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Formation of export-competent mRNP: escaping nuclear destruction

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In eukaryotic cells, primary transcripts are processed and bound by proteins before export to the cytoplasm. Nuclear production of export-competent messenger ribonucleoprotein particles (mRNPs) is a complicated process, and mRNP biogenic events that function sub-optimally are rapidly attacked by surveillance leading to degradation of the mRNA. Export of nuclear mRNAs is therefore constantly challenged by the opposing force of mRNA retention and decay. This balance ensures that only 'perfect' transcripts persist, and that non-functional and potentially deleterious transcripts are removed early in their biogenesis. Thus, eukaryotic systems of mRNP quality control can be viewed as simple Darwinian principles operating at the molecular level.

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Introduction

Transcripts generated by RNA polymerase II (RNAPII) are processed in several steps during maturation into functional messenger ribonucleoprotein particles (mRNPs). In eukaryotic cells, processing occurs in the nucleoplasm before export and in most cases even before mRNP release from the gene. Capping of the 5′-end, splicing of intronic sequences and 3′-end formation all contribute to the generation of transcripts that are packaged with proteins important for mRNP structure, nuclear export and cytoplasmic function [1]. mRNA processing and packaging events are connected to each other and to transcription. This coupling serves to increase the efficiency and specificity of mRNP formation [2]. Coupling of mRNP maturation to transcription occurs through at least three different kinds of contacts: first, interaction of processing factors with the C-terminal domain (CTD) of the large subunit of RNAPII; second, interaction of mRNP packaging factors with the elongating transcription complex; and third, interplay (at an ill-defined level) of mRNP maturation factors with chromatin. The interconnection between transcription, processing and packaging organizes mRNP maturation so that difficulties or delays in assembly events can easily be detected and offending transcripts degraded [3]. The best characterized mRNA surveillance system is nonsense-mediated decay (NMD), through which transcripts harboring a premature translational termination codon are rapidly degraded (see review by Conti and Izaurralde in this issue). However, recently other surveillance mechanisms have been shown to operate at earlier steps of mRNP biogenesis [1]. These processes, in contrast to most known cases of NMD, utilize nuclear mRNA turnover factors, and are often coupled to transcription.

In this review, we will give an overview of nuclear mRNA/mRNP transactions that are either known to be subject to quality control or potentially could be. Most of our inspiration originates from studies using the yeast *Saccharomyces cerevisiae*, and consequently this organism will form the basis of discussion.

mRNA turnover in the nucleus: the players

Bulk mRNA turnover takes place in the cytoplasm, where degradation pathways have been intensively studied. Recently, enzymes involved in nuclear mRNA decay have been identified that have activities comparable to cytoplasmic RNA decay enzymes and are capable of degrading the mRNA from its 5′- and 3′-ends. Which pathway predominates appears to depend on features of the substrate mRNA (e.g. whether it is intron-containing or not, and how far it has progressed in its biogenesis) [4–7].

Nuclear mRNA decay in the 3′–5′ direction involves the nuclear exosome, a complex containing several 3′–5′ exoribonucleases [8]. A core exosome is found in both the cytoplasm and nucleus; the nuclear exosome in yeast is distinguished by the associated 3′–5′ exoribonuclease Rrp6p as well as by Lrp1p/Rrp47p and the putative RNA helicase Mtr4p/Dob1p [9–11]. In addition to mRNA decay, the nuclear exosome is involved in processing of small nuclear and nucleolar RNAs (snRNAs and snoRNAs), ribosomal RNAs (rRNAs) and pre-rRNA spacer fragments.

Nuclear mRNA decay in the 5′–3′ direction is carried out by Rat1p, which has a cytoplasmic cousin in Xrn1p. Rat1p and Xrn1p can in fact functionally substitute for one another if artificially targeted to the compartment where

the other normally resides. However, only Rat1p is essential [12].

