Controlling nuclear RNA levels

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Abstract | RNA turnover is an integral part of cellular RNA homeostasis and gene expression regulation. Whereas the cytoplasmic control of protein-coding mRNA is often the focus of study, we discuss here the less appreciated role of nuclear RNA decay systems in controlling RNA polymerase II (RNAPII)-derived transcripts. Historically, nuclear RNA degradation was found to be essential for the functionalization of transcripts through their proper maturation. Later, it was discovered to also be an important caretaker of nuclear hygiene by removing aberrant and unwanted transcripts. Recent years have now seen a set of new protein complexes handling a variety of new substrates, revealing functions beyond RNA processing and the decay of non-functional transcripts. This includes an active contribution of nuclear RNA metabolism to the overall cellular control of RNA levels, with mechanistic implications during cellular transitions.

Cells are dynamic systems that constantly produce and degrade RNA. All RNA species are therefore subject to turnover, and work accumulating over the past few decades has delineated numerous contributing enzymes, factors and molecular mechanisms. A majority of research has focused on understanding mRNA decay, which takes place primarily in the cytoplasm. Beyond RNA processing reactions, interest in nuclear RNA turnover processes received attention only later, spurred by discovery of the 3′–5′ exonucleolytic and endonucleolytic RNA exosome and an appreciation of its broad utility in the processing and complete decay of nuclear RNAs[1,2] (BOX 1; BOX 2). The field of nuclear RNA decay research then gained further momentum with the realization that eukaryotic genomes are pervasively transcribed[3–5] and that a large fraction of such previously unknown transcription events produce RNAs that do not accumulate at detectable levels owing to their rapid degradation in the cell nucleus[6,7]. It is a standing debate how much of this RNA output is functional, and from an RNA decay-centric standpoint, it is an intriguing question how cells manage to sort functional RNAs from spurious transcripts that need to be repressed.

A common feature of nuclear RNA decay enzymes is that they target a wide variety of substrates and also partake in the processing of many stable RNA species, where 5′- or 3′-extensions are trimmed to mature the transcript[2,8]. This dual ability of nuclear degradation factors to be involved in both productive (processing) and destructive (complete decay) reactions presumably underlies their capabilities of imposing efficient RNA quality control[9]. Decay activities engage both during transcription and shortly after release of the nascent transcript, underscored, for example, by the intimate crosstalk between transcription termination and nuclear RNA decay (BOX 3). Cytoplasmic decay systems are instead often linked to the process of translation, which provides the direct possibility to readily identify aberrant and non-functional RNA. This opportunity is not offered to nuclear decay systems, which instead utilize RNA processing and export activities to assess RNA 'fitness' and degrade transcripts displaying non-optimal kinetics. Another obvious difference between nuclear and cytoplasmic RNA decay is that cytoplasmic decay is carried out mostly by 5′–3′ exonucleases, which makes sense as this prevents the untimely production of truncated proteins. In the nucleus, there are prominent contributions from both 5′–3′ and 3′–5′ decay activities, monitoring the state of RNA termini, which are typically needed for downstream function.

This Review concerns RNA polymerase II (RNAPII) transcripts that commonly receive 7-methylguanosine (m^7G) caps at their 5′-ends just after their emergence from the RNAPII exit channel. Such 5′-end capping protects transcripts from decay by nuclear 5′–3′ exonucleases (BOX 3). Similarly, RNA 3′-ends also require stabilizing characteristics, such as the poly(A) tail of mRNAs or the structured and protein-bound termini of small nuclear RNAs (snRNAs) or small nucleolar RNAs (snoRNAs), which are formed co-transcriptionally or shortly after transcription is complete. RNA substrates falling prey to nuclear decay typically lack such protective features, indicating that the cell nucleus is generally a hostile environment capable of swiftly removing transcripts with unprotected ends or that fail to exit the nucleus in a timely manner. We discuss nuclear RNA decay activities targeting RNAPII transcripts, focusing on the coding RNAs and non-coding RNAs (ncRNAs) produced within and around protein-coding transcription units of *Saccharomyces cerevisiae* and mammals, in which the major body of research has been carried out. We first go through the RNAPII cycle from transcription initiation to termination and focus on

RNA exosome
A multisubunit protein complex harbouring 3′–5′ exoribonucleolytic and endoribonucleolytic activities. The exosome is conserved in archaea and eukaryotic lineages (see also BOXES 1,2).

Transcription termination
The process whereby a transcribing RNA polymerase (RNAP) dissociates from its genome template.

Small nuclear RNAs
(snRNAs). Also termed U snRNAs due to their high uridine content, snRNAs are packaged with proteins into small nuclear ribonucleoprotein (snRNP) complexes and form part of the spliceosome complex (for example, U1, U2, U4, U5, U6, U11, U12, U4atac and U6atac snRNPs) or histone pre-mRNA processing complex (for example, U7 snRNP).

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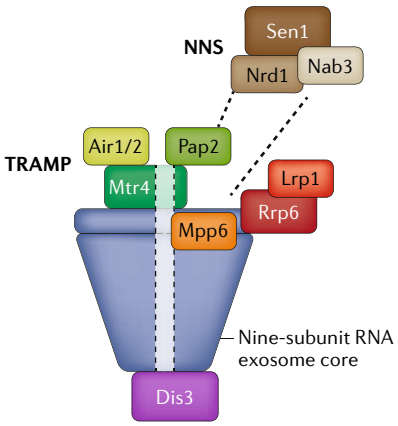
Box 1 | **The *Saccharomyces cerevisiae* nuclear RNA exosome and its cofactors**



The 3′–5′ exonucleolytic and endonucleolytic RNA exosome is a prominent player in eukaryotic nuclear RNA decay and interacts with a plethora of substrates[2,98]. The fundamental structure of the exosome complex includes a barrel-shaped nine-protein subunit core, associating with the processive exonuclease and endonuclease Dis3 situated at the bottom of the barrel and to which RNA can be threaded through the central cavity of the complex[98] (see the figure). This arrangement is similar for both nuclear and cytoplasmic exosomes. In *Saccharomyces cerevisiae*, the nuclear exosome contains a second active exonuclease, the distributive enzyme Rrp6, as well as accessory proteins Lrp1 (also known as Rrp47) and Mpp6. Rrp6 is situated at the top of the barrel, allowing for decay of structured RNAs that cannot be threaded through the central cavity. Lrp1 directly interacts with Rrp6 and facilitates its function, whereas Mpp6 also binds in the vicinity of Rrp6 but with a less established molecular function.

