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Review

RNA quality control in the nucleus: The Angels' share of RNA☆

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ABSTRACT

Biological processes are not exempt from errors and RNA production is not an exception to this rule. Errors can arise stochastically or be genetically fixed and systematically appear in the biochemical or cellular phenotype. In any case, quality control mechanisms are essential to minimize the potentially toxic effects of faulty RNA production or processing. Although many RNA molecules express their functional potential in the cytoplasm, as messengers, adaptors or operators of gene expression pathways, a large share of quality control occurs in the nucleus. This is likely because the early timing of occurrence and the subcellular partition make the control more efficient, at least as long as the defects can be detected ahead of the cytoplasmic phase of the RNA life cycle. One crucial point in discussing RNA quality control resides in its definition. A stringent take would imply the existence of specific mechanisms to recognize the error and the consequent repair or elimination of the faulty molecule. One example in the RNA field could be the recognition of a premature stop codon by the nonsense-mediated decay pathway, discussed elsewhere in this issue. A more relaxed view posits that the thermodynamic or kinetic aftermath of a mistake (e.g. a blockage or a delay in processing) by itself constitutes the recognition event, which triggers downstream quality control. Because whether inappropriate molecules are specifically recognized remains unclear in many cases, we will adopt the more relaxed definition of RNA quality control. RNA repair remains episodic and the degradative elimination of crippled molecules appears to be the rule. Therefore we will briefly describe the actors of RNA degradation in the nucleus. Detailed analyses of the mechanism of action of these enzymes can be found in several excellent and recent reviews, including in this issue. Finally, we will restrict our analysis to the yeast model, which is used in the majority of RNA quality control studies, but examples exist in the literature indicating that many of the principles of RNA quality control described in yeast also apply to other eukaryotes. This article is part of a Special Issue entitled: RNA Decay mechanisms.

1. The actors of RNA quality control in the nucleus

1.1. The nuclear exosome

The nuclear exosome is a major actor of RNA degradation and processing in the nucleus. Since this complex is described in more detail in another review of this special issue (Chlebowski et al., this issue), we will only briefly describe its general features. The exosome is involved in multiple pathways of RNA quality control, both in the nucleus (discussed below) and the cytoplasm. In the nucleus it is also involved in the maturation of ribosomal RNA and sno/snRNAs. The exosome is composed of a catalytically inert, six-subunit core in the shape of a doughnut. Three proteins, Rrp4p, Csl4p and Mtr3p, form a cap that associates with one end of the doughnut, while one of the two active catalytic subunits, Rrp44/Dis3p associates with the opposite end. The core (ten-subunits) exosome is composed by the six subunits composing the doughnut, the cap and Dis3p. The core exosome associates with specific subunits that define a cytoplasmic and a nuclear form. The latter is characterized by the association with the second catalytic subunit, Rrp6p. Dis3p and Rrp6p are both 3′–5′ exonucleases, but exhibit different catalytic properties. Dis3p is processive while Rrp6p is distributive. The two exonucleases have generally overlapping targets but several specificities have also been described [see Ref. 1 and references therein]. In addition to the exonucleolytic activity Dis3p also contains an endonucleolytic (PIN) domain that significantly contributes to its activity [1–4].

1.2. The TRAMP complex: an important exosome cofactor

The Trf–Air–Mtr4 polyadenylation complex (or TRAMP) is composed of a poly(A) polymerase (Trf4p or Trf5p), a zinc knuckle RNA binding protein (Air1p or Air2p) and the DExH-box RNA helicase Mtr4p [5–7]. Two different forms of the complex can be found *in vivo*: TRAMP4 (Trf4–Air2–Mtr4) and TRAMP5 (Trf5–Air1–Mtr4). TRAMP4 is the predominant form, likely due to the higher expression of Trf4p relative to Trf5p [8], and the two forms exhibit only partially redundant functions in the polyadenylation of specific substrates [9–11].

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Trf4p and Trf5p belong to the superfamily of non-canonical Pol ß RNA polymerases [12]. They contain a conserved catalytic core and N-terminal and C-terminal extensions with little predicted secondary structure [13]. Unlike other poly(A) polymerases, such as Pap1p, Trf4p and Trf5p do not possess an RNA binding domain and cannot polyadenylate their substrates in the absence of the associated Air1p or Air2p [7], which provide the RNA binding ability [14,15]. Polyadenylation by Trf4–Air2 is distributive *in vitro*, as opposed to the processive activity of Pap1p [6].

Mtr4p is a superfamily II helicase that belongs to the DExH-box family of RNA helicases. It was first described as an exosome cofactor that plays a prominent role in the degradation/processing of several RNA substrates, such as the 5.8S rRNA [16]. The closest homologue of Mtr4p is Ski2p (38% identity), which functions as a cofactor for the cytoplasmic form of the exosome [17].

