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REVIEW ARTICLE



Feedback to the central dogma: cytoplasmic mRNA decay and transcription are interdependent processes

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ABSTRACT

Transcription and RNA decay are key determinants of gene expression; these processes are typically considered as the uncoupled beginning and end of the messenger RNA (mRNA) lifecycle. Here we describe the growing number of studies demonstrating interplay between these spatially disparate processes in eukaryotes. Specifically, cells can maintain mRNA levels by buffering against changes in mRNA stability or transcription, and can also respond to virally induced accelerated decay by reducing RNA polymerase II gene expression. In addition to these global responses, there is also evidence that mRNAs containing a premature stop codon can cause transcriptional upregulation of homologous genes in a targeted fashion. In each of these systems, RNA binding proteins (RBPs), particularly those involved in mRNA degradation, are critical for cytoplasmic to nuclear communication. Although their specific mechanistic contributions are yet to be fully elucidated, differential trafficking of RBPs between subcellular compartments are likely to play a central role in regulating this gene expression feedback pathway.

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Introduction

Gene expression is often described as a linear pathway, beginning with transcription and processing of messenger RNA (mRNA), followed by export to the cytoplasm for translation and ultimately decay. Included in this pathway are many regulatory steps that alter transcript fate, including quality control-based surveillance, splicing, RNA modification, and translational control (Glisovic et al. 2008; Popp and Maquat 2013; Herzel et al. 2017). It is intuitive that changing an upstream step of the pathway will influence the cascade of subsequent downstream events. However, evidence emerging over the past decade indicates that the reverse is also true: alterations to cytoplasmic mRNA degradation lead to alterations to mRNA transcription, and vice versa (Haimovich, Medina, et al. 2013; Sun et al. 2013; Abernathy et al. 2015). Thus, these seemingly distal events in the gene expression cascade appear to be coupled and may coordinate to regulate the overall pool of mRNA in response to perturbations. This RNA abundance-transcription connection has been observed in diverse systems, including yeast, zebrafish and mammalian cells.

Coordination between mRNA degradation and transcription occurs both at a global scale and in a more

targeted fashion. Broad changes in mRNA abundance or transcription rates are sensed (Helenius et al. 2011; Haimovich, Medina, et al. 2013; Sun et al. 2013; Abernathy et al. 2015; Dumdie et al. 2018) and degradation of specific transcripts results in selective upregulation of genes with high sequence similarity (Rossi et al. 2015; El-Brolosy et al. 2019; Ma et al. 2019). Given the spatial separation of basal mRNA decay and synthesis in the cell, RNA binding proteins (RBPs) that shuttle between the nucleus and the cytoplasm are prime candidates for conveying RNA abundance information between these compartments. That said, many questions remain about the specific proteins involved and their functional roles, and insight into the potentially common nature of pathways between organisms remains elusive.

We begin with a brief overview of the mRNA lifecycle and then summarize the evidence supporting mRNA abundance feedback signaling, focusing on several outstanding questions in the field. These include whether the pathway that links mRNA degradation and transcription is conserved and similarly regulated in yeast and mammals, what factors are involved, and the bi-directional nature of the signaling. We also explore what the functional roles of feedback may be in a cell

or organism and address if accelerated decay during viral infection and homeostatic buffering of mRNA levels are converging on the same signaling pathway.

The mRNA lifecycle from synthesis to decay

RNA Polymerase II (Pol II) transcribes mRNA in the nucleus of cells upon its assembly on a gene promoter with a diversity of essential general transcription factors (GTFs) and gene specific transcription factors (Sainsbury et al. 2015). GTF recruitment and regulation determine Pol II escape from the promoter and initiation of productive elongation (Saldi et al. 2016; Chen et al. 2018). What DNA is transcribed is determined by binding of the GTFs to promoter regions, beginning with the TFIID subunit TATA Binding Protein (TBP) on which the subsequent GTFs assemble (Sainsbury et al. 2015). GTF binding and Pol II transcription are dependent upon chromatin accessibility, which can be modified by the cell to alter the transcriptional program. Generally, chromatin remodeling occurs by strengthening or weakening histone-DNA interactions with ATP-dependent changes, with post translational modifications (PTMs) to the histones themselves, or when these PTMs recruit additional remodelers (Venkatesh and Workman 2015). Histone eviction from the DNA or loosening of existing chromatin results in DNA that can be transcribed by Pol II while histone recruitment or chromatin compaction occludes Pol II.

The cycle of initiation and elongation is largely dictated by a series of phosphorylation events on the Pol II C terminal domain (CTD), which contains 52 repeats of the heptad YSPTSTS in mammals and 26 in yeast (Hsin and Manley 2012). The CTD further coordinates processing of the nascent mRNA through interactions with factors that orchestrate cotranscriptional splicing, capping, termination and poly(A) tail addition (Proudfoot et al. 2002; Hsin and Manley 2012; Herzog et al. 2017). As it is transcribed, each mRNA is bound by RBPs that coordinate its export through nuclear pore complexes (NPCs), mark exon-exon junctions, and protect the 5' cap and poly(A) tail. Nuclear surveillance of proper loading of these RBPs results in nuclear degradation of aberrantly loaded mRNAs, along with unadenylated and 3' unprocessed mRNAs by the Rps6-containing nuclear exosome (Burkard and Butler 2000; Libri et al. 2002; Stutz 2003; Houseley et al. 2006). mRNAs can also undergo a variety of additional modifications; the most prevalent of these is N⁶-methyladenosine (m⁶A), which is recognized and bound by m⁶A "reader" proteins that impact the fate and function of the mRNA in diverse ways (Hailing et al. 2019).