For exonucleases to gain access to the body of the mRNA, the 5′ cap and 3′ poly(A)-tail must first be removed. Yeast decappers in the nucleus are not well described; however, a fraction of both of the human decapping enzymes Dcp1p and Dcp2p are nuclear and functional homologues exist in yeast (see review by Fillman and Lykke-Andersen in this issue). Nuclear decapping is also stimulated by the Lsm2–8p complex [5]. In yeast, the major cytoplasmic deadenylase activity resides with the Ccr4p and Pop2p nucleases, complexed with several accessory factors [11]. Although genetic and biochemical data suggest that Ccr4p and Pop2p also have nuclear roles, nuclear deadenylase activity of these factors has not yet been demonstrated [13]. The Pan2p/Pan3p complex can also catalyze deadenylation, and one proposed nuclear function of Pan2p/Pan3p is the Pab1p-dependent trimming of the newly added poly(A) tail down to its species-specific size of 55–75 adenosines [14]. Interestingly, the deadenylase activity involved in nuclear mRNA decay appears to be processive, in contrast to the distributive nature of cytoplasmic deadenylation [5]. This suggests that although cytoplasmic and nuclear mRNA turnover may share common factors, the composition of the deadenylation complexes might differ in the two compartments. Additionally, nuclear endonucleolytic activities may provide entry sites for exonucleases. One such endoribonuclease is Rnt1p, which specifically cleaves double-stranded (structured) RNAs [15]. However, mRNAs targeted for endonucleolytic attack are probably few. The currently known nuclear mRNA degradation factors are listed in Table 1.

Transcription, mRNA processing and mRNA surveillance interfaces

Early transcription elongation and capping

Capping of the 5′-end occurs when the nascent transcript is ~30 nucleotides long. Efficient capping requires promoter-proximal pausing of RNAPII, and recruitment of capping enzymes is stimulated by the phosphorylation of Ser5-residues in the CTD heptad repeats characteristic of the early phase of transcription [16]. In turn, execution of capping triggers re-activation of RNAPII. This provides a possible capping checkpoint ensuring that only capped transcripts are further elongated. In the context of this review, it is of interest that the transcription elongation factor Spt5p is present at active yeast loci in these early phases of transcription [17]. *Drosophila* Spt5p physically interacts with the *Drosophila* nuclear exosome, and both are recruited with similar timing to transcriptionally active heat shock loci on polytene chromosomes [18]. The Spt5p/exosome relationship provides a possible link between early transcription and mRNA surveillance in that the presence of exosome activity early during transcriptional elongation could serve to degrade transcripts with a free 3′-end that arise from defective transition of RNAPII into the elongation phase. Such 'co-transcriptional degradation' could also deal with truncated transcripts arising more generally from abortive transcription events (Figure 1, stage 2).

What happens if transcription elongation proceeds without capping? Intriguingly, recent studies in yeast suggest that transcription termination occurs by the 'torpedo' model, in which Rat1p, after transcript cleavage, attacks the unprotected 5′-end and catches up with the transcribing RNAPII to dissociate it from the DNA template [19]. As Rat1p and its stimulatory factor Rai1p can both be detected at the 5′-end of coding regions, a similar mechanism might serve to eliminate transcripts with an exposed 5′-end arising from unsuccessful capping events (Figure 1, stage 1) [19]. Perhaps this contributes to the decrease in mRNA stability upon mutation of the Ceg1p capping enzyme [20].

