

## PERSPECTIVE

# The cytoplasmic fate of an mRNP is determined cotranscriptionally: exception or rule?

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**She2p is an RNA-binding protein that recognizes a zipcode on specific mRNAs necessary for the assembly of a protein complex that localizes them to the yeast bud tip. In this issue of *Genes & Development*, Shen and colleagues (pp. 1914–1926) demonstrate that She2p associates with RNAPII globally, but then recognizes the nascent chain only if it contains a zipcode. This demonstrates yet another case where the mRNA's cytoplasmic fate is determined by the RNAPII complex.**

The functional outcome of an mRNA is determined in the cytoplasm through binding proteins that specify its localization, translation, and degradation. It is a misperception that the fate of a localized mRNA is determined after nuclear export. In fact, evidence is accumulating to the contrary (Farina and Singer 2002). In this issue of *Genes & Development*, Shen et al. (2010) further this evidence by describing the nuclear component of localization in budding yeast, and demonstrating that localization of several mRNAs to the bud tip is determined cotranscriptionally. They show that the RNA-binding protein She2p, which is responsible for assembling a complex in the cytoplasm that contains myosin for RNA transport (Long et al. 1997, 2000), recognizes its target nascent mRNA in the nucleus. Importantly, Shen et al. (2010) show mechanistically that She2p interacts first with RNA polymerase II (RNAPII) throughout the entire elongation process via the association with the transcription factor Spt4–Spt5p/DSIF (DRB [5,6-dichloro-1-b-d-ribofuranosyl]benzimidazole] sensitivity-inducing factor), and then “hops” onto the nascent mRNA in a sequence-dependent manner. Interestingly, the initial interaction with RNAPII is general, but the move to the RNA presumably requires the presence of the She2p-binding sites on the RNA. These results, taken together with examples published over recent years and described below, shift our attention back into the nucleus, demon-

strating that the ultimate function of an mRNA can be determined in an RNAPII-dependent process.

## Cotranscriptional regulation of mRNA localization

In budding yeast, the She2 protein binds to specific RNA sequences called “zipcodes” (Kislauskis and Singer 1992) within the localizing transcript (Olivier et al. 2005), and directs localization of several mRNAs to the bud tip. When deleted, cells no longer properly localize their transcripts (Long et al. 1997). She2p recruits a type V myosin, Myo4p, through the She3p adaptor protein, thereby promoting the formation of the “locosome” (Long et al. 2000) needed for proper mRNA localization. The best understood example of a localized mRNA in budding yeast is *ASH1*, which encodes a transcriptional repressor of the HO locus and is a regulator of mating type switching in yeast. It is expressed in late mitosis and selectively represses mating type switching in the daughter cell so that only mothers can switch mating types after each cell division. She2p is a shuttling protein, and possesses a nonclassical nuclear localization sequence (NLS). When mutated, She2p became excluded from the nucleus, leading to mislocalization of *ASH1* mRNA (Shen et al. 2009), suggesting the possibility that She2p could bind localizing mRNAs in the nucleus.

Different regulators of mRNA localization have been shown to bind their targets in the nucleus in both *Saccharomyces cerevisiae* and in higher eukaryotes (Holt and Bullock 2009; Martin and Ephrussi 2009). For example, ZBP1 and ZBP2 are two RNA-binding proteins that regulate localization of  $\beta$ -actin mRNA to the leading edge of chicken embryo fibroblasts and growth cones in developing neurons. This localization is important for maintenance of cell polarity and directed cell motion (Shestakova et al. 2001; Farina et al. 2003). Using immunofluorescence and fluorescent in situ hybridization (FISH), ZBP1, ZBP2, and  $\beta$ -actin mRNA were found colocalized in nuclear foci identified as active  $\beta$ -actin transcription sites (Oleynikov and Singer 2003; Pan et al. 2007). However, it is unclear if RNAPII orchestrates ZBP1 and ZBP2 binding to their target mRNAs, or whether they bind to the  $\beta$ -actin mRNA independently.