The enzymatic activities of the TRAMP complex appear to be strongly coordinated. Polyadenylation by Trf4p–Air2p is modulated by the RNA helicase Mtr4p that limits the length of the poly(A) tails [18,19]. Conversely, the 3′–5′ duplex unwinding activity of Mtr4p [20,21] is significantly enhanced by the presence of Trf4p–Air2p [22]. This stimulation is independent from Trf4p polyadenylation activity since it also occurs in the presence of a catalytically dead Trf4p mutant (*Trf4-DADA*) [6]. Mtr4p requires a minimal 4–5 nt 3′ ssRNA overhang for duplex unwinding, and prefers oligo(A) extensions over other sequences [22,23]. Thus, addition of short poly(A) tails to structured RNAs, such as tRNAs, allows Mtr4p to interact and unwind its RNA substrate.

The TRAMP complex contributes to the stimulation of the exosome in many different ways. On one hand, the addition of short poly (A) tails may allow Mtr4p to unfold the RNA molecule *via* its RNA helicase activity, and, on the other hand, may provide an unstructured extension that can enter the exosome core channel to reach the catalytic site of Dis3p for processive degradation [24]. Indeed, the exosome displays very poor exonuclease activity *in vitro* on highly structured substrates, such as tRNAs, and efficient degradation requires both the presence of Mtr4p and Trf4p-dependent polyadenylation [6].

In addition to the stimulation of the exosome activity by polyadenylation of the RNA substrate, it has been shown that Trf4p can enhance Rrp6p-mediated degradation in a polyadenylation-independent manner *in vitro* [25]. However, the precise mechanism and the domains of Trf4p involved in Rrp6p stimulation are still to be elucidated.

TRAMP4 is the predominant exosome co-factor for nuclear quality control while TRAMP5 function appears to be restricted to rRNA processing [10].

1.3. The NNS complex and its involvement in transcription termination and RNA degradation

The Nrd1–Nab3–Sen1 (NNS) complex (Fig. 1) plays important roles in nuclear RNA quality control, both in the degradation of malformed RNAs and in transcription termination (discussed below). The NNS-complex is composed of the RNA-binding proteins Nrd1p and Nab3p and the Superfamily I helicase Sen1p. Nrd1p and Nab3p interact with specific RNA motifs (GUAA/G and UCUUG, respectively) that are often embedded within long AU-rich regions in the nascent RNA [19,26,27]. Sen1p is an essential and highly conserved DNA/RNA helicase that interacts with the nascent RNA in a sequence-independent manner [26, Porrua and Libri, submitted].

The NNS-complex copurifies with the TRAMP-exosome, but the precise physical contacts mediating these interactions have not yet been identified. It has been shown that recombinant Nrd1p can stimulate nuclear exosome-mediated degradation of RNA, but only in the presence of the NNS RNA binding sites, which suggests a role for the NNS-complex in recruitment of the nuclear exosome [28]. Therefore, the NNS complex is believed to work as an adaptor for the nuclear exosome to target specific classes of transcripts, including RNAs derived from pervasive transcription (see below).

An important role of the NNS complex is to terminate RNAPII transcription. Because of its connections with the exosome and TRAMP, transcription terminated by the NNS complex is linked to targeting of transcripts to the degradation pathway. The action of the exosome can generate functional RNAs, when exonucleolytic trimming is halted by protective ribonucleoprotein structures as observed for sno/snRNAs. However, in the vast majority of cases the function of the NNS complex is devoted to the elimination of non-functional RNAs and the control of pervasive transcription as discussed below.

2. RNA quality control actors at work: why, where and how

2.1. RNA quality control on transcription: from pervasive to hidden RNAs

The phenomenon of pervasive transcription was unveiled by transcriptome analyses in strains defective for nuclear RNA degradation pathways [for recent reviews see Refs. 29 and 30]. These studies have revealed widespread production of RNAs that are not associated with canonical transcriptional units, such as protein-coding or non-coding RNA genes. The ubiquitous presence of transcribing polymerases implies that the upstream chromatin control on transcription initiation is leaky. Also, the bidirectional nature of most promoters [31,32], at least in yeast, increases the potential for spurious initiation. Transcription through promoter regions in intergenes is generally disruptive, as it interferes with the normal function of the promoter, even though in some cases this provides an opportunity for regulation [33–35]. Therefore, downstream control mechanisms are required to limit the extent of spurious transcription and the consequent production of RNAs that might be toxic if translated or associated competitively with proteins or other nucleic acids. The cell adopts two strategies to limit pervasive transcription and its consequences: early termination of transcription and RNA degradation, which are frequently associated. RNAs produced by non-canonical transcription events are often rapidly degraded and present at very low steady-state levels in a wild type strain, hence the notion of hidden transcription. The largest share of these unstable non-coding RNAs (ncRNAs) is constituted by a class of RNAPII transcripts dubbed Cryptic Unstable Transcripts (CUTs) [7]. CUTs are short (200–500 nt) capped transcripts that are originated from both cryptic and bona fide promoters located in nucleosome free regions.