Basal mRNA decay begins with deadenylation in a process that is quite conserved between yeast and mammals. Deadenylation by the major deadenylases Pan2-Pan3 and Ccr4-Not initiate poly(A) tail shortening and removal of the stabilizing Poly(A) Binding Protein (Pabpc) from the 3' end of transcripts (Parker 2012; Mugridge et al. 2018; Webster et al. 2018; Yi et al. 2018). Protein-protein interactions link deadenylation to the core decapping complex, which is comprised of the Dcp2 decapping enzyme, the decapping activator Dcp1 and the scaffold protein Edc4 (Braun et al. 2012; Mugridge et al. 2018). Interactions of the core decapping complex with the deadenylation machinery stimulate Dcp2 to remove the protective 7^mG cap from transcripts. Decapping concludes in rapid degradation of the transcript, primarily by the 5'-3' exonuclease Xrn1, although 3'-5' exonucleases such as the exosome and Dis3L2 also participate (Garneau et al. 2007; Schoenberg and Maquat 2012; Lubas et al. 2013; Łabno et al. 2016). 3'-5' decay can be further stimulated by the nontemplated addition of uridines to the 3' end of the message by terminal uridylyl transferases (TUTases) (Lee et al. 2014; Lim et al. 2014).

In addition to basal mRNA decay, various cytoplasmic pathways exist to degrade aberrant mRNAs or to modulate transcript fate. During translation, mRNAs are surveyed by various quality control factors that detect faulty transcripts, including those with premature termination codons, those lacking termination codons or those with stalled ribosomes (Shoemaker and Green 2012; Radhakrishnan and Green 2016). Aberrant transcripts are generally triggered for rapid clearance through recruitment of specialized endonucleases followed by degradation by exonucleases involved in basal mRNA degradation (Shoemaker and Green 2012; Popp and Maquat 2013; D'Orazio et al. 2019). One of the best-characterized quality control mechanisms is nonsense mediated decay (NMD), in which the presence of a premature termination codon (PTC) directs the mRNA to rapid decay. Central to this pathway is the Upf1 protein, which binds in complex with the SMG1 kinase and the eukaryotic release factors eRF1 and eRF3 to the terminating ribosomes of NMD substrates, delaying termination. Subsequent Upf1 phosphorylation and further remodeling of the RNP complex leads to recruitment of mRNA decay factors to clear the aberrant transcript (Kim and Maquat 2019). The half-life of an individual mRNA can also be influenced by specific sequence elements often found in the 3' untranslated region (UTR), such as destabilizing AU-rich elements (AREs), which recruit RBPs that increase deadenylation (Barreau 2005), and binding sites for microRNAs, which

lead to mRNA cleavage and/or translational repression (Friedman et al. 2008).

Finally, cellular perturbations such as apoptosis and viral infection can result in large-scale alterations to mRNA decay. During the early stages of apoptosis, after mitochondrial outer membrane permeabilization but before DNA fragmentation, caspase 8 activation leads to release of the exoribonuclease Pnpt1 from the mitochondria. Pnpt1 coordinates with Dis3L2 and TUTases 4 and 7 to cause widespread degradation of cytoplasmic mRNA (Thomas et al. 2015; Liu et al. 2018).

Multiple diverse viruses, including alpha and gamma-herpesviruses, influenza A virus, poxviruses, and coronaviruses, also accelerate basal mRNA decay. These viruses express proteins that promote widespread decapping or endonucleolytic cleavage of mRNA, producing fragments that are degraded by the endogenous RNA decay machinery (Karr and Read 1999; Glaunsinger et al. 2005; Kamitani 2009; Huang et al. 2011; Jagger et al. 2012). This can benefit the virus by decreasing the abundance of immunostimulatory nucleic acids, reducing expression of host immune response genes, and liberating host translation machinery for viral use (Parrish and Moss 2007; Richner et al. 2011; Read 2013; Liu et al. 2014; Abernathy and Glaunsinger 2015). For DNA viruses such as alphaherpesviruses, accelerating turnover of virally derived mRNAs also sharpens the kinetic boundaries between stages of viral gene expression (Oroskar and Read 1987, 1989).

Given that an array of stimuli can alter the abundance of individual mRNAs or of the overall mRNA pool, what mechanisms exist that enable cells to sense and respond to such perturbations? Over the past decade, numerous studies have established that cells have compensatory mechanisms to deal with these gene expression changes, and research into these pathways has revealed surprising connections between cytoplasmic mRNA turnover and Pol II transcription (Haimovich, Medina, et al. 2013; Sun et al. 2013; Abernathy et al. 2015).

Maintaining consistency: connections between mRNA degradation and synthesis buffer against change

Global mRNA stabilization leads to a compensatory reduction in Pol II transcription

While it might be assumed that slowing mRNA degradation would cause a general increase in transcript abundance, early studies reported that mRNA levels remain relatively constant in *S. cerevisiae* strains lacking decay factors including the decapping enzyme Dcp1 and the

5' – 3' exonuclease Xrn1 (Muhlrad and Parker 1999; He et al. 2003). Indeed, several lines of evidence now indicate that cells respond in a compensatory manner to “buffer” against generalized reductions in either cytoplasmic mRNA degradation or transcription, thereby maintaining constant mRNA levels in the cell (Figure 1).

Studies by the Cramer lab comparing transcription rates by 4-thiouridine (4SU) incorporation with computationally inferred RNA decay rates in *S. cerevisiae* strains deleted for 46 different RNA decay factors including deadenylases, decappers, exonucleases and RNA processing enzymes revealed that cells with reduced mRNA decay generally compensate by decreasing transcriptional output (Sun et al. 2012, 2013). One notable exception was the strain lacking Xrn1, which did not cause a corresponding change in transcription, suggesting a direct role for Xrn1 in this buffering pathway (Sun et al. 2013). The Choder lab also measured RNA stability (by northern blot) and RNA synthesis (using genomic run-on (GRO) analysis) in a panel of decay factor null *S. cerevisiae* strains, including those lacking Xrn1, Ccr4, Dcp1 and Dcp2. In each of these mutant strains, RNA half-lives were increased while transcription rates were decreased, supporting a buffering model (Haimovich, Choder et al. 2013; Haimovich, Medina et al. 2013). A recent report further

