-transcriptional association of She2p with specific mRNAs has recently been demonstrated to affect localization of these mRNAs to yeast daughter cell.[17] It remains to be determined whether the co-transcriptional associations of the other above-mentioned factors with mRNAs are required for their post-transcriptional functions. Future work will reveal whether these factors affect all major stages in the life of the mRNA, as is the case of Rpb4/7, and whether there is a functional link between these factors. The issue of specificity, e.g., whether specific classes of mRNAs are differently imprinted, remains to be examined for most of the cases discussed above. She2p associates and affects localization of specific mRNAs.[17] Although Rpb4/7 heterodimer interacts with every mRNA examined today, deletion of *RPB4* affects the decay of only a specific class of mRNAs.[7] Specificity is an important issue, as regulatory mechanisms usually act on functional classes of mRNAs rather than individual mRNA molecules.

Epigenetics refers to inherent changes in phenotype caused by mechanisms other than changes in the underlying DNA sequence. Epigenetic studies focus on DNA and chromatin.[31] Recently this concept has been broadened to include also non-coding RNAs and association of components of transcriptional regulatory machinery with target genes on mitotic chromosomes.[32] For example, association of transcription factors with promoter elements during mitosis poises some genes for expression in progeny cells.[33] Here we propose to broaden the concept of epigenetics and to view mRNA imprinting as an additional element.

It is becoming clear that RNA molecules, including mRNAs, can be synthesized in one cell and transported by various mechanisms to another cell, thereby affecting the phenotype of the recipient cell. mRNA imprinting can play a role in this kind of cell-cell communication (see **Fig. 1**). This would be particularly significant in case the imprinting is regulated (see above). Transport of mRNAs from mother yeast cell to daughter cell (**Fig. 1A**) has been documented.[17] In addition to active transport, it is reasonable to assume that transport of many mRNAs occurs passively, before or after the daughter nucleus starts to synthesize its own mRNAs. There are many reports of active mRNA transport between neighboring cells. For example, in plant cells, mRNA is transported through special gates, called plasmodesmata (**Fig. 1B**).[34] Other, less trivial, cases involve the transport of secreted vesicles containing RNPs to neighboring or distant cells. The most studied ones are named "exosomes" (**Fig. 1C**), known to modulate immune function, angiogenesis, cell proliferation, and tumor-cell invasion.[35] Examples shown in **Figure 1** are by no means all cases of mRNA transfer between cells. Other possible cases include mating, sporulation, fertilization, maternal provision of sex determining mRNA in some insects, and mRNA storage in seeds. In all these cases, imprinting might have bearing on the mRNA transport itself, as well as on the phenotype of the recipient cells (see legend to **Fig. 1**). The effects last until the mRNA is degraded. Nevertheless, the encoded protein might leave long-term epigenetic effects (e.g., in case of a DNA methyltransferase or chromatin remodeler).

In summary, mRNA imprinting, whereby co-transcriptional association of factors with the transcript affects some or all major events that the mRNA undergoes, has been demonstrated in yeast.[4,5,17] mRNA imprinting, therefore, adds an additional level of regulation and seems to play a key role in gene expression. It also contributes to cellular logistics by regulating mRNA localization and possibly also orchestrating mRNA export, translation and decay at the right time and location. A corollary of this view is the possible impact of mRNA imprinting on cell-cell communication and in epigenetics (at least a short-term effect). Future work will determine how widespread mRNA imprinting is, how specific it is, and how many different components are involved.

Acknowledgments

I would like to thank all the lab members throughout the years for fruitful and stimulating discussions that helped shape all the ideas discussed here. Gal Haimovich and Adi Guterman comments on the manuscript are highly appreciated. I thank Yehudit Bergman for fruitful discussion. Work in the lab has been supported by the Israel Science Foundation, the Israel-American Binational Foundation, the Rappaport Foundation, Paamei Tikva, and the Israel Ministry of Health.