Transcription termination of CUTs is operated by the NNS pathway, which explains why these RNAs are unstable. Indeed, unlike sn/snoRNAs, CUTs do not contain a protective ribonucleoprotein core that halts exonucleolytic degradation by the exosome. CUTs generally contain sequences that are recognized by Nrd1p and Nab3p, which trigger transcription termination, presumably by recruiting Sen1p to provoke dissociation of the elongation complex (Fig. 1, Porrua and Libri, submitted). After termination, the released RNAs are polyadenylated by Trf4p and rapidly degraded by the exosome.

At least two elements ensure the specificity of this quality control mechanism that recognizes transcripts to be discarded, as opposed to RNAs that have to be retained for function. The first is the presence of the NNS binding sites in the transcript. The second is the position of these sites relative to the start site of transcription. Indeed, it has been shown that these sites are ignored by the NNS complex when located more than 900 bp from the transcriptional start site, and rather used by the mRNA termination pathway to generate stable RNAs [36]. One way to measure the distance from the transcription start site is to monitor the phosphorylation status of the C-terminal domain (CTD) of the largest RNAPII subunit. The CTD of RNAP II consists of tandem repeats of a hepta-peptide (YSPTSPS) that is subjected to different post-translational modifications throughout the transcription cycle. The CTD, in function of its modification status, serves as a landing pad for many proteins involved in key processes such as capping,