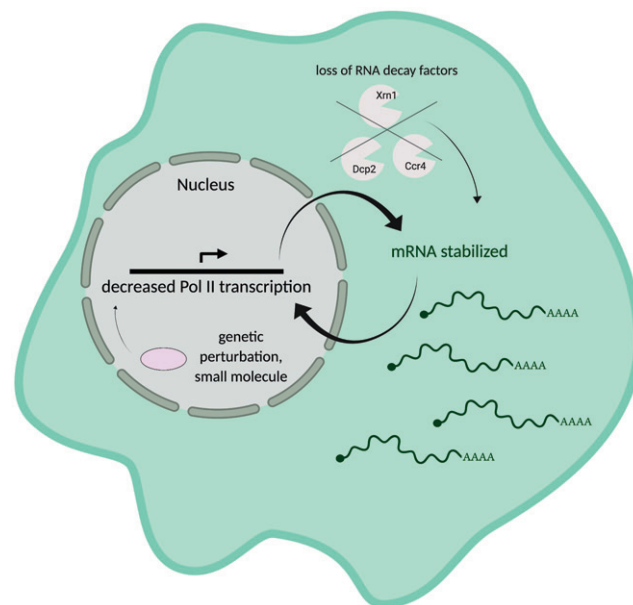


Figure 1. The homeostatic model of mRNA decay-transcription feedback proposes that appropriate mRNA levels are maintained by either altering mRNA decay or Pol II transcription. Removal of basal RNA decay factors results in decreased mRNA decay and stabilization of mRNAs; transcription then decreases to buffer against resulting increases in mRNA levels. Similarly, perturbations that cause reductions in transcription and thus less mRNA production are buffered through stabilization of mRNA.

demonstrates reduced elongating Pol II and reduced Pol II speed at *GAL* genes in an $\Delta xrn1$ strain (Begley et al. 2019). Each of these studies implicate Xrn1 in the transcriptional response to alterations in global mRNA stability, although the basis for differing transcriptional effects in the absence of Xrn1 is unclear. They may be attributed to different experimental approaches to measure transcription rates or potential differences in the $\Delta xrn1$ strains used. However, the convergence of these reports on Xrn1 highlights its central role in communicating RNA abundance information to the transcriptional machinery.

Experiments in mammalian cells that measure mRNA synthesis upon decay factor knockdown also suggest a buffering phenotype, although the connection is not as marked as it is in *S. cerevisiae*. Depleting the deadenylase PARN in mouse myoblasts showed a trend of decreased steady state RNA levels and increased half-lives using microarray analysis, but the differences are modest. For the small subset with two to three-fold increased half-lives, 4SU-based RT-qPCR measurements showed reduced transcription (Lee et al. 2012). Similarly, depleting Xrn1 from HepG2 cells resulted in reduced transcription for 8 out of 10 transcripts measured by RT-qPCR (Singh et al. 2019). Knockdown of the deadenylase CNOT6 impacted a smaller subset of the examined transcripts, caused less prominent buffering and in some cases increased transcription (Singh et al. 2019). Computational analysis comparing mRNA decay rates upon ActinomycinD treatment to total mRNA levels in induced pluripotent stem cells and human foreskin fibroblasts also provides evidence of a relationship between decay and transcription. Here, genes with faster decay rates were associated with increased steady state mRNA levels, as would be expected in a buffering model, although the genome-wide correlation is modest (Dori-Bachash et al. 2012). Extending these results in mammalian cells to additional global measurements of transcription under conditions of altered mRNA stability will be valuable, as will comparisons of the impact of an expanded set of mammalian mRNA decay pathway components.

Reduced Pol II transcription leads to compensatory increases in mRNA stability

As described above, eukaryotic cells can respond to global increases in mRNA half-life by decreasing transcriptional output. Notably, changes in transcription can be compensated in an analogous manner by altered mRNA stability, supporting the existence of a true feedback loop. In *S. cerevisiae*, a variety of strategies have been used to reduce transcription. For example, treatment

with the magnesium chelator 1,10-phenanthroline stalls all RNA polymerases (Johnston 1994) and phenocopies a genetic knockout of Rpb1-1 (Ray et al. 2013). Microarray-based time course measurements of mRNA levels during 1, 10-phenanthroline treatment were used to calculate decay rates, revealing a negative correlation between RNA stability and mRNA abundance (Shalem et al. 2011). This suggests that mRNAs are stabilized to buffer against the decreasing transcript levels resulting from transcriptional shutdown.

Similar trends were observed upon depletion of the SAGA subunits Sus1 and Spt20, which are key Pol II cofactors (Goler-Baron et al. 2008; Dori-Bachash et al. 2012; Baptista et al. 2017). Measurements of mRNA synthesis by GRO-seq as well as mRNA steady state levels during Sus1 depletion revealed that the stability of nearly all transcripts increased (García-Molinero et al. 2018). Sus1 depletion causes a gradient of reduced transcription rates where highly transcribed genes showed the most pronounced stabilization. Similarly, measurement of 4SU-labeled nascent RNA and total mRNA during loss of Spt20, which causes reduced transcription in close to 90% of genes, also showed that loss of mRNA synthesis was compensated by a decrease in decay rates for the vast majority of transcripts (Baptista et al. 2017). Thus, results from each of these genetic and chemical perturbations in yeast support a buffering model in which increasing mRNA stability is a mechanism used to compensate for reduced transcription, suggesting bidirectional decay/transcription communication.

While there are fewer relevant studies in mammalian cells, existing data largely support the buffering model. In particular, microarray analysis of cells depleted of the TFIIH kinase Mat1, which is involved in serine 5 phosphorylation of the Pol II CTD, showed that despite decreased elongating Pol II in the gene body, most mRNA steady state levels remained unchanged (Helenius et al. 2011). Additional experiments to directly evaluate how altering transcription impacts mRNA half-life will be important to confirm if reduced Pol II output results in reduced cytoplasmic mRNA decay in mammalian cells. One way to do this would be to test mRNA half lives while using a Pol II mutant with reduced elongation speed and correspondingly reduced transcription (Fong et al. 2014), or to globally test mRNA half lives during treatment with a drug that reduces transcription, like α -amanitin.