Table 1

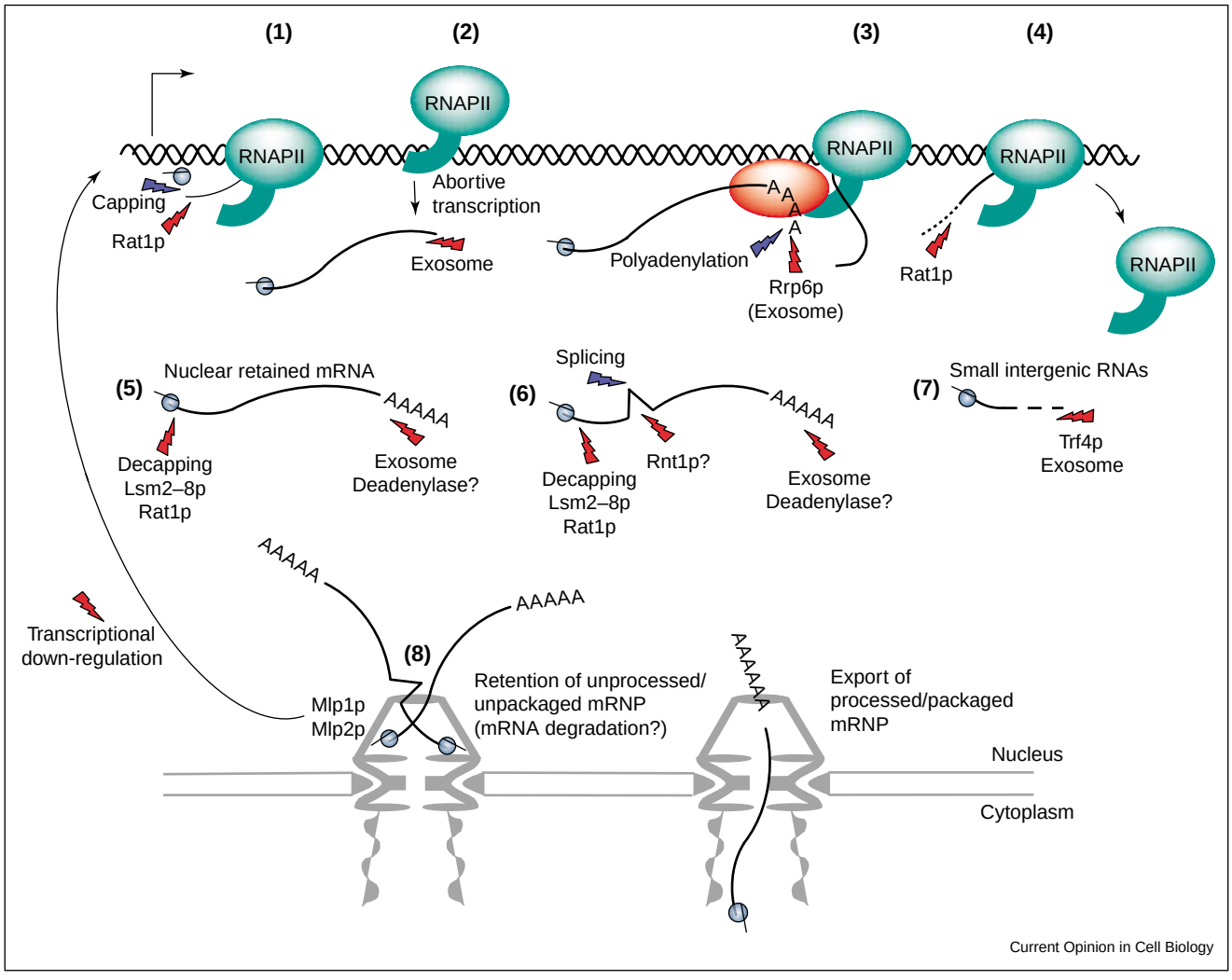
Nuclear degradation activities in yeast.

Enzyme	Activity
Rrp6p	3′–5′ exoribonuclease
Rrp47p/Lrp1p	Exosome cofactor
Core exosome	3′–5′ exoribonucleases
Mtr4p/Dob1p	Putative RNA helicase
Rat1p	5′–3′ exoribonuclease
Dcp1p	Decapping enzyme
Dcp2p	Decapping enzyme
Lsm2-8p	Stimulates nuclear decapping
Ccr4p/Pop2p	poly(A)-specific exoribonuclease
Pan2p/Pan3p	poly(A)-specific exoribonuclease
Rnt1p	Endoribonuclease

Splicing

Results from the Tollervey laboratory have demonstrated that levels of several pre-mRNAs in the yeast *prp2-1* late splicing mutant are stabilized up to 50-fold upon inactivation of nuclear exosome components and, to a lesser extent, upon inactivation of Rat1p [6]. Thus, extrapolating from these substrates, nuclear decay of pre-mRNA primarily occurs in the 3′–5′ direction, whereas 5′–3′ decay occurs to a minor extent. Increased pre-mRNA levels, in nuclear decay mutant backgrounds, are often accompanied by increases in mRNA levels, which suggests direct competition between splicing and nuclear degradation (Figure 1, stage 6). However, levels of reporter transcripts harboring 5′-splice-site or branchpoint mutations are not detectably affected by disruption of the nuclear decay machinery [6]. This most likely reflects short nuclear dwell times of these RNAs. Indeed, experiments initiated in the Rosbash laboratory 15 years ago showed that nuclear retention of pre-mRNAs appears to require the early stages of splicing commitment to occur — mutation of either the 5′ splice site or branchpoint