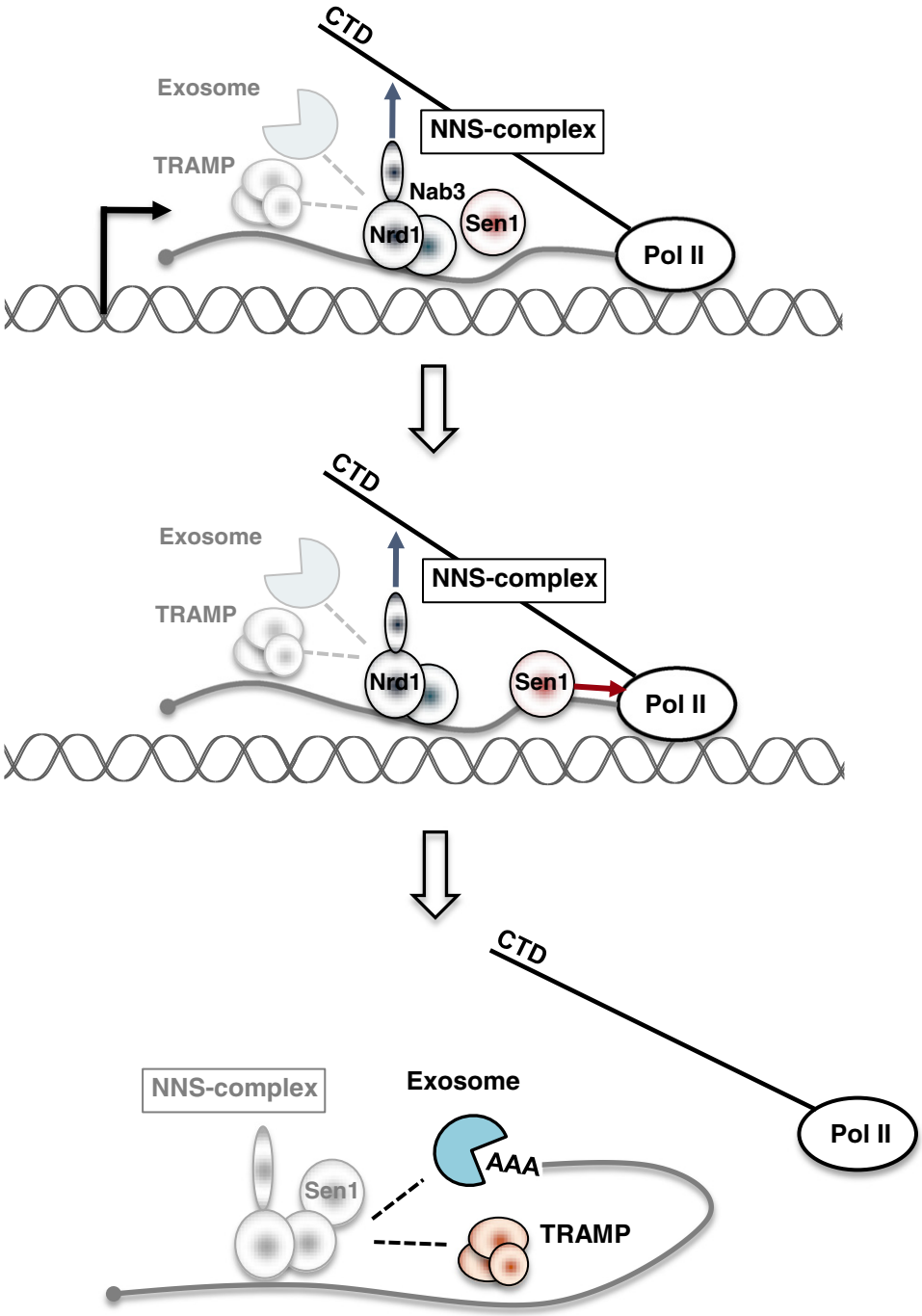

Fig. 1. The possible mechanism of transcription termination by the Nrd1–Nab3–Sen1 (NNS) complex. In the first step (top), Nrd1p and Nab3p bind to the RNA and recruit Sen1. At this and/or a later stage, Nrd1p might also interact with the carboxy terminal domain (CTD) of Rpb1p, the largest subunit of RNA Pol II (indicated by an arrow). The interaction of the NNS complex with the exosome and the TRAMP is indicated by dashed lines. The exosome and the TRAMP are dampened because they are not known to play a significant role at this stage. In the second step (middle panel) Sen1p directly contacts the nascent RNA and induces termination of transcription (red arrow). After termination (bottom), the RNA is released and degraded by the combined action of the TRAMP complex and the exosome.

elongation, termination, and splicing [37]. The serine at position 5 of each repeat (S5) is preferentially phosphorylated at early phases of transcription, while serine 2 (S2) is generally phosphorylated later. The read out of the CTD phosphorylation status is operated by Nrd1p, which recognizes the S5 phosphorylated forms via its CTD-interacting domain (CID) [38–40].

Quality control of pervasive transcription is a typical example of a mechanism that specifically recognizes defective molecules (i.e. RNAs with degradation signals produced by spurious transcription events) and activates downstream protective actions (i.e. transcription termination and degradation).

2.2. RNA quality control and mRNA nuclear retention

Once the RNA polymerase transcribes 3′-end processing signals at the end of a transcription unit coding for an mRNA, the nascent RNA is recognized by the cleavage and polyadenylation factor (CPF) and the cleavage factors (CF) Ia and Ib. The transcript is cleaved by factors in

the CPF/CF and polyadenylated by the associated poly(A) polymerase Pap1p [41]. Cleavage is associated with transcription termination, which also depends on the CPF–CF complex, by a mechanism that remains poorly understood. The release of the transcript does not appear to be a passive consequence of cleavage. In fact, several mutant conditions slow down or prevent release of the transcript, although cleavage (and possibly polyadenylation) is seemingly normal. The molecular mechanism underlying transcription site (TS) retention is largely mysterious. TS retention of transcripts occurs in cells that are mutated for components of the polyadenylation–termination complex or for factors that associate with the RNA early during transcription and are thought to mediate the transition from transcription to export (e.g. Yra1p, Sub2p, Mex67p, the THO complex) [42–50]. Strikingly, however, TS retention was also observed in cells expressing mutant versions of factors acting downstream in the mRNA export pathway, such as nuclear pore components and the Dbp5p helicase that mediates release of the RNA at the cytoplasmic face of the nuclear pore [42]. To explain these observations, it is necessary to postulate the existence of some form of feedback from the nuclear pore to the site of transcription or a physical connection at some point in the RNA export process between the site of transcription and the nuclear pore that persists in these mutants. Such a connection has indeed been revealed by the study of THO complex and Sub2p mutant strains [43]. Impairment of the THO complex, composed in yeast of Mft1p, Tho2, Hpr1, Thp2 and Tex1p, has been associated with several cellular phenotypes, ranging from genomic stability to mRNP biogenesis and export [44–53]. However, the molecular function of the complex remains rather mysterious. Interestingly it has been shown that an mRNP export intermediate accumulates in THO complex mutants that depends on the persistent association of the site of transcription with nuclear pore components and polyadenylation factors [43]. Thus, maintained proximity of the RNA to the site of transcription in THO complex mutants might be related to the extended contacts of the TS with the nuclear pore complex (NPC). The "malformed" RNA might then remain proximal to the TS, even after release from the transcription complex, i.e. in the absence of RNA polymerase.

Another striking and largely unexplained feature of transcription site retention is that it fully depends on the integrity of the nuclear exosome component Rrp6p. Deletion of the *RRP6* gene in THO or *sub2* mutants, for example, abolishes TS retention [47,50,54,55]. Deletion of *RRP6* also prevents formation of the DCF complex and the NPC-TS persistent association [43]. It has been suggested that partial degradation of the 3′-end of the newly transcribed RNA in THO complex mutants elicits transcript retention [51], but this option remains rather speculative and difficult to demonstrate.