Gene specific integration of mRNA decay and transcriptional responses

The above examples in yeast and mammals show buffering in situations in which genetic or chemical

perturbations globally impact transcription or RNA decay, thus creating an environment where compensation might be particularly pronounced. However, it is also relevant to ask if compensatory mechanisms are at play under steady state conditions — though this might be difficult to detect if the alterations are subtle. Interestingly, in human cell lines from the HapMap Project, RNA decay rates of a small subset of genes (~5%) were indeed anticorrelated with their transcription; higher transcription was associated with faster degradation in approximately half of this subset (Pai et al. 2012). These cell lines were assayed under steady state conditions without perturbation to mRNA decay rates or transcription. While it is difficult to ascertain the directionality of the association, it is intriguing to consider that even in the absence of direct perturbation, mRNA decay and transcription may be coordinated, at least for a subset of transcripts. This raises the possibility that a cell is constantly buffering mRNA levels, which becomes more apparent upon large-scale alternations to rates of decay or transcription.

More compelling evidence has recently emerged related to transcript-specific compensatory responses in mammalian cells and in zebrafish. These targeted changes are known as “genetic compensation” or “nonsense-induced transcriptional compensation” (NITC) (Wilkinson 2019), and have recently been proposed as a way for the cell to respond to the loss of a specific transcript by upregulating sequence-similar transcripts (El-Brolosy et al. 2019; Ma et al. 2019). NITC explains the previously observed discrepancies between gene knockouts and knockdowns and genetic mutants or chemical inhibition in mouse and *Arabidopsis*, where certain gene knockouts displayed less dramatic phenotypes due to induction of sequence-similar genes that could partially compensate (Rossi et al. 2015; Zhu et al. 2017).

NITC was recently shown to occur for specific premature termination codon bearing transcripts subject to NMD (Figure 2). (Genetic knockouts are most often generated by dsDNA cleavage followed by error prone non-homologous end joining repairing the lesion, frequently introducing a PTC (Lieber 2010; Carroll 2014)). Sequence-similar transcripts, often evolutionarily derived from the same precursor gene, become upregulated, as measured by global RNA sequencing of various knockouts (El-Brolosy et al. 2019; Ma et al. 2019). However, mutant genes defective in producing any mRNA are either not upregulated or only modestly so (Rossi et al. 2015; El-Brolosy et al. 2019), suggesting that the mRNA itself is involved in signaling—consistent with the targeted nature of this transcriptional response.

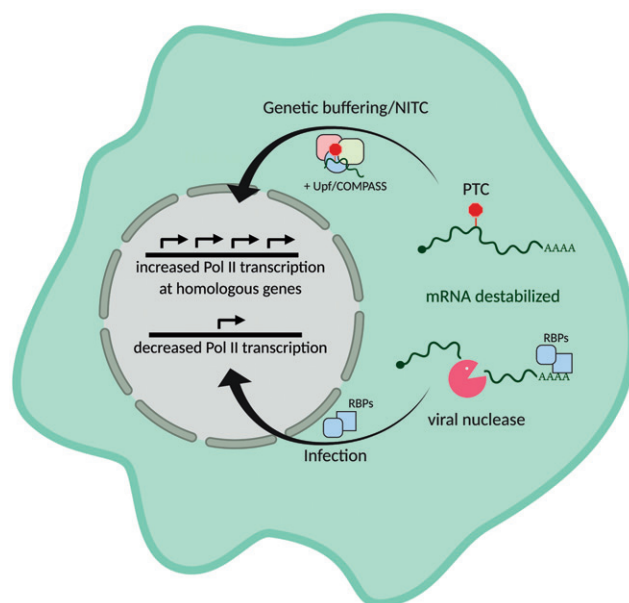


Figure 2. Cellular responses to mRNA destabilization include nonsense-induced transcriptional compensation (NITC) and decreased overall Pol II occupancy. mRNA with premature termination codons (PTCs) that are destabilized through the nonsense mediated decay pathway can recruit Upf proteins and members of the COMPASS complex. Upon translocation to the nucleus, they promote increased transcription of homologous genes by altering chromatin accessibility. Viral nucleases induce widespread mRNA decay by decapping or endonucleolytically cleaving mRNAs. Degradation of the resulting fragments by host exonucleases liberates previously bound RBPs, which are hypothesized to traffic into the nucleus in an mRNA concentration-dependent manner and decrease Pol II promoter occupancy.

Given the importance of PTCs for eliciting a transcriptional response, it is logical that NMD factors and basal RNA decay enzymes were implicated in the signaling. Indeed, removal of NMD components was found to diminish the transcriptional response, although the studies implicate different proteins in this pathway. El-Brolosy et al. showed that knockdown of Upf1, a central component of NMD, abrogated the transcriptional response, while Ma et al. observed that only knockout of Upf3a, a poorly understood NMD component, was sufficient to block the response (El-Brolosy et al. 2019; Ma et al. 2019). Both studies converged on a role for the COMPASS component Wdr5, which is responsible for deposition of H3K4me3 at the transcription start sites (TSS) of genes. Wdr5 depletion abolished transcriptional activation at compensating genes (El-Brolosy et al. 2019), and Upf3 was shown to interact with Wdr5 in an RNA-independent manner, providing a potential link between the NMD machinery and gene regulatory epigenetic modification (Ma et al. 2019). The basal RNA decay enzyme Xrn1 was also necessary for transcriptional compensation (El-Brolosy et al. 2019). While the

mechanistic link between RNA decay and transcriptional compensation remains unclear, RNA decay proteins could trim and modify RNA fragments, which might then be used to direct RBPs to complementary chromosomal locations.

It remains to be elucidated how fragments of PTC-containing mRNAs are directing chromatin remodeling in the nucleus. For example, how are these small RNAs generated and protected from complete degradation by basal RNA decay enzymes in order to be part of the signaling complex? Second, how are these small RNA fragments brought into the nucleus? Chaperoning RNAs could be a potentially novel role for Upf3a, or alternatively may be carried out by another protein, for example a component of the microRNA processing pathway.