Figure 1



Nuclear surveillance and degradation of mRNA/mRNP. Inefficient mRNA/mRNP reactions are readily attacked by degradative activities at various points in mRNP biogenesis. (1) Unsuccessful capping potentially allows co-transcriptional Rat1p-mediated degradation. (2) Abortive RNAPII transcription provides a possible entry site for 3′–5′ exosomal decay. (3) Inefficiently polyadenylated mRNAs are subject to Rrp6p-mediated degradation. (4) Presumably as a general means of transcriptional termination in yeast, RNAPII is chased down by Rat1p-mediated degradation of the downstream cleavage product of nascent RNA. (5–6) mRNAs and pre-mRNAs are unstable if restricted to the nuclear compartment. 3′-5′ degradation is mediated by the nuclear exosome, possibly initiated by a hitherto unidentified deadenylase activity. Degradation from the 5′-end involves an unknown decapping activity stimulated by the Lsm2–8p complex, followed by exonucleolytic decay by Rat1p. For some substrates the endoribonuclease Rnt1p may open the transcript for exonucleolytic decay. (7) Small RNAs transcribed from intergenic regions are degraded by the nuclear exosome triggered by Trf4p. (8) Improperly packaged, as well as unspliced, mRNAs are retained by Mlp proteins at the nuclear periphery. This negatively impacts transcription. See text for details. Blue or red lightning represents a productive or destructive impact on a given process, respectively. The red ellipse represents the mRNA 3′-end processing complex.

resulted in leakage of pre-mRNA into the cytoplasm [21,22].

As pre-mRNA splicing is not an obligate RNAPII co-transcriptional process, the exact localization of nuclear pre-mRNA decay remains elusive [23,24]. However, a recent effort from the Jacquier and Nehrbass laboratories has identified the perinuclear protein Mlp1p as a factor involved in pre-mRNA retention [25•]. In contrast to other mutants leading to nuclear pre-mRNA accumulation, deletion of Mlp1p, or its partner Mlp2p, has no direct effect on pre-mRNA splicing [25•]. Co-localization of over-expressed Mlp1p and an intron-containing reporter RNA suggests that pre-mRNA degradation could occur at Mlp1p/Mlp2p gates at the nuclear rim (Figure 1, stage 8). In support of this, a physical interaction between Mlp1p and Rrp6p has been reported [26]. However, recent data from the Stutz laboratory indicate that Mlp1p/Mpl2p might operate through transcriptional repression (Figure 1, stage 8; see also below) [27•].

Poly(A)-tail formation and release from the locus: a major quality control check point?

The 3′-end of nearly all eukaryotic mRNAs is generated by endonucleolytic cleavage followed by addition of a terminal poly(A)-tail [28]. Addition of the poly(A)-tail by poly(A) polymerases is a biphasic reaction with distinct kinetic properties *in vitro* [29]. During the first, distributive phase, 9–10 adenosine monophosphate (AMP) residues are gradually added, whereas the second phase is characterized by rapid and processive poly(A)-tail extension. During its synthesis, the poly(A)-tail is loaded with factor(s) that regulate tail length and protect it from degradation [14,30]. In yeast, two prominent poly(A)-binding candidates *in vivo* are Pab1p and Nab2p. Interestingly, the poly(A)-tail is also necessary for promoting mRNA release from its site of transcription. In yeast mutants affected in 3′-end processing, heat shock RNAs (hs-RNAs) are retained in close proximity to their site of synthesis [4,31–33]. Furthermore, a plasmid-produced mRNA containing a self-cleaving hammerhead ribozyme element in place of cleavage and polyadenylation signals is similarly inhibited in its progression away from the gene [34•]. If, however, the DNA template is modified to encode a stretch of 48 or more adenosines immediately upstream of the ribozyme element, the RNA is efficiently released. These data argue that, at least when the RNA is produced out of its normal chromatin context, the mere presence of a sufficiently long poly(A) tail is enough to enable mRNP release. The tail-length requirement likely reflects the need for a sufficient number of poly(A)-binding factors to decorate the tail, and interestingly mutation or deletion of Nab2p and Pab1p both result in nuclear mRNA retention [35,36].