A general consequence of RNA quality control for THO complex and *sub2* mutants is the nuclear degradation of the transcript shortly after transcription, although the penetrance of this phenotype is rather variable, depending on the mutant under scrutiny. The nuclear exosome is responsible for degradation, because transcript abundance is generally restored in degradation defective double mutants (e.g. *sub2-rrp6*Δ). The TRAMP complex is also involved in this facet of nuclear control, but the poly(A) polymerase activity of Trf4p is not required [50].

Interestingly, Nrd1p and Nab3p have been shown to crosslink to over one thousand mRNAs *in vivo* [19] although transcription termination of mRNA coding genes does not depend on the NNS complex. Thus binding to these transcripts might suggest a role for Nrd1p–Nab3p in targeting mRNAs that are not properly assembled into functional mRNPs for degradation by the exosome.

Another actor in the nuclear quality control of mRNA is the cap binding complex (CBC). The CBC is composed of two proteins, Cbc1p and Cbc2p and recognizes directly the cap structure of mRNAs. The CBC is involved in splicing and export. Surprisingly, it was shown that deletion of the *CBC1* gene in yeast suppresses the growth phenotype associated with mutation of Rat7p, a nuclear pore component. Several mRNAs produced in the *rat7-1* context (or another nuclear pore mutant, *nup116*Δ) have decreased stability, which is partially restored by deletion of *CBC1* [56,57]. Thus, Cbc1p participates in the degradation of these RNAs in export mutants, and possibly in the degradation of a few specific transcripts in a wild type strain [58], in an Rrp6p-dependent manner. The mechanistic basis of the implication of Cbc1p in this RNA quality control pathway remains however poorly understood.

Quality control by transcription site retention and degradation in THO complex and other mRNP export mutants is a typical case in which the existence of a specific recognition system that detects an error has not been demonstrated. For instance, it is not completely clear whether mRNPs produced in THO complex or Sub2p mutant strains are significantly different (and therefore recognizable) from those produced in wild-type cells. Conceivably, events at the transcription-export interface are slow in these mutants and it is this kinetic failure that brings about the quality control action of competing discard and retention pathways (see Fig. 2 for a similar case).

2.3. Quality control and mRNA processing

The number of genes containing at least one intron in yeast is low (approximately 3%) but since intron containing genes are highly expressed, roughly one in every three mRNAs derives from a spliced precursor [59,60]. Splicing is a complex process that requires the co-transcriptional assembly of a large ribonucleoprotein particle, the spliceosome, within which intron excision takes place. Because of the complex and multistep nature of splicing, the overall efficiency of the process is a function of the efficiencies of each individual step. Delays or mistakes that stochastically arise at each step are expected to accumulate and conceivably generate a substantial fraction of defective molecules. These are substrates for nuclear and cytoplasmic quality control. Indeed, the first evidence for nuclear quality control of RNA came from studies on splicing in yeast. Mutation of Prp2p prevents splicing but allows spliceosome assembly. However, in *prp2* mutant cells, the expected decrease in the steady-state level of spliced mRNAs is not accompanied by an increase in the steady state levels of pre-mRNA, unless the exosome (or to some extent the nuclear 5′–3′ exonuclease Rat1p) is defective [61]. These early experiments suggested the occurrence of nuclear degradation, which prevents the accumulation and translation of potentially toxic pre-mRNAs. Interestingly, in exosome mutants the overall levels of some spliced mRNAs are also increased relative to wild type cells, indicating that at least a fraction of the stabilized pre-mRNAs can be converted to mature products, albeit with low efficiency [61]. This result is important because it shows that the unspliced transcripts that are degraded are not dead end products and, therefore, that RNA degradation and splicing pathways compete for a common pool of molecules. One important facet of these findings is that competition between splicing and degradation also occurs in strains that do not exhibit specific RNA processing defects. Indeed, in these pioneer studies, it was shown that metabolic depletion of the exosome can increase the steady state levels of a few model pre-mRNAs and mRNAs even in the absence of splicing defects. Recently, genome-wide analyses of exosome targets have confirmed and extended these results, using several combinations of exosome catalytic mutants [1,62]. Strikingly, it was found that, even in splicing-proficient strains, the exosome degrades precursors derived from at least half of all intron-containing genes, with a strong preference for large introns (roughly 80% of pre-mRNAs are stabilized more than two fold). Dis3p is the main factor responsible for the degradation of intron-containing precursors, which involves both its endo- and exonucleolytic activities. Failure to degrade the pre-mRNA generally leads to increased mRNA levels, which generalizes the concept of competition between RNA processing and degradation [1].