Responding to a threat: transcriptional repression upon accelerated mRNA decay

While it is clear that cells can sense changes to mRNA levels and alter transcription or mRNA stability to buffer against these perturbations, there are circumstances in which a buffering response may not be beneficial. One prominent example is during intracellular pathogen infection, which can cause large-scale alterations to the cellular mRNA pool. Indeed, many diverse viruses accelerate basal mRNA decay as an integral part of their life-cycle. These include gammaherpesviruses such as Epstein-Barr virus (EBV) (Rowe et al. 2007), Kaposi's sarcoma-associated herpesvirus (KSHV) (Glaunsinger and Ganem 2004; Glaunsinger et al. 2005) and murine gammaherpesvirus 68 (MHV68) (Covarrubias et al. 2011; Richner et al. 2011), alphaherpesvirus such as herpes simplex virus (HSV) (Kwong and Frenkel 1987; Smibert et al. 1992; Everly and Read 1997), influenza A (Jagger et al. 2012; Desmet et al. 2013; Gaucherand et al. 2019), vaccinia virus (Parrish et al. 2007; Liu et al. 2014), and SARS and MERS coronavirus (Kamitani et al. 2006; Kamitani 2009; Lokugamage et al. 2015). Most of these viruses encode broad acting, mRNA specific endonucleases (EBV BGLF5, KSHV SOX, MHV68 muSOX, HSV-1 vhs, influenza A PA-X) or decapping factors (vaccinia D9, D10), which create mRNA cleavage products that are directly accessed and cleared by the basal mRNA decay machinery such as Xrn1 (Gaglia et al. 2012; Abernathy and Glaunsinger 2015). In addition to the infection context, expression of these viral nucleases alone in mammalian cells is sufficient to drive widespread mRNA decay, reducing cytoplasmic mRNA populations by 50–70% (Glaunsinger and Ganem 2004;

Gaglia et al. 2012; Gaucherand et al. 2019; Rodriguez et al. 2019).

A connection between accelerated mRNA decay and transcription was first shown during infection with the gammaherpesviruses MHV68 (Figure 2). Wild type MHV68 causes reduced Pol II recruitment to mammalian promoters. However, infection with a decay-deficient mutant virus does not, as measured by Pol II ChIP qPCR or 4SU pulse labeling at the *Gapdh* and *Rplp0* promoters. Similarly decreased Pol II promoter occupancy at representative loci was observed upon transfection of the gammaherpesvirus endonucleases muSOX and SOX or the alphaherpesvirus endonuclease vhs, indicating that widespread mRNA degradation in the absence of other infection signatures is sufficient to reduce Pol II promoter occupancy. Notably, depletion of Xrn1 or Dis3L2 from these cells restored Pol II occupancy, and the reduction could be reinstated by complementation with wild type but not catalytically dead Xrn1 (Abernathy et al. 2015). This suggests that degradation of the cleaved mRNA fragments (rather than the initial endonucleolytic cleavage) is critical for reducing Pol II occupancy. These data have recently been extended through Pol II ChIP-seq experiments, revealing widespread decreases in Pol II at nearly all host promoters in murine fibroblasts infected with wild type but not RNA decay-deficient MHV68 (Hartenian et al. 2019). It is notable that infection with the alphaherpesvirus HSV-1 also dramatically reduces Pol II occupancy and nascent mRNA production (Rutkowski et al. 2015; Abrisch et al. 2016; Dremel and DeLuca 2019), although as yet there is no evidence linking this to mRNA degradation.

Although gammaherpesviruses are double-stranded DNA viruses that are transcribed by mammalian Pol II, in the environment of widespread mRNA decay, viral transcription remains robust and the Pol II ChIP signal on the viral genome is not significantly reduced compared to that of an RNA decay-deficient virus (Abernathy et al. 2015; Hartenian et al. 2019). However, viral promoters become susceptible to decay-induced transcriptional repression when integrated into the host genome, suggesting that either the structure of the replicating viral DNA and/or its location within viral replication compartments protects them from this cellular pathway (Hartenian et al. 2019).

The viral data suggest that mammalian cells do not attempt to buffer against widespread mRNA decay (Figure 3). Instead, the loss of Pol II from promoters may represent an antiviral response to a pathogen-associated signature, akin to other “patterns of pathogenesis” (Vance et al. 2009) like disruption of the actin cytoskeleton and damage to the plasma membrane,

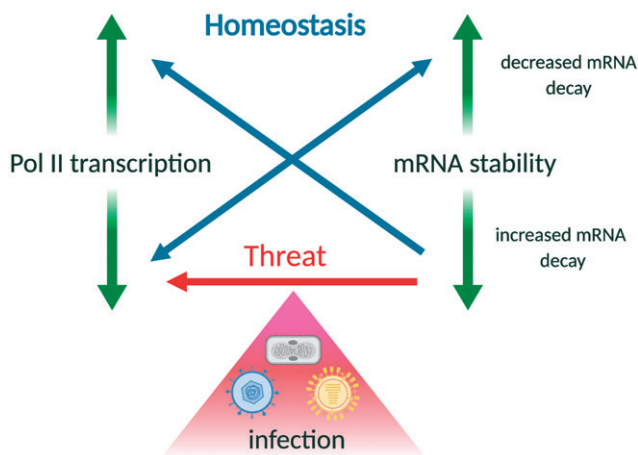


Figure 3. mRNA stability and Pol II transcription are linked processes, but may be differentially controlled during homeostasis or upon cellular threats. To maintain homeostatic mRNA levels and buffer against global mRNA abundance changes, eukaryotic cells modulate Pol II transcription or mRNA stability in a compensatory manner. However, during infection with mRNA decay-inducing viruses, the loss of mRNA is exacerbated by reduced Pol II transcription, perhaps as a component of a stress or antiviral response.

which trigger innate immune responses (Legrand-Poels et al. 2007; Lamkanfi and Dixit 2009). An observation from apoptotic cells may be in line with this hypothesis: overexpression of the apoptotic RNA decay factor Pnpt1 increases apoptosis. While the link between RNA decay and induction of apoptosis is unclear, cell death is a conserved pathway manipulated by pathogen infection (Jorgensen et al. 2017). Future work exploring the downstream consequences of dramatically accelerated mRNA decay in other contexts, including RNase L activation or noninfectious stresses will help define the generalizable nature of this transcriptional response to globally accelerated RNA decay.

mRNA decay-dependent transcription silencing during oocyte-to-embryo transition

The maternal to zygotic transition (MZT) is a well-recognized time of maternal mRNA decay followed by zygotic transcriptional activation, when ~35% of maternal mRNAs are destabilized by a series of direct targeting mechanisms (Tadros and Lipshitz 2009). Although there is no evidence that decay elicits transcription during the MZT, there is a connection between mRNA decay and transcription during the oocyte to embryo transition, a stage of development prior to the MZT (Dumdie et al. 2018). The mechanism involves degradation of mRNAs encoding transcriptional regulators by the essential protein Zfp36L2, which is part of a protein family associated with ARE-dependent mRNA decay.