Transcription-site retention of hs-RNAs produced in polyadenylation-malfunctioning mutants, as well as strains deleted for Pab1p, is dependent on the nuclear exosome component Rrp6p [4,32,35]. The molecular mechanism underlying Rrp6p-dependent retention is not well-defined. However, part of the answer may be that this is yet another example of the struggle between productive mRNP maturation events and mRNP surveillance. We favor a suggestion, first put forward by the Butler laboratory, that Rrp6p challenges Pap1p during polyadenylation, creating a competition between Pap1p-mediated polymerization and Rrp6p-mediated degradation (Figure 1, stage 3) [37]. In this view, transcription-site retention of mRNA in 3′-end formation mutants in the presence of Rrp6p is the result of antagonized poly(A)-tail synthesis, and mRNA release upon Rrp6p removal occurs as a result of unchallenged residual polyadenylation activity in these mutants. This model fits nicely with data showing that read-through mRNAs produced in yeast pre-mRNA cleavage and polyadenylation mutants are retained and degraded in the presence of Rrp6p, or polyadenylated and released in its absence [4,38].

Another class of factors important for transcript progression away from the gene is the THO complex. This complex was initially implicated in transcription elongation, but has more recently been shown to function at the interface between transcription and mRNP formation by recruiting the mRNA export factor and TREX (transcription/export) complex member Sub2p to nascent RNA [39,40]. In strains in which a THO component is deleted or Sub2p is mutated, *HSP104* transcripts exhibit two interesting phenotypes: a *HSP104* RNA-FISH signal can be detected at or near its site of transcription, and a pool of transcripts appear to be 3′-end truncated [4,33]. Both phenotypes are reversed upon deletion of Rrp6p, suggesting that 3′-end truncation results from incomplete degradation [4]. The localization of 3′-end truncated *HSP104* species in a THO deletion strain is unclear; however, complete — or nearly complete — transcripts are retained, as determined by an RNA-FISH signal from a probe targeted immediately upstream of the *HSP104* RNA stop codon [4]. Interestingly, the genetic interactions of a strain deleted for the THO complex member Mft1p cluster with mutant alleles of factors of the 3′-end formation machinery (C Saguez, JR Olesen and TH Jensen, unpublished). Perhaps retention and degradation of transcripts in THO/Sub2p mutants is also a consequence of the competition between Pap1p and Rrp6p.

The ability of Rrp6p and of the nuclear exosome to target inefficiently polyadenylated species recalls recent results on the nuclear degradation of stable structured RNAs by the Trf4p poly(A) polymerase system. Trf4p, and its paralog Trf5p, are related to DNA polymerase β and belong to the nucleotidyl transferase superfamily, which also includes canonical poly(A) polymerases [41]. In a recent study, the Anderson laboratory showed that aberrant (hypomethylated) $tRNA^{met}$, caused by mutation of a tRNA methyltransferase, is unstable as a result of polyadenylation by Trf4p and subsequent degradation by Rrp6p and the exosome [42••]. It was noted that this degradation pathway is reminiscent of the bacterial system, where oligo-adenylation of structured RNAs provides a tag for 3′–5′ exonucleolytic decay [43]. It is now clear that degradation of other eukaryotic RNAs, such as snRNAs, snoRNAs, 5SrRNA and small RNAs transcribed from hitherto un-annotated genomic regions, also proceeds through a polyadenylation-dependent exosome pathway (A Jacquier, D Libri and B Seraphin, personal communication; [44,45]). The evidence that Trf4p-mediated polyadenylation destabilizes RNAs is opposed to the more traditional notion of the poly(A)-tail as an mRNA stability element. However, it was previously suggested that such a 'bacterial-like' degradation mechanism could account for Rrp6p-mediated quality control at eukaryotic transcription sites [46]. Intriguingly, oligo(A)-addition by Trf4p shows some similarities with adenosine polymerisation by Pap1p during its distributive phase (A Jacquier, D Libri, and B Seraphin, personal

communication). A fascinating possibility is therefore that Rrp6p might be able to gain access to inefficiently polyadenylated substrates during this phase of polyadenylation. Perhaps by entering the processive phase with subsequent addition of poly(A)-binding proteins, an mRNP escapes degradation.

Physiological roles of nuclear mRNA surveillance systems

The question remains whether nuclear mRNA surveillance has a physiological function besides ridding the cell of aberrant transcripts. The NMD machinery, for example, functions not only to degrade aberrant mRNAs, but also to regulate levels of several physiological messages [47,48]. Two recent examples suggest that the nuclear exosome might also be involved in the organization of normal gene expression.