These findings point to the notion that quality control occurs in wild type cells and might target errors occurring stochastically during

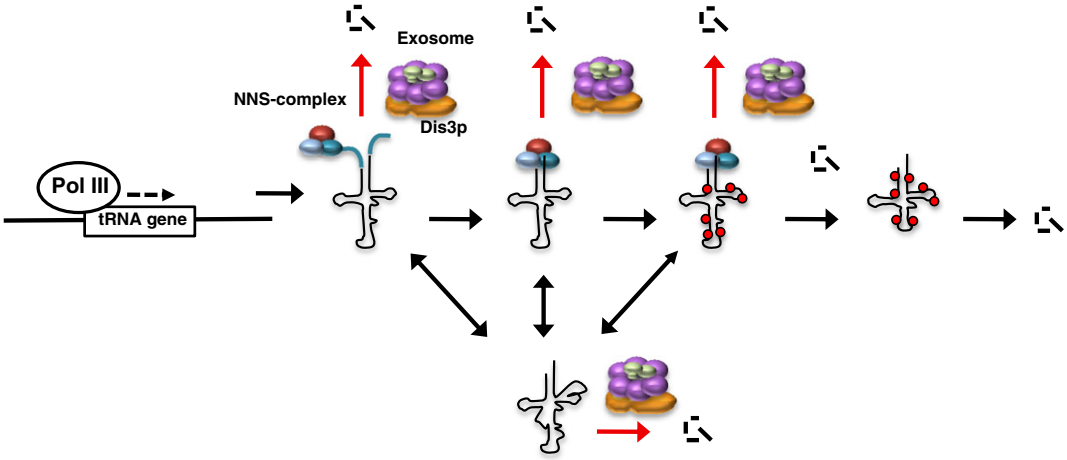

Fig. 2. Scheme illustrating a possible model for nuclear tRNA quality control. After transcription, pre-tRNAs rapidly associate with the NNS complex, which functions as an exosome adaptor. Stochastic errors that occur during processing (double headed arrows, bottom) could lead to the generation of misfolded or hypomodified tRNAs (tRNA modifications indicated by red dots) that are degraded by the exosome. When misfolded or hypomodified tRNAs can re-enter the normal processing pathway (double headed arrows), production of mature tRNAs compete with degradation. Top: competition between processing and degradation (red arrows) does not necessarily involve the production of defective molecules. In this view, degradation occurs by default unless processing occurs faster. Quality control by kinetic competition obviates the need for specific recognition of defective tRNAs, but implies that normal molecules are subject to destruction (referred to as the Angels' share in the text).

processing. However, since apparently normal molecules can be formed when degradation is impaired, this suggests either that defective pathways are reversible, allowing transcripts to enter the right processing pathway if not degraded, or that degradation also targets normal molecules.

Another player in the quality control of pre-mRNAs was recently identified in the poly(A) binding protein Nab2p in *Saccharomyces cerevisiae* [63] and similar findings were reported for the *Saccharomyces pombe* Pab2p [64]. Strains depleted of Nab2p displayed increased levels of intron containing transcripts, suggesting that Nab2p binds to the poly(A) tails of these RNAs and recruits Rrp6p for degradation. Pre-mRNAs derived from ribosomal protein genes are preferential Nab2p targets [63], presumably because they contain large introns, which are more sensitive to degradation [1]. However, no increase in the levels of mature mRNAs was detected upon depletion of Nab2p, suggesting that these pre-mRNAs might be dead-end intermediates that cannot re-enter the splicing pathway when degradation is impaired.

Quality control by competition also applies to defects in 3′-end processing and transcription termination. Deletion of the gene coding for the nuclear exosome component Rrp6p suppresses the growth defect associated with mutation of *PAP1*, encoding the mRNA poly(A) polymerase [65]. Since mRNAs are restored to normal levels in these strains, this suggests that these transcripts are degraded as a result of defective polyadenylation. Similarly, mutation of *RNA14* and *RNA15*, encoding CF1A components, leads to defective termination and consequent production of extended transcripts that are targeted for degradation by the nuclear exosome [47,66]. When degradation is impaired in exosome mutants, not only are extended RNAs stabilized, but also normally sized RNAs (i.e. cleaved and polyadenylated at the correct position) are partially restored. This observation either suggests that normal mRNAs can be generated from the extended precursors via an alternative 3′-end processing pattern [66] or that, more likely, preventing degradation allows more time for the crippled 3′-end processing pathway to function [47], underscoring the notion of competition between RNA processing and disposal.

In the case of both unspliced pre-mRNAs and extended read-through transcripts, the mechanism by which aberrant RNAs are recognized remains unclear. These aberrant transcripts are different from the functional, mature forms, and could be specifically recognized by an RNA quality control "alert" system. However, currently, the evidence for such a specific recognition mechanism is still missing.