A prominent role of the *S. cerevisiae* nuclear exosome is to completely degrade so-called cryptic unstable transcripts (CUTs) and to trim 3′-extensions of stable non-coding RNAs (ncRNAs), such as small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs). All these transcripts are co-transcriptionally bound by the Nrd1–Nab3–Sen1 (NNS) complex, which elicits RNA polymerase II (RNAPII) transcription termination and at the same time recruits the RNA exosome (see main text for details). In the case of CUTs, this leads to complete degradation of the RNA, whereas 3′-ends of snRNAs or snoRNAs are trimmed until obstructed by the highly stable mature 3′-end protected by proteins of the small nuclear ribonucleoprotein (snRNP) or small nucleolar ribonucleoprotein (snoRNP) complexes[2,98].

The NNS complex can recruit the exosome via direct interaction of Nrd1 and Nab3 with Mpp6 and Rrp6 (REFS[99–101]). In addition, Nrd1 directly binds the Pap2 (also known as Trf4) subunit of the Trf4–Air2–Mtr4 polyadenylation (TRAMP) complex, which provides polyadenylation and helicase activities to the exosome[2,98,99,102]. The TRAMP complex associates directly with the top of the RNA exosome core, where Mtr4 occupies and extends the central exosome channel[2,98]. Consequently, the helicase domain of Mtr4 is positioned to thread RNA into the exosome core, and, consistently, TRAMP acts as a cofactor for most functions of the *S. cerevisiae* nuclear exosome. Polyadenylation by the Pap2 subunit of TRAMP presumably facilitates channelling of RNAs that would otherwise have difficulties in accessing Dis3 or Rrp6. The TRAMP subunits Air1 and Air2 provide RNA-binding capacity.

Small nucleolar RNAs (snoRNAs). RNAs containing conserved H/ACA or C/D box motifs that are packaged with proteins into small nucleolar ribonucleoprotein (snoRNP) complexes that guide the pseudouridylation or methylation of ribosomal RNAs (rRNAs) and other RNAs. Despite their name, snoRNAs are not necessarily restricted to the nucleolus.

Cryptic unstable transcripts (CUTs). Highly unstable short *Saccharomyces cerevisiae* RNAs that are often encoded upstream and antisense of protein-coding genes.

nuclear RNA decay opportunities intersecting this process at its various stages. Thereafter, we discuss physiological roles of nuclear RNA decay systems in imposing efficient RNA quality control, in tuning gene expression levels in response to external cues and in providing means for cells to maintain constant RNA levels. Finally, we briefly touch on pathological consequences when these RNA decay processes are defective.

Nuclear RNA decay: substrates and mechanisms

Decay of premature termination products

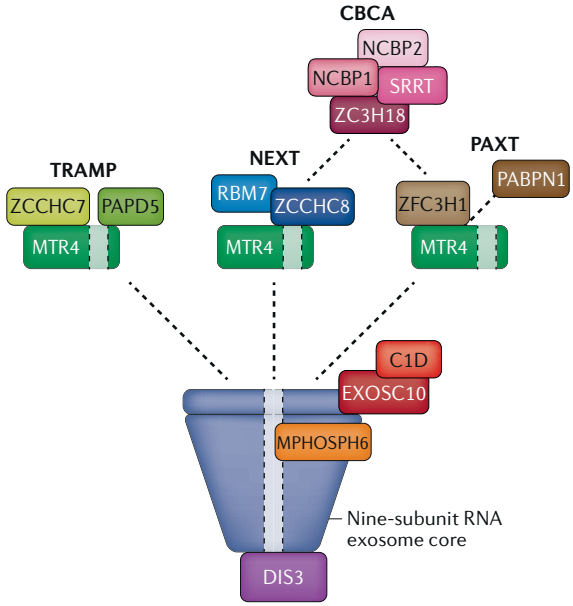
Progression of RNAPII through a regular protein-coding transcription unit is frequently considered to be highly processive, producing pre-mRNA that is matured into a stable transcript. However, numerous studies have challenged this view by demonstrating that RNAPII often terminates before reaching gene ends, producing unstable RNA by-products in the process. Interestingly, such 'gene-body termination-coupled RNA decay' can employ the machinery that also targets transcripts produced from short cryptic transcription units in the genome, including those running upstream of gene promoters, as well as more remotely positioned loci[10–15]. This holds true from *S. cerevisiae* to mammals, suggesting an evolutionarily conserved principle despite the distinct factors engaged.

The S. cerevisiae Nrd1–Nab3–Sen1 system. The best understood transcription-termination-coupled RNA decay system targets cryptic unstable transcripts (CUTs) in *S. cerevisiae*. These RNAs are co-transcriptionally bound by the Nrd1–Nab3–Sen1 (NNS) complex, which elicits RNAPII transcription termination while simultaneously recruiting the Trf4–Air2–Mtr4 polyadenylation (TRAMP) complex, in turn bridging to the RNA exosome (BOX 1). The RNA-binding proteins Nrd1 and Nab3 both recognize short sequence-specific RNA motifs, which are encoded abundantly in the *S. cerevisiae* genome but largely depleted from mRNA coding strands[13,16]. Nrd1 also interacts specifically with the Ser5-phosphorylated carboxy-terminal domain (CTD) of RNAPII. This modification is characteristic of early elongation by RNAPII, restricting NNS action primarily to short transcription units[17]. In combination, this allows for promiscuous targeting of cryptic transcripts, typically those containing NNS binding sites, at positions with high levels of Ser5-P RNAPII, without grossly affecting mRNA production. Although transcription of CUT transcription units may be functionally relevant for their regulation of adjacent or overlapping protein-coding genes[5], no CUT RNA has so far been assigned any function. The NNS complex therefore appears to act as a transcriptome gatekeeper, preventing the interference of short cryptic transcripts with normal RNA metabolism.

Targeting of premature transcription termination products in mammals. Premature transcription termination within gene units also occurs commonly in human cells. A first possible location for RNAPII to terminate transcription is at the negative elongation factor (NELF)-dependent and DRB sensitivity-inducing factor (DSIF)-dependent stall site 30–50 bp downstream of the transcription start site (TSS) of the gene[18]. From here, RNAPII can either progress into productive elongation or terminate transcription entirely. Although the relative numbers of RNAPII complexes undergoing these respective fates are being debated[19,20], some fraction of RNAPII terminates, necessitating RNA removal (FIG. 1a). Here, the 5′–3′ exonuclease XRN2 (BOX 3) and the RNA exosome (BOX 2) have both been reported to play a role[19,21]. XRN2 is of particular interest because it can degrade the nascent RNA while it is still associated with RNAPII and thereby trigger transcription termination. Indeed, RNAPII stalling at this position was reported to be impacted both by decapping and XRN2 activities[22]. Such early RNA targeting might provide a means by which RNAPII complexes that fail to proceed from their 5′-stall positions or transcribing RNA that fails to be capped can be cleared off the DNA template, creating so-called TSS-RNAs in the process (FIG. 1a).