References

1. Armache KJ, Kettenberger H, Cramer P. Architecture of initiation-competent 12-subunit RNA polymerase II. Proc Natl Acad Sci USA 2003; 100:6964-8.
2. Bushnell DA, Kornberg RD. Complete, 12-subunit RNA polymerase II at 4.1-A resolution: implications for the initiation of transcription. Proc Natl Acad Sci USA 2003; 100:6969-73.
3. Ujvari A, Luse DS. RNA emerging from the active site of RNA polymerase II interacts with the Rpb7 subunit. Nat Struct Mol Biol 2006; 13:49-54.
4. Goler-Baron V, Selitrennik M, Barkai O, Haimovich G, Lotan R, Choder M. Transcription in the nucleus and mRNA decay in the cytoplasm are coupled processes. Genes Dev 2008; 22:2022-7.
5. Harel-Sharvit L, Eldad N, Haimovich G, Barkai O, Duek L, Choder M. RNA polymerase II subunits link transcription and mRNA decay to translation. Cell 2010; 143:552–63.
6. Farago M, Nahari T, Hammel C, Cole CN, Choder M. Rpb4p, a Subunit of RNA Polymerase II, Mediates mRNA Export during Stress. Mol Biol Cell 2003; 14:2744-55.
7. Lotan R, Goler Bar-On V, Harel-Sharvit L, Duek L, Melamed D, Choder M. The RNA polymerase II subunit Rpb4p mediates decay of a specific class of mRNAs. Genes Dev 2005; 19:3004-16.
8. Lotan R, Goler-Baron V, Duek L, Haimovich G, Choder M. The Rpb7p subunit of yeast RNA polymerase II plays roles in the two major cytoplasmic mRNA decay mechanisms. J Cell Biol 2007; 178:1133-43.
9. Selitrennik M, Duek L, Lotan R, Choder M. Nucleocytoplasmic shuttling of the Rpb4p and Rpb7p subunits of yeast RNA polymerase II by two pathways Eukaryotic Cell 2006; 5:2092-103.
10. Jorgensen P, Tyers M. How cells coordinate growth and division. Curr Biol 2004; 14:R1014-27.
11. Daulny A, Geng F, Muratani M, Geisinger JM, Salghetti SE, Tansey WP. Modulation of RNA polymerase II subunit composition by ubiquitylation. Proc Natl Acad Sci USA 2008; 105:19649-54.
12. Breitkreutz A, Choi H, Sharom JR, Boucher L, Neduva V, Larsen B, et al. A global protein kinase and phosphatase interaction network in yeast. Science; 328:1043-6.
13. Choder M. Rpb4 and Rpb7: subunits of RNA polymerase II and beyond. Trends Biochem Sci 2004; 29:674-81.
14. Khazak V, Sadhale PP, Woychik NA, Brent R, Golemis EA. Human RNA polymerase II subunit hsRPB7 functions in yeast and influences stress survival and cell morphology. Mol Biol Cell 1995; 6:759-75.
15. Khazak V, Estojak J, Cho H, Majors J, Sonoda G, Testa JR, et al. Analysis of the interaction of the novel RNA polymerase II (pol II) subunit hsRPB4 with its partner hsRPB7 and with pol II. Mol Cell Biol 1998; 18:1935-45.
16. Uversky VN, Dunker AK. Understanding protein non-folding. Biochim Biophys Acta 2010; 1804:1231-64.
17. Shen Z, St-Denis A, Chartrand P. Cotranscriptional recruitment of She2p by RNA pol II elongation factor Spt4-Spt5/DSIF promotes mRNA localization to the yeast bud. Genes Dev 2010; 24:1914-26.
18. Oleynikov Y, Singer RH. Real-time visualization of ZBP1 association with beta-actin mRNA during transcription and localization. Curr Biol 2003; 13:199-207.
19. Kohler A, Hurt E. Exporting RNA from the nucleus to the cytoplasm. Nat Rev Mol Cell Biol 2007; 8:761-73.
20. Lin CL, Evans V, Shen S, Xing Y, Richter JD. The nuclear experience of CPEB: implications for RNA processing and translational control. RNA 2010; 16:338-48.
21. Dermody JL, Dreyfuss JM, Villen J, Ogundipe B, Gygi SP, Park PJ, et al. Unphosphorylated SR-like protein Npl3 stimulates RNA polymerase II elongation. PloS One 2008; 3:e3273.
22. Cuenca-Bono B, Garcia-Molinero V, Pascual-Garcia P, Garcia-Oliver E, Llopis A, Rodriguez-Navarro S. A novel link between Sus1 and the cytoplasmic mRNA decay machinery suggests a broad role in mRNA metabolism. BMC Cell Biol 2010; 11:19.
23. Rother S, Burkert C, Brunger KM, Mayer A, Kieser A, Strasser K. Nucleocytoplasmic shuttling of the La motif-containing protein Sro9 might link its nuclear and cytoplasmic functions. RNA 2010; 16:1393-401.
24. Yisraeli JK. VICKZ proteins: a multi-talented family of regulatory RNA-binding proteins. Biol Cell 2005; 97:87-96.
25. Windgassen M, Sturm D, Cajigas IJ, Gonzalez CI, Seedorf M, Bastians H, Krebber H. Yeast shuttling SR proteins Npl3p, Gbp2p, and Hrb1p are part of the translating mRNPs, and Npl3p can function as a translational repressor. Mol Cell Biol 2004; 24:10479-91.
26. Sobel SG, Wolin SL. Two yeast La motif-containing proteins are RNA-binding proteins that associate with polyribosomes. Mol Biol Cell 1999; 10:3849-62.
27. Le Hir H, Seraphin B. EJCs at the heart of translational control. Cell 2008; 133:213-6.
28. Isken O, Kim YK, Hosoda N, Mayeur GL, Hershey JW, Maquat LE. Upf1 phosphorylation triggers translational repression during nonsense-mediated mRNA decay. Cell 2008; 133:314-27.
29. Cho EJ, Kobor MS, Kim M, Greenblatt J, Buratowski S. Opposing effects of Ctk1 kinase and Fcp1 phosphatase at Ser 2 of the RNA polymerase II C-terminal domain. Genes Dev 2001; 15:3319-29.
30. Rother S, Strasser K. The RNA polymerase II CTD kinase Ctk1 functions in translation elongation. Genes Dev 2007; 21:1409-21.
31. Cedar H, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. Nat Rev Genet 2009; 10:295-304.
32. Zaidi SK, Young DW, Montecino M, Lian JB, Stein JL, van Wijnen AJ, Stein GS. Architectural epigenetics: mitotic retention of mammalian transcriptional regulatory information. Mol Cell Biol 2010; 30:4758-66.
33. Ali SA, Zaidi SK, Dacwag CS, Salma N, Young DW, Shakoori AR, et al. Phenotypic transcription factors epigenetically mediate cell growth control. Proc Natl Acad Sci USA 2008; 105:6632-7.
34. Kehr J, Buhtz A. Long distance transport and movement of RNA through the phloem. J Exper Bot 2008; 59:85-92.
35. Simons M, Raposo G. Exosomes--vesicular carriers for intercellular communication. Curr Opin Cell Biol 2009; 21:575-81.
36. Tan Q, Li X, Sadhale PP, Miyao T, Woychik NA. Multiple mechanisms of suppression circumvent transcription defects in an RNA polymerase mutant. Mol Cell Biol 2000; 20:8124-33.