2.4. Quality control of tRNAs in the nucleus

The making of a mature tRNA is a complex process that requires a high number of modifications, in addition to 5′- and 3′-end processing and, often, intron splicing. In the yeast *S. cerevisiae* every tRNA species is modified on average at 13 positions. Similarly to pre-mRNA splicing the complexity of the process is susceptible to generating mistakes that trigger quality control mechanisms. The first evidence of a nuclear quality control mechanism for tRNAs was reported by Anderson and colleagues [67], who showed that mutations in Trf4p and Dis3p can suppress the growth phenotype of mutants defective in the 1-methyladenosine 58 tRNA methyltransferase (m1A58 Mtase). Lack of this important modification leads to instability of the initiator $tRNA_i^{Met}$, which is degraded by the nuclear exosome. An increase in the levels of the hypomodified $tRNA_i^{Met}$ in *trf4Δ* or nuclear exosome mutants is the molecular event that underlies suppression. In degradation defective strains, polyadenylated forms of hypomodified $tRNA_i^{Met}$ were observed and they were dependent on Trf4p integrity and levels. It was shown that Trf4p can discriminate between native (fully modified) and unmodified tRNAs for polyadenylation, providing a clue on how degradation specificity is achieved [13].

Another tRNA quality control pathway, dubbed rapid tRNA degradation (RTD) that also targets hypomodified tRNAs, is dependent on the 5′–3′ exonucleases Rat1p (nuclear) and Xrn1p (cytoplasmic). It was shown that a critical element of target recognition is the stability of the tRNA structure, particularly the T-stem and the acceptor stems, which are affected by the missing modification [68,69].

A more recent genome-wide analysis [1] has revealed that even in cells that are fully proficient for tRNA processing and modification, a consistent fraction of the tRNAs that are produced get degraded by the exosome, using both Dis3p and Rrp6p activity. This phenomenon concerns the vast majority of tRNAs as transcripts derived from 80 to 90% of tRNA genes are stabilized at least two-fold (depending on the specific exosome mutation). Pulse chase experiments have shown that impairing exosome activity affects the levels of tRNA intermediates early after transcription, suggesting that pre-tRNA are the targets of exosome quality control in wild type cells. Strikingly, stabilization of intermediates leads to increased mature tRNA levels at later time points, suggesting that the pre-tRNAs targeted for degradation are functional, at least as far as processing is concerned.

Similarly to pre-mRNAs, the question arises as to why such a large fraction of transcribed tRNAs (roughly 50%) are degraded in the apparent absence of defects (Fig. 2). It is possible that a consistent

fraction of defective molecules is generated by stochastic errors occurring at each individual step in the processing-modification pathway. The probability of having at least one error in a given molecule would thus be expected to be relatively high, considering the number of steps. However, it is also possible that a consistent fraction of normal tRNAs are degraded (see below).

How does the cell recognize molecules that are to be degraded? It has been shown by *in vivo* crosslinking experiments that Nrd1p and Nab3p associate with tRNA intermediates, suggesting that these factors might specifically target exosome degradation targets [19]. However, whether association of Nab3p/Nrd1p reflects the existence of a processing or structural defect remains unclear as they may associate by default to all (healthy and unhealthy) molecules. Considering the presumably large variability in the degradation substrates, it is difficult to imagine that every tRNA molecule bearing a given defect is recognized specifically, either directly or *via* an adaptor (e.g. Nrd1p/Nab3p). However, it is possible that some defects (e.g. the lack of modifications) somehow translate into a common recognition tag for degradation. Indeed, it has been suggested that hypomodified tRNAs undergo structural alterations that somehow make them preferred substrates for degradation [68,69].

2.5. Quality control of rRNAs

Ribosomal RNAs (rRNAs) undergo several quality control steps in the cytoplasm that are generally linked to the occurrence of translation [see for instance Ref. 70 and references therein]. However, degradation of rRNA species has also been reported in the nucleus as a result of defective nuclear export. In an *sda1-2* mutant strain, where nuclear export of both 60S and 40S ribosomal particles is impaired, the levels of all rRNA intermediates leading to the production of mature 25S rRNA were strongly decreased. However, in the *sda1-2*, *rrp6* Δ and *sda1-2*, *trf4*Δ double mutant strains the levels of rRNA intermediates were restored [71], indicating that, as a consequence of the block in nuclear export, exosome and TRAMP-dependent quality control leads to rRNA degradation. As in the many cases described above, rRNA processing is not affected in the *sda1-2* mutant and decreasing the efficiency of degradation restores mature rRNA levels. The molecular mechanisms triggered as a consequence of the nuclear export block that induce rRNA degradation remain, however, unknown. Importantly, an increase in rRNA levels (all species) was not detected in strains that are deficient for exosome activity but not impaired in ribosome biogenesis [1], indicating that, unlike in the case of tRNAs, the exosome does not degrade a substantial fraction of rRNAs in a wild-type strain.