Early transcription termination can also occur downstream of the TSS-proximal stalling site through the

Box 2 | **The mammalian nuclear RNA exosome and its cofactors**



The basic structure of the nuclear RNA exosome is conserved between yeast and mammalian cells. A noticeable difference from the *Saccharomyces cerevisiae* RNA exosome (BOX 1) concerns its active subunits, as EXOSC10 (the human homologue of Rrp6) is not restricted to the nucleus, and three functionally distinct DIS3 homologues (DIS3, DIS3L and DIS3L2) exist in mammals, where only DIS3 associates with the nuclear exosome[103]. Mammals also encode homologues of Lrp1 (C1D), Mpp6 (MPHOSPH6) and Mtr4 (MTR4). Moreover, the human TRAMP complex is similar to the *S. cerevisiae* Trf4–Air2–Mtr4 polyadenylation (TRAMP) complex. Besides MTR4, it is composed of PAPD5 and a proposed functional analogue for Air1 (ZCCHC7), and TRAMP appears to conduct activities in mammalian nucleoli[15]. Additional mammalian cofactor complexes evolved independently and have no direct protein homologues in *S. cerevisiae*. Instead, many are conserved in *Schizosaccharomyces pombe*. In addition to its participation in the TRAMP complex, MTR4 is part of the nuclear exosome targeting (NEXT) complex, which also consists of the RNA-binding protein RBM7 and the large zinc-finger protein ZCCHC8 (REF.[15]). NEXT is a so-called exosome adaptor complex, as it provides exosomal access, via its RNA-binding activity, to a range of nuclear transcripts, such as a fraction of promoter upstream transcripts (PROMPTs) and enhancer RNAs (eRNAs), as well as the 3′-extended regions of small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs)[15,32]. The related poly(A) exosome targeting (PAXT) connection contains MTR4 and another large zinc-finger protein, ZFC3H1 (REF.[12]). PAXT also connects to PABPN1, although this interaction is weaker and partially RNA-dependent (hence the term 'PAXT connection')[12]. The presence of PABPN1 implicates the PAXT pathway in decay of poly(A)$^+$ RNA.

Commonly, the NEXT and PAXT assemblies can, via their zinc-finger proteins, make contact to the cap-binding complex (CBC)[12,32]. This interaction is mediated by yet another zinc-finger protein, ZC3H18, which interacts with the CBC and serrate RNA effector molecule homologue (SRRT) to form the CBCA complex in a mutually exclusive manner with the RNA transport factor PHAX[32]. Consistently, the CBCA complex contributes to the exosomal decay of some PROMPTs, eRNAs, and snRNA and histone RNA 3′-extensions[11,32]. Interestingly, CBCA components, but not NEXT, PAXT or exosome subunits, are engaged in transcription termination at these transcription units[11]. This indicates that transcription termination and RNA decay are also intimately linked in mammalian cells.

process of premature cleavage and polyadenylation (PCPA) at cryptic polyadenylation sites (PASs)[11,23–26]. PCPA is normally dampened by the U1 small nuclear ribonucleoprotein (snRNP) binding at upstream canonical, or cryptic, splice donor sites[23,24,26,27]. Consistently, widespread PCPA was first discovered in cells with compromised U1 snRNP function[23]. Yet, PCPA also occurs in unperturbed cells, perhaps because of insufficient U1 snRNP levels or because U1 snRNP action is inefficient or actively suppressed at such sites. As PCPA appears to coincide with a second RNAPII stalling event at stable nucleosomes downstream of CpG island promoters[26], it is tempting to speculate that slow RNAPII progress allows for cleavage at these cryptic PASs. Consistently, the majority of PCPA-induced transcription termination events occur early within the first introns of genes, and the resulting RNAs are often unstable owing to their decay by the RNA exosome[11,25,26] (FIG. 1b). In this sense, PCPA-derived transcripts are quite similar to promoter upstream transcripts (PROMPTs), also termed upstream antisense RNAs (uaRNAs), which often terminate at PASs, yielding exosome-sensitive RNAs[6,28–30] (FIG. 1c). We speculate that PAS usage for PCPA-derived transcripts and PROMPTs, while eliciting transcript cleavage, might not allow for efficient polyadenylation or poly(A) tail protection, explaining why RNA decay prevails in these cases. As the majority of PROMPTs are unspliced and because PCPA often occurs inside first introns, none of these transcripts have undergone splicing. Given the stimulatory effect of splicing on 3′-end processing, this may contribute to such inefficient polyadenylation[31]. Moreover, owing to the relatively short lengths of PCPA-derived RNAs and PROMPTs, their 3′-ends are close by the cap-binding complex (CBC) and its associated SRRT (also known as ARS2) protein (forming the CBCA complex) (BOX 2), which are mechanistically involved in both transcription termination and rapid decay of these transcript types[11,32]. This may contribute to reduced polyadenylation and/or directly favour RNA decay owing to the connection between the RNA exosome and the CBCA complex through the nuclear exosome targeting (NEXT) complex (BOX 2). Finally, as increased RNAPII travelling on the DNA changes its CTD modification pattern, which in turn regulates interactions with RNA processing factors[33], it is also possible that a promoter-proximal CTD configuration is not compatible with efficient 3′-end polyadenylation.

PCPA–PROMPT termination-coupled RNA decay shares similarities with the NNS-dependent pathway from *S. cerevisiae*. Both employ the RNA exosome for transcript decay, both target PROMPTs as well as

Small nuclear ribonucleoprotein (snRNP). A particle consisting of a small nuclear RNA (snRNA) and its protein-binding partners.

Nuclear exosome targeting (NEXT). This complex is a mammalian nuclear RNA exosome adaptor containing the MTR4, RBM7 and ZCCHC8 proteins. It is involved in targeting the exosome to short and non-sequence-specific RNAs.

Box 3 | **Nuclear 5′–3′ decay in *Saccharomyces cerevisiae* and human cells**

All eukaryot′ic model organisms contain a nucleus-specific 5′–3′ exonuclease, termed Rat1 in *Saccharomyces cerevisiae* and XRN2 in humans. Rat1 and XRN2 partake in the 5′-end trimming of stable non-coding RNAs (ncRNAs)[104] and quality control of the RNA capping process[34]. Moreover, these enzymes are known for their roles in degrading the uncapped 3′-fragment arising after mRNA 3′-end cleavage, contributing to RNA polymerase I (RNAPI) and RNAPII transcription termination[44,45,47]. The exact mechanistic impact of exonucleolytic decay on transcription remains unclear. Moreover, even though exonuclease activity is required for transcription termination, it is not sufficient, as artificial targeting of the homologous cytoplasmic 5′–3′ exonuclease Xrn1 to the nucleus rescues decay of the downstream RNA fragment without reinstalling transcription termination in a *rat1-1 S. cerevisiae* mutant background[105].

