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Escaping nuclear decay: The significance of mRNA export for gene expression

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ABSTRACT

Control of protein-coding gene expression in eukaryotes employs the regulated production of mRNA, its intra-cellular transfer to cytoplasmic ribosomes and final transcript degradation. Despite a rich body of literature on these events, an involvement of nuclear RNA decay systems remains largely unexplored. Instead, nuclear RNA degradation is often considered a quality control precaution engaged primarily in ridding cells of aberrantly processed transcripts and spurious non-coding RNA. Recent research from human and budding yeast cells now demonstrates that even protein-coding transcripts fall prey to nuclear decay and that this is countered by their nuclear export. Here, we highlight these results, addressing the interplay of mRNA maturation with its nuclear export kinetics and decay.

Nuclear polyA-binding proteins are moderators of nuclear mRNA stability

A rich body of evidence illustrates that the proper processing of nascent transcripts is vital not only for their downstream function, but also for the mere survival of mRNA within cells. For example, in budding yeast the failure to add a polyA tail to the 3' end of the nascent transcript results in its immediate degradation (Burkard and Butler, 2000). Consequently, a minimum length of 3' end polyadenosines is required for mRNA stability and export (Dower et al, 2004). More recently, the protective function of this RNA modification was shown to be mediated by the yeast polyA-binding protein Nab2, which coats the pA tail and physically blocks 3'-5' exonucleolytic decay by the nuclear RNA exosome complex (Schmid et al., 2015). Given the universal binding of Nab2 to mRNA (Tuck and Tollervey, 2013; Batisse et al., 2009; Baejen et al., 2014), the protein is expected to act as a global regulator of gene-expression. That is, changes in Nab2 protein concentration and/or its subcellular localization are expected to globally impact nuclear mRNA decay rates. Interestingly, mutation or depletion of Nab2 also cause accumulation of unspliced pre-mRNAs (Schmid et al., 2015; Soucek et al., 2016), suggesting that the protein may also play a role in the removal of nuclear-retained transcripts. Human cells encode a sequence homolog of Nab2, called ZC3H14, but its role in nuclear polyA tail biology and RNA decay remains unclear. In contrast, the main human nuclear polyA-binding protein, PABPN1, which lacks a budding yeast homolog, appears to carry out two - seemingly opposing - roles. On the one hand, PABPN1 stimulates the polyadenylation efficiency of RNA polyA polymerase (PAP) and protects the nascent polyA tail (Kühn and Wahle, 2004). On the other hand, PABPN1 also interacts with RNA exosome co-factors to facilitate the nuclear decay of multiple non-coding RNAs (Meola et al., 2016; Bresson et al., 2015). Thus, polyA-binding proteins in both yeast and human cells are important determinants of nuclear mRNA stability that can act in protective as well as destructive manners.

A role of mRNA export kinetics in tuning gene expression

Nuclear retained RNA is generally turned over, which is evidenced by the highly unstable nature of pervasive transcripts in both yeast and human nuclei (Wyers et al., 2005; Preker et al., 2008). In contrast, long-lived functional non-coding RNAs, such as snRNAs, snoRNAs and highly abundant long non-coding RNAs such as NEAT1 and MALAT, persist in the nucleus for longer periods of time, presumably owing to their specific tight packaging, secondary structures and/or usage of specialized protective features to prevent decay (Schmid and Jensen, 2018). Protein-coding RNAs and short-lived pervasive transcripts, on the other hand, share some characteristics, yet, only the former generally escape nuclear decay. What explains this difference? Apparently, the 5' cap and the 3' polyA tail can only yield protection for a limited time, or in well-organized combination with other features of the RNA. We therefore