2.6. The "Angels' share" of RNA

The Angels' share is the fraction of alcoholic beverages that evaporates during storage in barrels and amounts to roughly 2% per year. Over 10 years the overall loss can be as much as 18%. The Angels' share of RNA appears to be much higher, probably as much as 50% for tRNAs (Fig. 2). This might not look economical, but the generation of functional and properly shaped molecules has an entropic cost that might be partially paid by the seeming waste of excess production. Indeed, "choosing" among many possible solutions (i.e. discarding the wrong ones) might be more economical than "spending" free energy to improve the accuracy of the production process to generate fewer but better molecules.

There are basically two possible sources for the Angels' share of RNA in normal (non-mutant) conditions: the first is the random occurrence of errors that generates malformed molecules either in their sequence, shape or associated factors. These molecules are probably generally discarded, but if degradation is impaired, they can often re-enter the normal pathway, implying a late competition between the two events (Fig. 3).

How defective molecules are recognized is generally poorly understood. Although specific recognition occurs in some cases (e.g. termination-degradation signals in CUTs or the recognition of some non-modified tRNAs), this is presumably not the rule. For instance, many possible alterations in the structure of tRNAs can be generated by missing modifications and, although some might create analogous perturbations, it is unlikely that all converge towards a common degradation-inducing state recognized by the exosome. We suggest that the energy-requiring discrimination events in many cases of quality control are operated by the maturation process itself. A defective molecule might be judged as such by its inaptitude to proceed

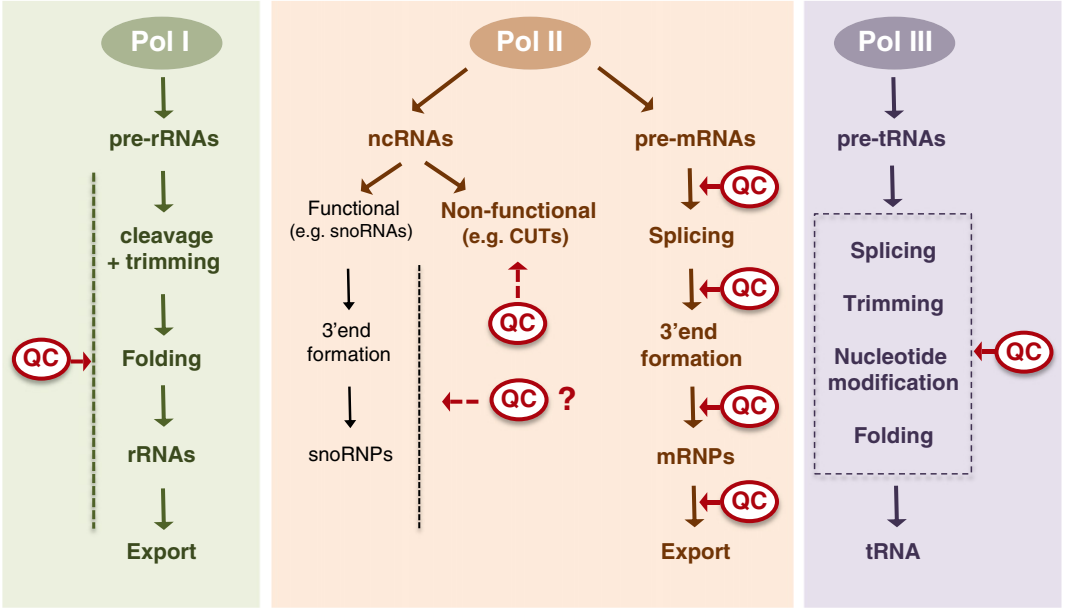

Fig. 3. A summary of quality control (QC) pathway for transcripts produced by the three polymerases. The main processing steps for each class of transcripts are indicated. The occurrence of QC at a specific processing step is indicated when explicitly demonstrated. Otherwise the occurrence of QC is indicated for the whole process. In the case of snoRNAs the occurrence of quality control is not formally demonstrated (indicated by a question mark). This is because mutants affecting quality control by the NNS-exosome pathway also affect normal 3′-end formation and processing of snoRNAs.

sufficiently fast to the next processing step and, as a consequence, would be degraded (Figs. 2 and 3). In this scenario, the exosome would not be activated or recruited to defective substrates, but would target all RNA molecules by default and degrade those that would not go efficiently through processing. Here, sorting of the wrong molecules would not require more energy than that spent to recognize the right processing substrates. The drawback is that degradation would then compete with maturation independently from the generation of defective molecules and certainly eliminate a share of normal molecules before they had the chance to enter the processing pathway. Such a default "destruction timer" would generate the second possible Angels' share of RNA, i.e. fully functional molecules that are discarded as a by-product of cheap quality control.

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