Single cell RNA sequencing of a conditional Zfp36L2 knockout revealed that its targets are enriched for chromatin regulation and transcription-associated processes, including histone demethylases. In the absence of Zfp36L2, the oocyte does not undergo transcriptional repression, highlighting the potential role of RNA decay of specific transcripts in regulating transcription (Dumdie et al. 2018).

Role of RNA binding proteins in connecting mRNA decay to transcription

A central question is which factors are involved in sensing mRNA levels and effecting the corresponding changes in transcription and mRNA stability? This remains unclear in both the global and the specific instances of transcriptional or mRNA decay modulation. Multiple reports linking mRNA abundance and transcription implicate RNA binding proteins (RBPs) – particularly RNA decay enzymes like Xrn1, Dis3L2 and Ccr4-Not – as key messengers for conveying information on mRNA abundance between the nucleus and the cytoplasm. RBPs are natural candidates for this role given their ability to shuttle in an RNA-concentration dependent manner (Piñol-Roma and Dreyfuss 1992; Krecic and Swanson 1999; Gilbertson et al. 2018; Timmers and Tora 2018). Furthermore, there is precedent for RBPs having distinct functions in the cytoplasm versus the nucleus. Examples include hnRNPs, whose nuclear binding influences transcriptional activation and splicing while their binding to mRNAs and RBPs in the cytoplasm influences translation, stability and localization (Shyu and Wilkinson 2000). The heterodimeric Pol II subunits Rpb4/7 are another example, as they also play a role in determining cytoplasmic mRNA fate (discussed below) (Haimovich, Choder, et al. 2013; Lotan et al. 2005, 2007; Harel-Sharvit et al. 2010; Shalem et al. 2011; Duek et al. 2018).

Decay factors play a central role in activating mRNA decay-dependent signaling

The majority of proposed models converge on the importance of Xrn1 for signaling to the nucleus to convey information on mRNA decay or abundance, although hypotheses differ as to the specific role it plays in this feedback (Figure 4). The Choder lab found that wild type but not catalytically dead Xrn1 shuttles into the nucleus in an mRNA decay-dependent manner and directly binds chromatin. CHIP-exo analysis further showed that additional decay factors, including Dcp2 and Lsm1, also bind chromatin and elicit a transcriptional response. In the presence of an Xrn1 catalytic

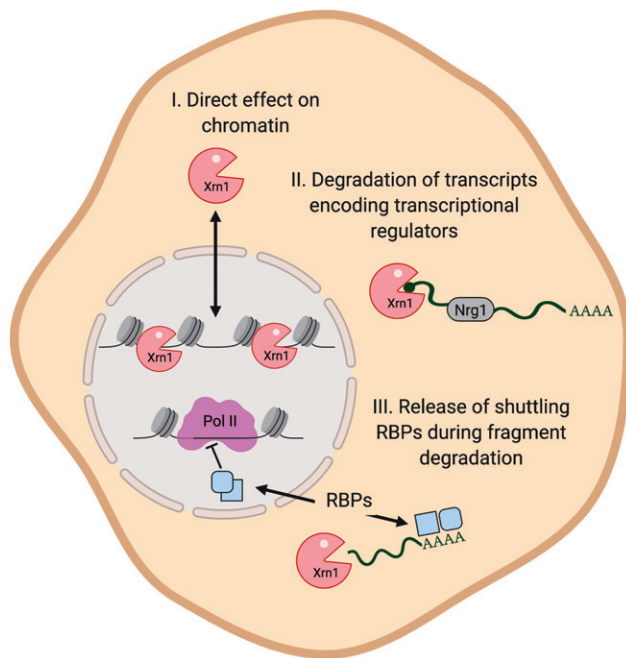


Figure 4. Multiple models have been proposed to explain how Xrn1 connects mRNA stability with transcription. Xrn1 may act directly by shuttling into the nucleus to bind chromatin and reduce transcription. It may alternatively (or additionally) indirectly impact transcription by degrading transcripts encoding transcriptional regulators such as Nrg1, or by causing release of RNA binding proteins (RBPs) during accelerated mRNA decay, which traffic into the nucleus and result in reduced transcription.

mutant, they observed that Pol II elongation is reduced (Haimovich, Choder et al. 2013; Haimovich, Medina et al. 2013).

Several recent reports further implicate Xrn1 in transcription elongation at yeast genes. In a $\Delta xrn1$ strain less elongating Pol II was found at GAL loci by transcriptional run on (Begley et al. 2019) and genome wide by Native Elongating Transcript (NET) sequencing (Fischer et al. 2019). Furthermore, the $\Delta xrn1$ strain showed increased recruitment of the Pol II release factor TFIIIS to the body of the GAL genes (Begley et al. 2019). TFIIIS is important for resolving backtracked Pol II, as it stimulates the intrinsic endonucleolytic activity of Pol II on mRNA (Cheung and Cramer 2011). This raises the possibility that under conditions of reduced mRNA decay, Pol II backtracking and nascent chain release are stimulated.