Shen et al. (2010) offer the first account that provides a mechanistic interpretation of how this association is

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achieved, and how it is coupled with the transcription machinery. They show that nuclear association of She2p with the nascent mRNA occurred cotranscriptionally through the interaction with the RNAPII-bound Spt4–5p transcriptional elongators. This interaction was necessary for proper localization of *ASH1* mRNA to the bud tip and subsequent localization of Ash1p in the daughter nucleus to inhibit mating type switching (Jansen et al. 1996). Consequently, mutation of either Spt4 or Spt5 strongly reduced the efficiency of *ASH1* localization to the bud tip, phenocopying the She2p deletion. Remarkably, chromatin immunoprecipitation (ChIP) experiments showed that She2p associated with the actively transcribing RNAPII of nonlocalizing transcripts *ACT1*, *FBA1*, *PMA1*, *ASC1*, or *PGK1* as well. However, the ChIP signal was greatly enriched when the localizing mRNAs *ASH1*, *IST2*, or *EAR1* were transcribed, because She2p could apparently “hop” from an RNAPII-bound Spt4p–5p/DSIF directly onto the zipcode sequence in the nascent mRNA. Enrichment of the She2p signal was RNase-sensitive, while RNase treatment had no effect on the level of She2p associated with the nonlocalizing *ACT1*, *FBA1*, *PMA1*, *ASC1*, and *PGK1* genes. Finally, ChIP experiments also revealed that the presence of a zipcode inserted into the nonlocalizing LacZ mRNA resulted in the RNA-dependent interaction of She2p with the LacZ gene.

The “hopping” of the She2p regulator from the Spt4/5p-associated RNAPII onto the mRNA in a sequence-dependent manner is a conceptual teaser. When the zipcode sequence that forms a secondary structure on the mRNA (there are four of them in *ASH1*: three in the ORF, and one in the 3′ untranslated region [UTR]) (Chartrand et al. 1999) first emerges from the elongating RNAPII, the affinity of She2p for the RNA relative to Spt4p–Spt5p has to be greater than for the RNAPII in order to switch. This jump is specific for the zipcode, since it does not occur when a nonlocalizing mRNA is transcribed. Here, Spt4p and Spt5p could act as a binding adjuvant to increase the likelihood that each zipcode will be occupied. How the specific mRNA sequence is recognized and how the transfer of the RNAPII-bound She2p onto the nascent chain is achieved remain to be addressed. Does the reading of the mRNA sequence occur concomitantly with the elongation process, or does the RNAPII pause every so often, giving RNAPII-bound She2p adequate time to find its target? In case of the latter, this mechanism would recall the capping reaction of the newly transcribed mRNA, and, possibly, this mechanism could serve as a model for She2p interaction with transcribing zipcode RNA.

The capping process begins soon after transcription initiation, when RNAPII is paused ~20–30 nucleotides (nt) into the pre-mRNA synthesis by the joint action of DSIF (an Spt4p–Spt5p complex) and NELF (a four-protein complex) (Hartzog et al. 1998; Wu et al. 2003). This promoter-proximal pausing provides a temporal window for capping execution as soon as the nascent transcript emerges from the RNAPII (Chiu et al. 2001; Pei and Shuman 2002). After the relief of the pause, RNAPII engages in a productive elongation, and Spt4–5/DSIF re-

mains associated with it throughout this process (Andrulis et al. 2000).

It is possible that, similar to the capping reaction, She2p could be transferred onto the nascent mRNA in an RNAPII-induced transcriptional pause. Such a scenario could occur farther downstream into the coding sequence because elongating RNAPII is known to pause away from the transcription start site (Darzacq et al. 2007). The pause sites are usually found in mammalian cells and are less well characterized in budding yeast but are not unlikely. With the help of Spt4p and Spt5p, a stalled RNAPII would have adequate time to search for a zipcode sequence to allow She2p to “hop” onto it.

For instance, the RNAPII–Spt4/5p–She2p complex could recognize its target mRNA if the RNAPII would proceed with transcription more slowly on genes coding for localizing mRNAs. *ASH1* zipcodes, for example, are long, 118- to 250-nt sequences that form strong secondary structures (Chartrand et al. 1999); the ones located in the ORF could slow down the elongating polymerase. A slower (or paused) RNAPII could assure that each and every zipcode will be identified as a binding site, and enable She2p to disassociate from the RNAPII and bind to the mRNA in a timely fashion. This dissociation step may alternatively provide a resistance to polymerase elongation. In the case of nonlocalizing mRNAs that do not have a zipcode, the RNAPII with the bound She2p would therefore move through the gene more rapidly. This mechanism is reminiscent of alternative splicing, where the recognition of the alternative splice site depends on the velocity of the elongating RNAPII (Muñoz et al. 2009). In this case, it was described that the transcribing RNAPII will recognize the weaker splice site only if it traverses through the coding sequence slowly enough for the splicing machinery to assemble on the newly synthesized transcript. If the velocity of the RNAPII is too high, spliceosomal assembly occurs only on stronger splice sites.