Rat1 and XRN2 target RNAs with mono-phosphorylated 5′-ends and are thus inactive towards the capped or tri-phosphorylated 5′-ends of nascent RNAPII transcripts[106,107]. Instead, Rat1 and XRN2 act on 3′-fragments produced by endonucleolytic cleavage. In addition, decapping enzymes (Dcp1–Dcp2 in *S. cerevisiae*; DCP1–DCP2 in humans) can produce substrates for Rat1/XRN2-targeted 5′-ends. Indeed, while these decapping enzymes are present mostly in the cytoplasm, they also have a nuclear phase and can partake in the XRN2-dependent quality control of unspliced pre-mRNAs in human cells[43].

Interestingly, the Rat1 cofactor Rai1 was found to be an unconventional decapping enzyme, producing Rat1/XRN2-sensitive RNA 5′-ends by virtue of two enzymatic capabilities: pyrophosphohydrolyase activity, which converts triphosphate to monophosphate, and decapping activity, which removes unmethylated 5′-caps[108,109]. Thus, Rai1 not only stimulates the exonuclease activity of Rat1 but also specifically converts RNA 5′-ends that did not properly form a canonical 7-methylguanosine (m^7G) cap. The *S. cerevisiae* genome encodes a second Rai1 homologue, Dxo1, which lacks phosphohydrolase activity but possesses an even more general decapping activity that is capable of removing both unmethylated and methylated 5′-caps. Dxo1, which is present in both the nucleus and the cytoplasm, also displays exonuclease activity, suggesting that it acts independently of Rat1 or Xrn1 (REF.[110]). Consistent with their similar enzymatic activities, co-deletion of Rai1 and Dxo1 leads to accumulation of incompletely capped RNAs[110]. Human cells contain only a single Rai1/Dxo1 homologue, DXO (also known as DOM3Z), which localizes to both the nucleus and the cytoplasm and shares enzymatic characteristics with Dxo1. Consistently, aberrantly capped RNAs accumulate in human cells depleted of DXO. Interestingly, these transcripts are often unspliced and inefficiently 3′-end cleaved, suggesting an important quality control function of the enzyme[111].

transcripts arising from early transcription termination within mRNA genes and both appear to be specific for short transcription units. This argues for an evolutionarily conserved need to suppress short spurious transcription events. Even so, the employed molecular machineries are different (BOX 1; BOX 2), prompting the question about their origins. Most *S. cerevisiae* genes are short (<3 kb) and lack introns. It is likely that sorting of functional from non-functional termination events in this context requires molecular criteria other than the distance to gene TSSs or the presence of introns. This could explain the evolution of the sequence-specific NNS system, compared with the sequence-independent TSS-distance measuring pathway in higher eukaryotes. A more detailed description of these pathways in model systems other than *S. cerevisiae* and human cells would facilitate a better understanding of the evolution of nuclear decay systems suppressing cryptic transcripts.

Competition between RNA processing and decay

The examples discussed so far concern non-conventional RNAs, set apart from regular mRNAs, which usually show nuclear stability and are exported to the cytoplasm owing to their efficiently added 5′-caps and 3′-poly(A) tails (FIG. 1d). This is evident in *S. cerevisiae* mutants for the capping or cleavage and polyadenylation machineries, in which RNA production is severely impaired owing to degradation by Rat1 (the *S. cerevisiae* homologue of XRN2 (BOX 3)) or the nuclear RNA exosome (BOX 1), respectively[34–37]. In the case of a capping deficiency, such decay is likely to be initiated early during transcription as outlined above[21,34]. In the case of 3′-end RNA targeting, given the intimate coupling between RNA biogenesis steps, inefficient polyadenylation may also explain the nuclear decay observed in splice-defective and mRNA export-deficient mutants[38–42]. As a common principle, it is unlikely that dedicated cofactors are targeting inefficiently processed RNAs. Rather, it appears that the inherent effectiveness of nuclear RNA decay systems allows them to rapidly scavenge any RNA with unprotected ends.

Less is known about nuclear decay of processing-defective transcripts in higher eukaryotes. Conceptually, detection of processing defects must be more flexible and able to tolerate the adaptable usage of alternative splice sites and polyadenylation sites. Indeed, clearance of aberrantly processed RNA may often be cytoplasmic, in which the process of translation allows for a direct monitoring of mRNA utility. Even so, reporter mRNAs with defective splice sites or 3′ processing sites were found to be subject to nuclear 5′–3′ decay[43]. Although it is unclear how these RNAs might be targeted from their 5′-ends, this indicates that a lack of processing can promote nuclear decapping, leading to decay[43].

Decay of RNA downstream of 3′-end processing sites

A functional role of RNA decay: the torpedo model. mRNAs are normally synthesized as 3′-extended precursors, requiring an initial endonucleolytic cleavage event carried out by CPSF3 (also known as CPSF73; Ysh1 in *S. cerevisiae*). The RNA 3′ fragment produced, which carries an exposed 5′-hydroxyl group, is then typically degraded by the 5′–3′ exonuclease XRN2 (Rat1 in *S. cerevisae*)[44–46], which 'chases' after the still transcribing RNAPII, forming the basis for the suggested torpedo model for transcription termination (FIG. 1e). Although this provides an

Torpedo model
A model that suggests that transcription termination is caused by the nuclear 5′–3′ exonuclease (Rat1 in *Saccharomyces cerevisae*; XRN2 in mammals) degrading the nascent RNA attached to RNA polymerase (RNAP) after an endonucleolytic cleavage event, akin to a torpedo chasing after a target.