The Cramer lab did not detect a ChIP signal for Xrn1 on transcriptionally active loci, and proposed an alternate model in which the activity of Xrn1 on specific transcripts, such as the transcriptional repressor Nrg1, leads to broad changes in mRNA synthesis (Sun et al. 2013). There is an additional kinetic component to this model, in which a delay in the mRNA decay-induced

transcriptional signaling reflects the time it takes for altered Nrg1 mRNA levels to influence Nrg1 protein abundance. Xrn1 auto-regulation is also a factor, as high Xrn1 protein levels lead to increased decay of its own transcript (presumably dependent on decapping), ultimately lowering Xrn1 protein levels and thus influencing mRNA decay (Sun et al. 2012, 2013). Presumably, Xrn1 decay of its own transcript would be dependent on decapping. How this connection is coordinated is not yet clear, although it has been established that interactions occur between Xrn1 and Pat1 and Xrn1 and Dcp1 (Nissan et al. 2010; Braun et al. 2012). Measurements of the specific transcription, translation and decay rates of Xrn1 and Nrg1 would further bolster these arguments.

Xrn1 is also required for cytoplasmic to nuclear communication in mammalian cells undergoing viral nuclease-driven transcriptional repression (Abernathy et al. 2015). This is notable because in cells expressing a viral endonuclease, mRNA will be cleaved and translationally inactivated prior to exonuclease-mediated degradation of the cleaved fragments. In addition to Xrn1, analysis of a subset of individual mammalian loci suggested that the 3'-5' decay factor Dis3L2 and the Ccr4/Pan2 deadenylases were also involved in the cytoplasmic decay to transcriptional repression signaling (Abernathy et al. 2015). These data suggest that degradation of the endonuclease-cleaved fragments by multiple mRNA decay factors contributes to decay-transcription signaling.

“mRNA coordinators” help direct mRNA fate

Connections between transcription rates and cytoplasmic mRNA decay have also been linked to the heterodimeric Pol II subunits Rpb4 and Rpb7, which traffic from the nucleus to the cytoplasm and have been termed “mRNA coordinators” (Haimovich, Choder, et al. 2013; Lotan et al. 2005; Lotan et al. 2007; Harel-Sharvit et al. 2010; Shalem et al. 2011; Duek et al. 2018) (Figure 5). Rpb7 interacts with mRNAs as they leave the exit tunnel of the polymerase during *in vitro* transcription (Újvári and Luse 2005), and Rpb4 binds the primarily cytoplasmic RNA decay proteins Pat1 and Lsm2 (Lotan et al. 2005). Both Rpb4 and Rpb7 can localize to P-bodies (Lotan et al. 2005, 2007) and loss of Rpb4 is associated with aberrant decay of a subset of mRNAs involved in protein synthesis (Lotan et al. 2005). Given the association of Rpb4 and Rpb7 with multiple stages of the mRNA lifecycle, they have been proposed to act as a platform for regulation, imprinting mRNAs with transcriptional information that can be read out during decay. Although experiments in which covalently linking Rpb4 to a core subunit of Pol II (and thus

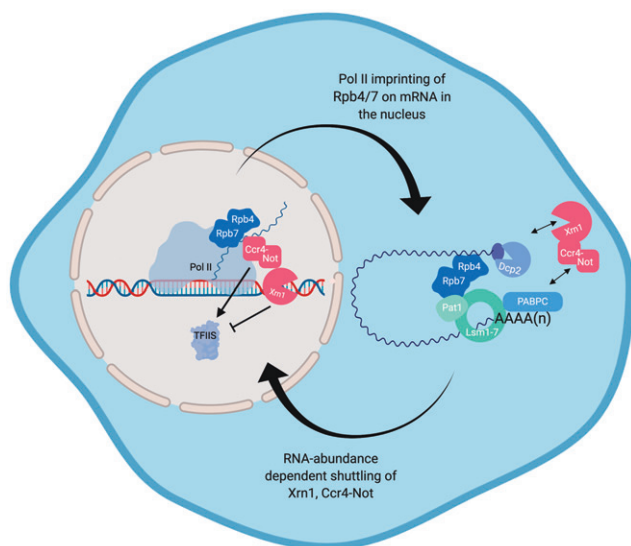


Figure 5. mRNA coordinators Rpb4/7 are implicated in imprinting mRNAs in the nucleus to convey transcription rate to the cytoplasm. Rpb4/7 have been shown to interact with the nascent mRNA chain in the nucleus and Pat1 and members of the Lsm1-7 complex in the cytoplasm. RNA decay factors Ccr4-Not and Xrn1 degrade mRNA in the cytoplasm and may affect Pol II elongation by either coordinating with (Ccr4-Not) or antagonizing (Xrn1) the Pol II cofactor TFIIIS, which is responsible for disengaging stalled Pol II.

preventing its trafficking) did not change basal levels of RNA decay (Schulz et al. 2014), it was subsequently shown that a portion of the covalently tethered Rpb4 could still be found in the cytoplasm, potentially retaining the capability to imprint mRNAs (Villanyi et al. 2014; Villanyi and Collart 2015; Duek et al. 2018). Furthermore, a Pol II mutant unable to recruit the Rpb4/7 subunits (*rpb6*^{Q100R}) does not stabilize mRNA to buffer against reduced transcription (Shalem et al. 2011), consistent with the hypothesis that Rpb4/7 is required for nuclear-cytoplasmic communication during buffering.

The Ccr4-Not complex may also be involved in “mRNA coordination”, as these factors play multiple roles in transcription and RNA decay (Villanyi and Collart 2015). The Ccr4-Not complex contains nine subunits in yeast comprising the proteins with deadenylase activity (Ccr4 and Caf1), the Not1 scaffold and additional regulatory subunits. It is these additional subunits that are proposed to coordinate RNA decay and transcription (Villanyi and Collart 2015). The Ccr4-Not complex interacts with TBP, TFIIIS, SAGA, Pol II, and the nascent transcript (Collart 2003; Kruk et al. 2011). Its interaction with TFIIIS is proposed to stimulate nascent chain cleavage, causing Pol II backtracking and reengagement of Pol II on stalled transcripts *in vitro* and *in vivo* (Kruk et al. 2011; Gupta et al. 2016; Begley et al.