The first example concerns regulation of *NAB2* RNA levels, which are increased three- to five-fold upon disruption of the nuclear exosome [49]. Strikingly, this effect requires a sequence of 26 consecutive adenosines in the *NAB2* 3′-UTR that, perhaps by mimicking a Trf4p-produced substrate, sensitizes the transcript to exosome degradation. Inactivation of Nab2p also leads to increased *NAB2* RNA levels, suggesting that Nab2p itself might be involved in this regulation of its expression. This provides the first indication that mRNA export and mRNA degradation might intersect at the regulatory level, and that Nab2p may have dual functions in facilitating mRNA export in one situation and initiating degradation of the message in another.

The second example concerns the aforementioned RNAs transcribed from intergenic regions of the yeast genome. In wild-type cells, these RNAs are rapidly removed by the Trf4p/exosome pathway, whereas in *Δrrp6* cells they are remarkably stable (A Jacquier, D Libri, B Seraphin, M Ares and M Rosbash, personal communication). On the basis of these findings, one might speculate that a major surveillance role for the nuclear exosome under physiological conditions is to degrade these RNAs. Whether such an activity in preserving transcriptional fidelity relates to the recent proposal that Rrp6p and Lrp1p/Rrp47p might be involved in RNA removal under DNA- and RNA-damaging conditions remains to be seen [50].

Given the many roles of the nuclear exosome, what else could be in store for us? Whole-genome yeast microarray experiments demonstrate that very few conventional mRNAs are affected upon *RRP6* deletion, and instead the fraction of polyadenylated stable RNAs and RNAs derived from intergenic regions increases substantially (A Jacquier, D Libri, B Seraphin, M Ares and M Rosbash personal communication; [50]). The *NAB2* regulatory loop may therefore be an exception rather than a general finding. These results also pose an experimental challenge; as fascinating as the myriad nuclear functions of Rrp6p are, they complicate our ability to delineate the direct consequences of its disruption.

Nuclear mRNA surveillance and feedback on transcription

Some of the co-transcriptionally recruited mRNA export factors are also directly, or indirectly, involved in transcription. This gives the mRNP potential to modulate transcription rate. Possible entities involved are the THO/TREX and Sac3p/Thp1p complexes, both of which contain components implicated in transcription and mRNA export, and whose mutation results in defects in both processes [4,51–53]. For some genes, the molecular network extends from transcription factors to components of the nuclear membrane, and sometimes this connection can be paralleled by relocation of the gene from the nuclear interior to the nuclear periphery upon transcriptional induction [4,51–54].

Functional evidence of a connection between the nuclear periphery and mRNP transcription comes from the Stutz laboratory, which reported that levels of intronless mRNAs produced in a strain temperature-sensitive for the mRNP assembly factor Yra1p increase upon Mlp1p and/or Mlp2p deletion [27•]. Interestingly, the level of Mlp2p-mediated decrease of a nucleus-retained mRNA correlated with the degree of transcriptional inhibition of the corresponding gene. The authors therefore suggest that a negative feedback loop exists in response to an mRNP assembly defect [27•]. The potential benefit deriving from such regulation was recently illustrated by the finding that slowing down transcription in yeast mutants where mRNP maturation is crippled partially restores mRNA quality [55]. Interestingly, loss of Mlp1p/Mlp2p also restores growth of strains mutated for mRNP factors Yra1p and Nab2p. This suggests that Mlp-proteins might act as 'mRNP sensors' for these important mRNP constituents at the nuclear periphery (Figure 1, stage 8). Whether this activity of the Mlp barrier is also important for recognizing pre-mRNAs remains an open question. A full understanding of the fascinating layer of quality control at the nuclear periphery and its relationship to transcription awaits further clarification.

Conclusions

A huge proportion of mRNA is turned over in the nuclear compartment, and although most of this is in the form of spliced-out introns, a fair share of exonic sequence is presumably lost to quality control. As exemplified in this review, we propose that most, if not all, nuclear quality control is based on kinetic competition between opposing processes. In this way, the intimate connection between productive and destructive mRNA transactions may serve not only to eliminate unwanted molecules but also to generally optimize gene expression.

At least in the context of the relatively simple and compact genome of *S. cerevisiae*, it seems the strategy is to compromise somewhat on the precision of mRNP synthesis and to compensate for this by investing in efficient quality control. Characterization of mRNA surveillance pathways in higher eukaryotes will reveal whether this is also a strategy chosen by cells with a more complex genomic organization.

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