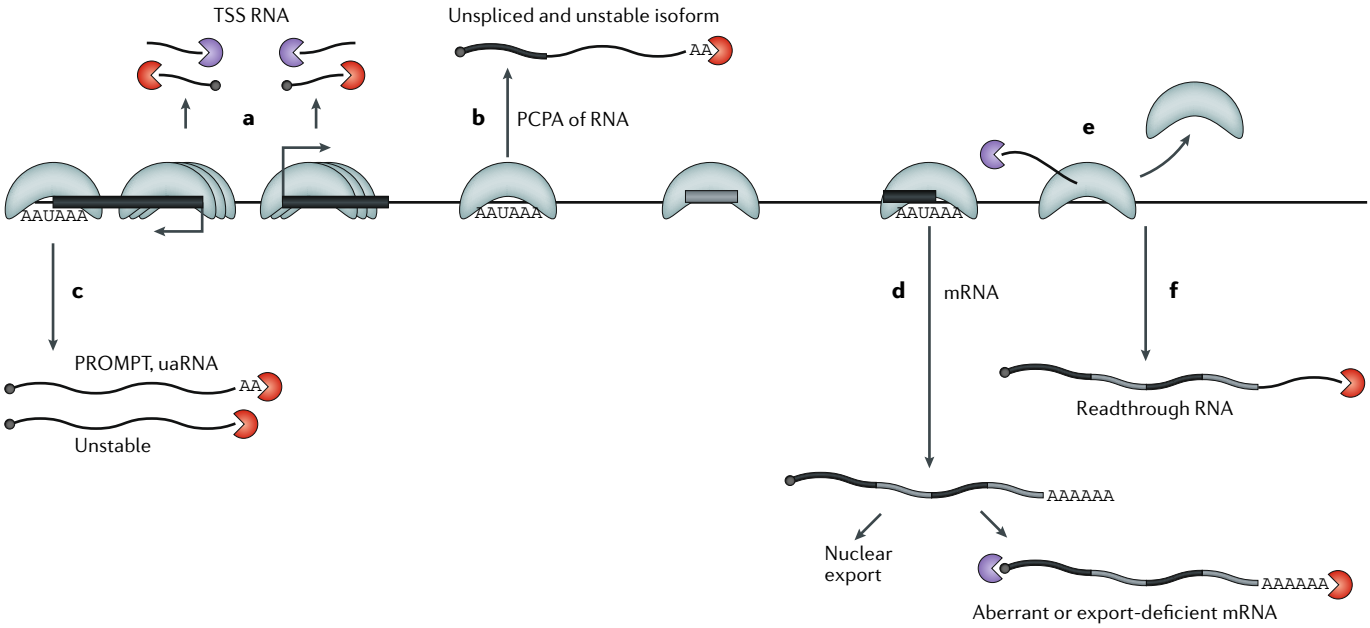


Fig. 1 | **Nuclear RNA decay opportunities around protein-coding genes.** Nuclear decay opportunities around a typical protein-coding gene in higher eukaryotes. **a** | Promoters are divergent, and transcription in both directions is accompanied by promoter-proximal stalling of RNA polymerase II (RNAPII; shown as the grey crescent shape), which provides a first opportunity for termination. The enzymes responsible for degrading the resulting so-called transcription start site (TSS) RNAs are not known, but both XRN2 (5′–3′ exonuclease activity, shown as the purple 'PacMan' symbols) and the nuclear exosome (shown as the red 'PacMan' symbols) have been implicated. **b** | Cryptic polyadenylation sites (PASs), primarily inside first introns, provide other regions where premature transcription termination can occur. (PASs are shown as the AAUAAA sequences.) The resulting premature cleavage and polyadenylation (PCPA) of RNAs produces substrates of the nuclear RNA exosome, which is assisted by the nuclear exosome targeting (NEXT) or poly(A) exosome targeting (PAXT) assemblies. **c** | Bidirectional transcription produces upstream reverse-oriented transcripts known as promoter upstream transcripts (PROMPTs) or upstream antisense RNAs (uaRNAs). These transcripts are not spliced but are terminated by PASs or other termination sequences. Such TSS-proximal termination does not support stable RNA production but leads to decay by the RNA exosome. The exact polyadenylation status of these transcripts before decay is unclear. **d** | Cleavage at the annotated PAS normally leads to the production of a stable mRNA, whereas abnormalities, such as poor termini processing or lack of nuclear export, elicits nuclear decay by XRN2 or the nuclear exosome. **e** | Decay of the downstream fragment created by endonucleolytic cleavage is carried out co-transcriptionally by XRN2, which also participates in transcription termination. **f** | If endonucleolytic cleavage is delayed, termination may occur at downstream fail-safe terminators. The resulting readthrough RNAs are metastable, owing to decay by the RNA exosome. See the main text for more details.

example of a direct functional contribution of nuclear RNA decay, its exact mechanistic contribution to transcription termination remains poorly described[47] (BOX 3). As RNAPII often progresses several kilobases past mammalian PASs, this mode of transcription termination produces high amounts of seeming by-products. Integrating their decay with transcription termination therefore not only ensures that the eviction of RNAPII from the DNA template is coupled to transcript cleavage but also prevents release into the nucleoplasm of the downstream RNA, thereby avoiding any interference with normal RNP metabolism (see below).

3′–5′ decay of downstream RNA. Although the described 5′–3′ decay of RNA downstream of 3′-end processing sites appears to be the norm, studies have also demonstrated a contribution of 3′–5′ decay (FIG. 1f). Although controversial, this may even contribute to transcription termination, as suggested by an observed termination defect upon depletion of RNA exosome factors in *Schizosaccharomyces pombe*[48]. In any case, a role for 3′–5′ exonucleolytic activity in downstream RNA decay is well established in situations when 3′-end cleavage is delayed or defective but transcription termination still occurs, for example, by fail-safe transcription termination at co-transcriptional cleavage sites (CoTCs) or at roadblock terminators[47]. For example, *S. cerevisiae* pre-mRNA cleavage mutants produce readthrough RNA that is degraded by the RNA exosome[35,37,39]. A similar process occurs in human cells, in which the exosome degrades extended histone mRNA species[11,32]. Even though they are not polyadenylated, these transcripts undergo endonucleolytic cleavage by CPSF3 and are therefore subject to XRN2-dependent decay[49]. The exosome-dependent forms are therefore unlikely to be canonical precursors of histone mRNAs. Rather, they represent uncleaved transcripts that may either be 3′-trimmed to produce mature RNA or be fully degraded.

Nuclear decay of poly(A)⁺ RNA

The majority of processing and packaging of RNAs occurs during transcription. RNAs released from RNAPII are therefore often fully mature and swiftly exported to the cytoplasm. Hence, if they overcome the risk of being degraded during transcription or shortly

Co-transcriptional cleavage sites
(CoTCs). Regions positioned downstream of annotated poly(A) sites that are subjected to endonucleolytic cleavage to facilitate transcription termination. CoTCs are poorly defined and the mechanisms underlying RNA cleavage remain to be uncovered.

Roadblock terminators
Genomic regions that cause 'roadblock' transcription termination, for example, when they are occupied by a tightly bound DNA-binding protein that prevents RNA polymerase (RNAP) from transcribing beyond this site.

after their termination, mature transcripts are only in danger if they reside in the nucleus for a prolonged time. This might occur, however, and is illustrated by work on both *S. cerevisiae* and mammalian cells, which implies that even seemingly functional mRNAs are sometimes turned over, placing nuclear decay systems as post-transcriptional regulators of gene expression similar to the long-established role of their cytoplasmic counterparts.