She2p is only one of the components of the locosome structure (Long et al. 2000) that is responsible for bud-specific transport of mRNAs. Other components, like She1p–5p, are essential for proper localization of *ASH1* mRNA and Ash1p sorting (Long et al. 1997). Interestingly, when the She2p NLS is mutated so that it can no longer enter the nucleus, it can still bind to the target mRNA on its own in the cytoplasm (Du et al. 2008), but with a diffuse RNA phenotype rather than bud tip-anchored mRNA localization (Shen et al. 2009). Furthermore, when the N terminus of She3p, an adapter of She2 interaction with Myo4p, was fused with She2p to prevent its nuclear import, *ASH1* mRNAs could localize to the bud tip as in wild-type cells, but, remarkably, failed to redistribute Ash1p asymmetrically (Du et al. 2008). While in the nucleus, She2p also recruits the Loc1p and Puf6p proteins that are involved in the translational repression of the *ASH1* transcript (Deng et al. 2008; Shen et al. 2009). Although cytoplasmic association of *ASH1* mRNA with She2p does occur (and these mRNAs can localize to the bud tip with a reduced efficiency), nuclear mRNA processing mediated by the activity of nuclear She2p appears

to be critical for coordination of cellular localization and appropriate Ash1p translation. This demonstrates that the order of binding of regulatory proteins to the mRNA involved in both processes is paramount to achieve a highly efficient localization and translation, and She2p appears to orchestrate these events. However, it is unclear whether an entire locosome assembles on the nascent mRNA chain (plausibly also in the Spt4p/5p-dependent manner), or if some of the components of the locosome interact in the cytoplasm. While association of some components, like Loc1p and Puf6p, could occur cotranscriptionally together with She2p, others, like Myo4p, appear to bind in the cytoplasm. Of course, there could be other factors that are mRNA-specific or are found to be involved in locosome formation only during specific stages of the cell cycle. Controlling which proteins would participate in mRNA localization would provide a cell with greater control over the formation of an mRNP and its cytoplasmic fate. It is tempting to hypothesize that such regulation could begin cotranscriptionally by controlling which locosome components would be recruited to the RNAPII and later bind to the target mRNA in response to a cellular hierarchy. A cell could synthesize mRNPs of different compositions from a specific gene, depending on the phase of the cell cycle or its stage during development or differentiation, and this could increase the number of localizing patterns and, potentially, other mRNA functions. Such regulatory mechanisms would become more important in higher eukaryotes, where temporal and spatial regulation of localization occurs during differentiation and development.

In the *Drosophila* oocyte, for example, *oskar* mRNA travels to the posterior pole of an oocyte during oocyte development. This process is regulated through nuclear splicing, rather than recognition of a specific zipcode sequence in the mRNA itself (Hachet and Ephrussi 2004). In this case, the nuclear shuttling proteins Y14/Tsunagi and Mago nashi, which are also components of the exon-junction complex (EJC), are required for *oskar* mRNA localization. As with other zipcode-binding proteins, these travel with the localizing mRNA, and are found to colocalize with *oskar* mRNA at the posterior pole of an oocyte. Intronless *oskar* mRNA not only mislocalized, but was also poorly translated relative to the wild-type *oskar* mRNA. Therefore, splicing in the nucleus not only regulated localization of *oskar* mRNA, but also the efficiency of its translation in the cytoplasm. Hence, alternative splicing could give rise to different mRNA-protein complexes with diverse cytoplasmic localization patterns that could participate in regulating embryo development. During *Drosophila* embryogenesis, 71% out of 3370 mRNAs investigated were found to be localized, and several dozen new localization patterns were identified (Lécuyer et al. 2007), indicating that different mRNAs interact with different protein regulators to assure variability. As with *oskar* mRNA, the locosome assembly could begin cotranscriptionally in a regulated manner to maximize regulation of localization patterning.

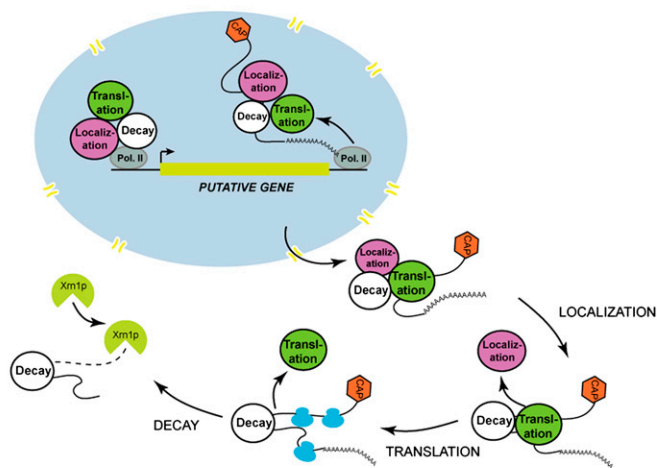
Cytoplasmic processes as diverse as mRNA decay and cellular localization were shown to be regulated on