2019). Furthermore, a Δ *ccr4* strain showed a global reduction in TFIIIS/Pol II DNA occupancy, which is consistent with Ccr4-Not participation in TFIIIS recruitment (Babbarwal et al. 2014). It is worth noting that this same study saw a global increase in TFIIIS/Pol II occupancy in a Δ *xrn1* strain (Begley et al. 2019), implying a different role for Xrn1 and Ccr4-Not in influencing Pol II occupancy. Future mechanistic studies will be key to understand how these decay factors interface with the transcriptional machinery to integrate decay rates with mRNA synthesis rates.

Additional data provide intriguing links between the Ccr4-Not complex, Xrn1 and the putative mRNA coordinators Rpb4/7. Rescue of arrested Pol II by Ccr4-Not from a yeast reconstitution system requires Rpb4/7 (Babbarwal et al. 2014). It is therefore possible that Rpb4/7 participates in cotranscriptionally recruiting mRNA decay factors such as Ccr4-Not to the nascent transcript. Supporting this model is the observation that Ccr4-Not binding to Pol II is significantly weakened in the absence of Rpb4/7 (Babbarwal et al. 2014). Notably, Ccr4-Not was also shown to bind the C-terminal unstructured domain of Xrn1 in human and drosophila cells (Chang et al. 2019). Thus, one possibility is that through this interaction, both Ccr4-Not and Xrn1 may be recruited to elongating Pol II, thereby connecting transcription and decay. Yet, how the localization of these proteins and their roles in transcription versus mRNA decay are coordinated remains largely enigmatic, particularly in response to stimuli that lead to buffering.

Cytoplasmic poly(A) binding protein accumulates in the nucleus during accelerated mRNA decay

A recent report identified proteins that accumulate in the nucleus during accelerated mRNA decay using a tandem-mass tag (TMT) mass spectrometry approach. mRNA decay was induced with the gammaherpesviral ribonuclease muSOX in the presence or absence of Xrn1 to identify proteins that may shuttle in an mRNA-concentration dependent manner (Gilbertson et al. 2018). The primary class of proteins that accumulated in the nucleus in an Xrn1-dependent manner are associated with RNA 3' UTRs and poly(A) tails, including Pabpc1, Pabpc4 and Larp4.

Pabpc1 and 4 had previously been identified as indicators of widespread RNA decay and shown to translocate to the nucleus upon viral nuclease-induced mRNA degradation (Piron et al. 1998; Glaunsinger and Ganem 2004; Harb et al. 2008; Kumar and Glaunsinger 2010; Salaun et al. 2010; Kumar et al. 2011; Park et al. 2014). Cytoplasmic to nuclear trafficking of Pabpc is

dependent on its RNA-recognition motifs (RRMs). These RRM motifs contain noncanonical nuclear localization signals (NLS) (Kumar et al. 2011) which are exposed upon release of Pabpc from mRNA. As a result, Pabpc shuttles into the nucleus in an mRNA-concentration dependent manner (Kumar et al. 2011). In cells depleted of Pabpc1 and Pabpc4, Pol II occupancy was no longer reduced in muSOX expressing cells relative to control cells, and over-expression of Pabpc1 in the nucleus was sufficient to decrease Pol II occupancy in the absence of viral nuclease expression (Gilbertson et al. 2018). These data support an RNA-concentration dependent shuttling model and suggest that nuclear accumulation of Pabpc can influence transcription, though the mechanism(s) underlying this phenotype remain unclear.

One can imagine various possible scenarios by which RBPs such as Pabpc might “switch” functions from directing cytoplasmic mRNA fate to altering nuclear processes. As mRNA concentrations change, the proportion of mRNA-bound versus unbound RBPs may change commensurately. The unbound RBP population would then be available to participate in regulating another cellular process, like transcription, by altering the chromatin state either through directly binding to promoters or complexing with other proteins that would perform remodeling. A recent report identified many RBPs with DNA-associated regulatory roles, providing precedence for such a hypothesis (Xiao et al. 2019). Alternatively, RBPs could bind another factor(s) involved in transcription, titrating that factor away from the DNA and thereby regulating transcription in a more indirect fashion or, like Ccr4-Not, RBPs could coordinate directly with the transcription complex. Finally, they might compete with resident nuclear RBPs for binding nascent mRNA, thereby disrupting the mRNA processing environment.

Conclusions and future perspectives

In summary, there is consensus across various systems that mRNA decay and transcription are linked processes, such that disruption of one can result in compensatory or antagonistic responses by the other. This is manifest in cells in several ways, including a feedback loop that buffers against changes to overall mRNA levels, NITC that compensates for the loss of specific transcripts, and decreased Pol II promoter occupancy in response to virally accelerated mRNA decay. However, many questions remain in this relatively young field. It is unclear if global increases and decreases in mRNA half-lives are communicated to the nucleus via the same pathway, either during buffering or potential

pathogenic threat responses. Similarly, by which mechanism(s) are increases and decreases in transcription sensed and does the cell respond to both by buffering its mRNA populations? Additional perturbations to each system, namely destabilizing mRNA in yeast and altering transcriptional output in mammalian cells, will be important to determine the extent of convergence of the mammalian and yeast models. Further identification and mechanistic understanding of the factors involved in nuclear to cytoplasmic communication will be key to unraveling the connection between these spatially disparate processes and will provide additional insights into system-to-system commonalities.

Coordination of mRNA abundance appears to be conserved across eukaryotes, highlighting the importance of such communication and suggesting an evolutionary advantage to coupling transcription and decay. It also raises the question of whether mRNA buffering is a continuously active process or if it is specifically initiated upon perturbation. The buffering model has been extrapolated to have benefits in maintaining cellular homeostasis (Haimovich, Medina, et al. 2013; Sun et al. 2013; Timmers and Tora 2018), although there is no evidence indicating the cost to a cell unable to buffer mRNA levels. In the viral context, responding to infection by restricting Pol II recruitment to promoters may be a cellular defense strategy to spare the organism at the cost of an individual cell, akin to antiviral translational shutdown pathways activated by protein kinase R. This hypothesis could be explored by examining whether innate immune or cell-death signaling is initiated downstream of accelerated mRNA decay. While we are still at the early stages of understanding signaling between mRNA decay and transcription, the conservation of this connection across species and contexts indicates that it is an important aspect of gene regulation.

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