A timing model for post-transcriptional nuclear decay. An early hint that prolonged nuclear residence results in nuclear decay stems from *S. cerevisiae*, where a pathway termed decay of RNA in the nucleus (DRN) was proposed to target a specific subset of nuclear-retained mRNA[50]. Similarly, unspliced mRNAs, which are generally poor nuclear export substrates, are degraded by the nuclear exosome[38,51]. These examples illustrate the general concept that, like newly produced transcripts, nuclear-retained RNAs are also at risk of being turned over. What mechanism underlies this phenomenon? Depletion of the *S. cerevisiae* nuclear poly(A) binding protein (PABP), Nab2, triggers the global post-transcriptional disappearance of newly produced poly(A)-tailed RNA, suggesting a critical need for their immediate 3′-end protection[52]. Interestingly, *S. pombe* cells contain two nuclear PABPs, Pab2 and Nab2, where the former acts as an exosome cofactor and the latter antagonizes Pab2 and thereby serves a protective function[53]. Similarly, whereas *S. cerevisiae* Nab2 protects newly synthesized mRNA, it also facilitates the decay of nuclear-retained unspliced pre-mRNAs[51,54]. Hence, Nab2 appears to be a dual-purpose protein managing two opposing functions. Finally, the mammalian homologue of *S. pombe* Pab2, PABPN1, is also a cofactor for the RNA exosome in a pathway coined PABPN1 and PAP-mediated RNA decay (PPD)[55–57]. As the human Mtr4 homologue MTR4 (also known as SKIV2L2 or MTREX) interacts with the zinc-finger protein ZFC3H1 and PABPN1 to form the poly(A) RNA exosome targeting (PAXT) connection (BOX 3), and as ZFC3H1 is also required for the decay of PPD targets[12], the PPD and PAXT pathways may overlap, or at least cooperate, in degrading nuclear polyadenylated RNA. As for *S. cerevisiae* Nab2, the role of PABPN1 is paradoxical because all nascent mRNA poly(A) tails are supposedly bound by this protein as part of their biogenesis and initial protection[58].

A 'nuclear timer' model has therefore been invoked to rationalize the double-faced activity of PABPs: it is protective of newly produced poly(A)$^+$ RNA, while eliciting decay of transcripts with longer nuclear residence times[59,60]. How would such timing be achieved? Importantly, PAXT-mediated and PPD-mediated decay of poly(A)$^+$ RNA do not appear to be closely coupled to transcription termination, which would otherwise place all protein-coding messages at risk. Moreover, whereas the interaction between MTR4 and ZFC3H1 is strong, their connections to PABPN1 and the exosome are more transient[12,15]. Full assembly of a decay-promoting complex may therefore take time, in line with a timer model. In analogy to the mechanism of cytoplasmic mRNA turnover, it is also possible that PAXT does not assemble on RNAs with mature poly(A) tails but only acts after the initial deadenylation by another slow-acting nuclear deadenylase. Indeed, PABPN1 also interacts with the deadenylase PARN[61]. However, this interaction has so far only been shown to be required for maturation of the human telomerase RNA component (TERC) and to antagonize TERC decay by the RNA exosome[61]. Any role of PARN in the timed nuclear decay of poly(A)$^+$ RNA still needs to be established.

How to escape nuclear decay

The above considerations suggest that the most efficient way for any RNA to escape nuclear decay is to swiftly evacuate from its degradative environment (FIG. 2). But how do RNAs with functions in the nucleus then cope? Hints may come from long non-coding RNAs (lncRNAs) that are functional in the nucleus and thus sufficiently stable. Interestingly, known examples are often associated with chromatin, such as the XIST RNA on the inactive X chromosome[62], or enriched within subnuclear structures, such as the nuclear-enriched abundant transcript 1 (NEAT1) in paraspeckles[63]. De-protection or decay of full-length RNAs may thus be most efficient in the nucleoplasm, allowing RNAs to escape by aggregating in subnuclear assemblies or structures that lack sufficient decay capacity (FIG. 2).

Another conceptually related way to prevent nuclear decay is to wrap the sensitive RNA termini in RNA–protein complexes or stabilizing RNA structures. The CBC and PABPs are prime examples of the protective roles of RNA-binding proteins for the 5′-ends and 3′-ends, respectively. However, as outlined above, these measures will not prevent decay during longer nuclear exposure. Lasting protection may therefore require stronger assemblies, such as the RNPs formed on snRNAs by the Sm proteins[64] and those formed on snoRNAs by the H/ACA and D-box snoRNA-binding snoRNP factors[65]. Moreover, proteins may not necessarily be required, as protective triple-helical RNA structures have also been suggested to form and stabilize the two stable nuclear lncRNAs, metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) and NEAT1 (REF.[66]) (FIG. 2). Finally, it was recently suggested that cytoplasmic RNA isoforms, which are U-rich upstream of their PASs, are especially stable owing to the suggested back-folding of the poly(A) tail onto this upstream sequence[67]. Whether this also allows for protection against decay in the nucleus is not known.

Physiological relevance of nuclear RNA decay

Cellular RNA hygiene

It is an ongoing debate of how many of the promiscuously transcribed RNAs from eukaryotic genomes are functional. Although this will ultimately have to be settled using a case-by-case approach, most targets of nuclear RNA decay systems may serve no immediate function. This concerns RNA products of promiscuous transcription or cases of quality control where aberrantly processed or packaged transcripts are eliminated. In addition, nuclear RNA decay is also active in situations where the transcription event itself, but not its

Poly(A) RNA exosome targeting
(PAXT). Connection made up of a mammalian nuclear RNA exosome adaptor that contains the MTR4, ZFC3H1 and PABPN1 proteins and is involved in recruiting the exosome to polyadenylated RNAs.

Long non-coding RNAs
(lncRNAs). RNAs that are most often defined as non-protein-coding transcripts longer than 200 nucleotides.