a transcriptional level in both yeast and higher eukaryotes (Goler-Baron et al. 2008; Holt and Bullock 2009; Martin and Ephrussi 2009). To date, such examples are rare, and therefore the regulatory path that cotranscriptionally defines the composition of the cytoplasmic mRNP is still an exception rather than a rule. However, these cases will likely become more prominent in the future. Experimental evidence presented by Shen et al. (2010) and bolstered by the similar findings reported for other genes suggests a scenario as shown in Figure 1. An mRNA becomes equipped with all of the critical regulatory proteins cotranscriptionally, and then progressively sheds them in the cytoplasm with the completion of each successive step in the mRNA life cycle (Fig. 1A). This cotranscriptional view of the mRNP assembly is markedly different from the one that is generally assumed to exist for a "typical" mRNA, where mRNP assembly is regarded as a step-by-step process that depends on the successive, diffusion-driven association and disassociation of individual regulatory proteins in the cytoplasm (Fig. 1B).

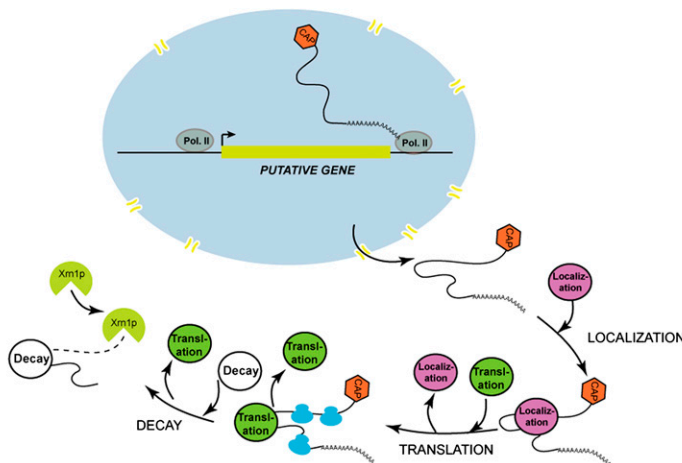
### Perspective

Transcription of nascent mRNA molecules is a highly dynamic and regulated process, and many processes that affect mRNP configuration occur cotranscriptionally and are independent of the cytoplasmic association of protein regulators with the mRNA. This brings the RNAPII enzymatic complex into the center of mRNP regulation, where it can be viewed as both a catalyst of mRNA synthesis and a scaffold onto which regulatory proteins assemble. According to the *Saccharomyces* genome database, Rpb1p, the large RNAPII subunit, interacts physically with 129 other proteins, and each one of these proteins further interacts with a number of other proteins. For example, the TFIID subunit Tef14p interacts with Rpb1p directly and with 88 other proteins, illustrating that RNAPII holds a huge capacity to engage in a variety of unique complexes, all of which can ultimately determine the functional outcome of a cytoplasmic mRNA. She2p, Spt4p, and Spt5p are fairly abundant proteins, and were reported to be present in ~4070, 4490, and 2340 protein molecules per cell, respectively (Ghaemmaghami et al. 2003). The majority of their protein is concentrated in the nucleus, and they can potentially interact with the RNAPII. It would be interesting to know whether every RNAPII associates with the Spt4p-Spt5p-She2 complex, or if there are RNA polymerases that do not. If so, are there zipcodes that are not occupied by the She2 protein, and how would this affect localization (and translation) efficiency of an mRNA such as *ASH1*? Varying the scope of these interactions during the synthesis of a single gene could vary the way a single mRNA species is localized, translated, and decayed, by carrying with it into the cytoplasm a regulatory mechanism that could play an important role during differentiation and development. Looking forward, it will be exciting to see whether this type of regulation becomes accepted as a general mechanism of mRNP assembly.

## A: TRANSCRIPTION DETERMINES THE CYTOPLASMIC FUNCTION OF AN mRNA



## B: CYTOPLASMIC FUNCTION OF AN mRNA IS INDEPENDENT OF TRANSCRIPTION



**Figure 1.** (A) An mRNP is fully configured in the nucleus cotranscriptionally. It exits the nucleus equipped with proteins that define its localization, translation, and decay patterns. After each completed step, individual regulatory proteins are shed away in a step-by-step fashion. (B) An mRNP structure changes dynamically throughout the lifetime of an mRNA in the cytoplasm through the diffusion-driven association and disassociation process. Transcriptional events in the nucleus are separated physically and temporally from those that occur in the cytoplasm, and have little effect on the formation of a functional mRNP.

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