speculate that timing is a key factor such that the generally rapid export, and consequential evacuation of mRNA from the degradative environment of the nucleus, secures their stability. In contrast those non-coding RNAs, which lack export competence, remain nuclear long enough to fall prey to decay. Consistent with this idea, blocking of yeast mRNA export was long known to trigger the decay of highly expressed heat-shock mRNA (Libri et al., 2002; Assenholt et al., 2008). Our recent results generalize this notion by demonstrating that newly made protein-coding transcripts are abundantly degraded in response to an inhibition to nuclear export, triggered by depletion of main mRNA export adaptors or perturbations of the nuclear pore structure (Tudek et al., 2018). Interestingly, the severity of the decay phenotype was proportional to the amount of nuclear retained polyA RNA and it was therefore suggested to be due to the sequestering of Nab2 onto nuclear trapped RNAs, preventing its protection of nascent polyA tails. Such titration may not be specific for Nab2, but likely also involves other RNA binding proteins, which may explain the diverse RNA biogenesis phenotypes observed in RNA export mutants (Tudek et al., 2018; Tran et al., 2007).

Recent studies in human cells point to a similar competition between nuclear export and decay, with a dedicated role of the nuclear exosome co-factor ZFC3H1 (Meola and Jensen, 2017). This large Zn-finger protein connects to PABPN1 to facilitate the exosomal removal of polyadenylated transcripts (Meola et al., 2016). Interestingly, ZFC3H1 also actively prevents the export of polyA RNAs marked for decay (Silla et al., 2018; Ogami et al., 2017). Other exosome co-factors may in a similar manner compete with export factors for RNA binding (Fan et al., 2018). While such direct competition between proteins involved in export vs. decay has not been directly described in yeast, spliceosome-associated proteins appear to promote export and decay of spliced and unspliced RNA, respectively (Hackmann et al., 2014). It is therefore interesting to note, that Nab2 co-operates with the Mlp1/2 subunits of the nuclear basket to retain unspliced RNA (Soucek et al., 2016; Galy et al., 2004).

A regulatory role for nuclear export in unperturbed cells?

In normal conditions, most mRNAs probably associate with protective proteins and undergo swift nuclear export. However, given the vast number of proteins, that can interact with RNA and the numerous inter-connections between RNA processing, packaging and nuclear export factors, it is likely that mRNA export is the target for regulation, and that this may impact transcript half-lives. Consistently, in yeast a few specific nuclear-retained mRNAs are targeted to exosome-mediated decay in a process depending on the nuclear cap-binding protein Cbc1 (Kuai and Sherman, 2005). Similarly, in human cells RNAs exhibit different export kinetics, which was proposed to limit transcriptional noise from gene activation bursts (Bahar Halpern et al., 2015). Finally, nuclear decay was shown to specifically target poorly expressed transcripts during mouse embryonic stem cell differentiation, contributing to their regulation (Lloret-Llinares et al., 2018). As expression of several RNA export factors vary during development and tumorigenesis (Domínguez-Sánchez et al., 2011; Li et al., 2016), it is plausible that regulation of export, with the inevitable impact on nuclear decay, is widespread during health and disease processes. Unfolding such regulation will be an exciting research direction for the future.

RNA decay is critical for nuclear homeostasis

Why do cells conduct such rigorous decay of nuclear RNA? Co-transcriptional RNP formation relies on RNA:protein interactions of the nascent RNA and we speculate that maintaining a balanced concentration of nuclear transcripts is key to preserve productive interactions. That is, any unsolicited accumulation of RNA has the deleterious potential to titrate limiting RNP factors and disturb nascent RNP formation. In support of this idea, numerous studies have highlighted that increasing nuclear RNA levels via impairment of decay or disruption of export leads to misregulation of transcription termination, RNA splicing and the accumulation of DNA:RNA R-loops, inducing genomic instability (Hammel et al., 2002; Jimeno et al., 2002; Fox et al., 2015; Gavalda et al., 2013; Tudek et al., 2018). These diverse phenotypes may all result from sequestration of RNA binding subunits of termination and splicing complexes (Tudek et al., 2018; Tran et al., 2007), underscoring the importance of keeping nuclear RNAs in check by a 'hyper-active' nuclear decay environment.

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