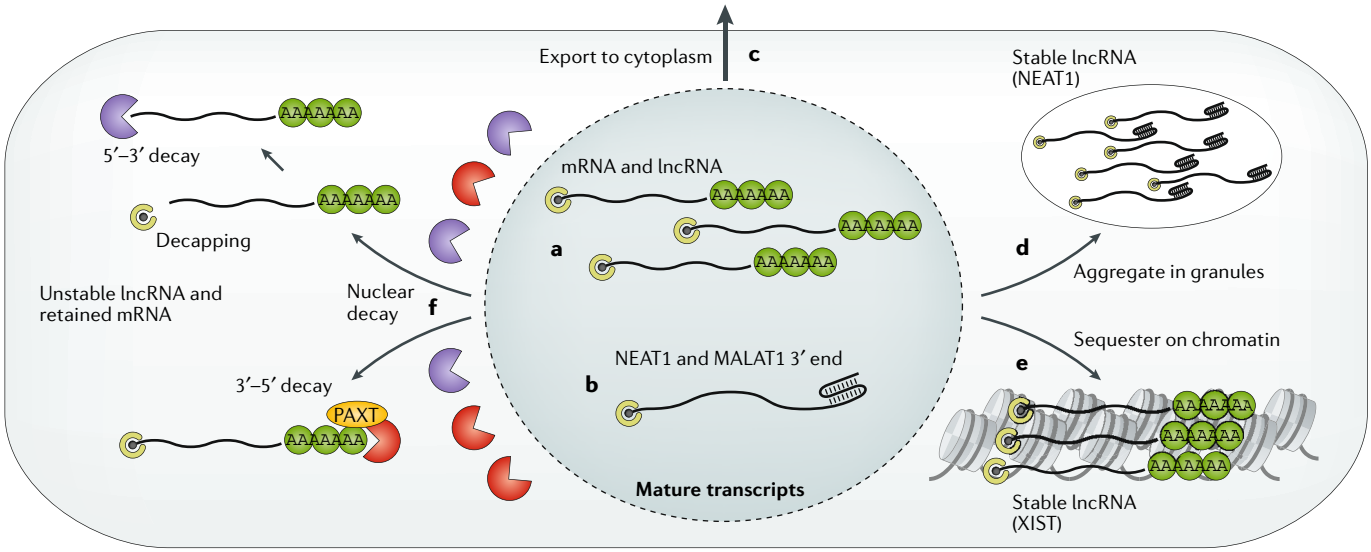


Fig. 2 | **Escaping nuclear decay.** RNA survival in the degradative environment of the cell nucleus requires end-protective features. These might be the 5′-cap, bound by the cap-binding complex (CBC; shown as the light green 'c' shape), and the poly(A) tail, bound by poly(A) binding proteins (PABPs; shown as green circles) (part **a**), although long-term protection will not be conferred. To achieve this, more specialized RNA structures, such as the triple-helical 3′-ends of the long non-coding RNAs (lncRNAs) nuclear-enriched abundant transcript 1 (NEAT1) and metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) (part **b**) can be established. Long-term RNA survival is further provided by its export to the cytoplasm (part **c**) or its sequestration in ribonucleoprotein (RNP) granules such as paraspeckles (part **d**), as exemplified by the NEAT1 lncRNA, or on chromatin (part **e**), as exemplified by the accumulation of XIST on the inactive X chromosome. RNAs that lack such features get degraded by XRN2 (purple 'PacMan' symbols) or the nuclear RNA exosome (red 'PacMan' symbols), assisted by their respective decapping and poly(A) RNA exosome targeting (PAXT) cofactors (part **f**). See the main text for more details.

resulting RNA, serves a regulatory purpose. Finally, cells also produce large amounts of superfluous RNA from the parts of primary transcripts that are not included in the mature RNA. Given the diversity and multitude of such non-functional RNA, a crucial role of nuclear RNA decay is to keep an acceptable nuclear hygiene in order to avoid interference with regular cellular function, such as through unsolicited aggregation or sequestering of RNA-binding proteins. This is especially critical for nuclear RNA biogenesis factors that are available in limiting amounts. On a related note, it was recently shown that the absence of the PAXT components MTR4 and ZFC3H1 causes some of their substrates to leak into the cytoplasm and interfere with mRNA translation[68]. Thus, nuclear RNA control also plays an important role for normal cell function. This is further emphasized by the observation that the nuclear RNA export factor ALYREF interacts with the CBC and PABPN1, bound at RNA 5′- and 3′-ends, respectively[69,70]. Both CBC and PABPN1 also interact with MTR4 (BOX 2), corroborating the idea of mutually exclusive recruitment of export and decay factors to prevent nuclear export of transcripts targeted by the decay machinery[69].

Another threat posed by unwanted accumulation of nuclear RNA is the formation of RNA:DNA hybrids, so-called R-loops. R-loops have been proposed to partake in transcription initiation and termination[71], but they also pose a hazard, triggering increased genomic instability. Recently, the exosome was shown to diminish R-loop formation and its consequential mutagenicity and induction of translocation hot spots in B cells[72–74]. The underlying mechanism remains elusive, but it is interesting to note that the exosome can interact with the R-loop resolving RNA helicase senataxin (SETX)[75].

Gene regulation

Owing to their efficacies in maintaining a clean environment, nuclear RNA decay systems can be leveraged to contribute to mRNA expression regulation with several independent examples reported from *S. cerevisiae*, *S. pombe* and mammalian cells.

S. cerevisiae. Gene expression regulation by nuclear RNA decay is perhaps best signified by the numerous mRNA biogenesis factors that are subject to auto-regulation. One example concerns the *NAB2* mRNA, which harbours a genome-encoded homopolymeric A stretch in its 3′ untranslated region (UTR). Binding of the Nab2 protein to this motif depends on Nab2 availability and prevents canonical mRNA 3′-end processing, instead funnelling the RNA to nuclear decay[76]. A more general role for nuclear decay in shaping the *S. cerevisiae* transcriptome was recently demonstrated for cells undergoing glucose depletion[77]. During this 'diauxic shift', the authors measured an altered binding of the NNS component Nab3 and the exosome cofactor Mtr4 to mRNAs, which did not simply parallel the observed changes in transcription of the implicated genes. Instead, changed protein binding appeared to augment the transcriptional programme by increasing or decreasing nuclear decay for downregulated or upregulated transcripts, respectively. This phenomenon seems to be purely post-transcriptional, suggesting a role of Nab3 outside of its regular transcription

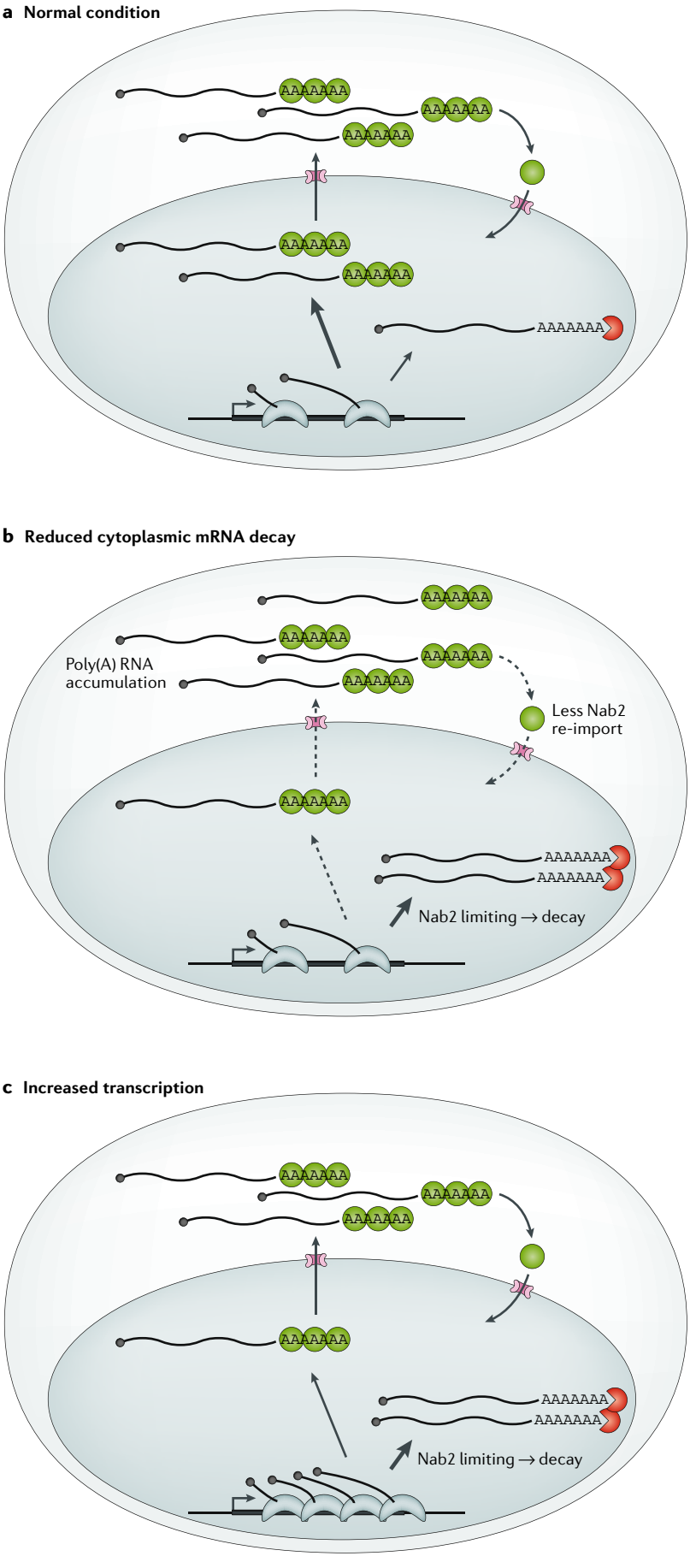


Fig. 3 | **RNA homeostasis model.** A potential role for nuclear RNA decay in cellular RNA homeostasis control, exemplified for the Nab2 protein (green circles). **a** | During normal growth, newly produced mRNAs are bound by Nab2 and exported to the cytoplasm. **b** | When cytoplasmic decay is reduced, cytoplasmic mRNA levels increase, in turn delaying nuclear re-import of Nab2. This leads to a shortage of nuclear Nab2 and an ensuing increased decay of nascent transcripts, thereby counteracting the cytoplasmic mRNA overabundance. **c** | Increased transcription will be intrinsically buffered, as only the pool of nascent RNA that can be bound by Nab2 escapes to the cytoplasm, whereas excess transcripts are nuclear degraded.

termination function within the NNS complex. It also provides the first comprehensive systems biology example that nuclear RNA decay systems regulate mRNA expression changes with a potential to amplify transcriptional gene expression responses, comparable to the more established contribution from cytoplasmic RNA decay. However, in contrast to cytoplasmic decay, nuclear RNA removal ultimately prevents any cytoplasmic appearance and translation, begging the question of the benefits of such a rigorous system. Transcription is usually regulated at the level of initiation. Thus, nuclear decay may provide a more stringent gene expression response that is capable of rapidly preventing transcriptional output even from polymerases that are already engaged when the response is triggered. Cytoplasmic decay, conversely, is the only means to remove RNAs that are already present in the cytoplasm before a specific stimulus is encountered.

S. pombe. A function for nuclear decay in mRNA expression control is well established in *S. pombe* and concerns the suppression of meiotic gene expression during vegetative growth. Here, the sequence-specific RNA-binding protein Mmi1 plays a central role by tagging meiotic mRNAs for decay by the exosome[78,79]. When cells enter meiosis and need to express meiotic transcripts, Mmi1 gets sequestered away by binding to the meiosis-specific lncRNA, meiRNA[79]. Mmi1 is part of the so-called Mtl1–Red1–Core (MTREC) complex[80–82], which contains homologues of several human exosome cofactors (BOX 2). Nuclear RNA decay by the MTREC complex also depends on the canonical polyadenylation machinery and Pab2. This reminiscence of the PPD and PAXT pathways in mammalian cells is striking, but an obvious Mmi1 homologue has not been identified in higher eukaryotes.

MTREC target genes are often embedded in heterochromatin, where MTREC not only facilitates exosomal decay but also regulates the heterochromatic methylation of histone H3 lysine 9 (H3K9)[80,81]. Interestingly, some targets are also subject to the canonical RNA interference (RNAi)-dependent gene silencing pathway[83]. Moreover, a recent report suggested that a third distinct pathway, contingent on the 5′–3′ exonuclease Dhp1, the *S. pombe* homologue of XRN2, contributes redundantly with the above pathways to silencing at centromeres and mating type loci[84]. Hence, suppression of RNA from inactive genomic regions seems to be a key function of diverse nuclear decay systems.

Table 1 | **Nuclear RNA decay factor mutations and links to disease**

Gene	Function	Disease	Evidence	Refs
EXOSC3 (also known as *RRP40*)	Essential exosome core component	Pontocerebellar hypoplasia (PCH) subtype 1b	Homozygous or compound heterozygous *EXOSC3* mutations are found in roughly 50% of patients with PCH subtype 1	89,112
EXOSC8 (also known as *RRP43*)	Essential exosome core component	PCH subtype 1c	Homozygous *EXOSC8* mutations were found in ten affected individuals	89,113
EXOSC2 (also known as *RRP4*)	Essential exosome core component	Novel syndrome[a]	Three affected individuals: two patients with homozygous mutations and one with compound heterozygous mutations in *EXOSC2*	89,114
DIS3 (also known as *RRP44*)	Essential nuclear exosome exonuclease	Multiple myeloma (MM) and plasma cell leukaemia (PCL)	>10% somatic mutation rate in MM and primary and secondary PCL	93
RBM7	RNA-binding factor of the NEXT complex	Spinal motor neuropathy (SMN)	Homozygous mutation in one individual	90
PABPN1	Nuclear PABP of the PAXT connection	Oculopharyngeal muscular dystrophy (OPMD)	Homozygous or compound heterozygous triplet repeat expansion of polyalanine-encoding sequence in affected individuals	92
ZCCHC14	Nuclear PABP	Non-syndromic autosomal recessive intellectual disability (NS-ARID)	Homozygous mutation in six individuals from two different families	91

EXOSC, exosome component; NEXT, nuclear exosome targeting; PABP, poly(A) binding protein; *PABPN1*, poly(A) binding protein nuclear 1; PAXT, poly(A) exosome targeting; *RBM7*, RNA-binding motif protein 7; *ZCCHC14*, zinc-finger CCHC-type containing 14. [a]Novel syndrome with compound phenotype, including retinitis pigmentosa, hearing loss, premature ageing, short stature and mild intellectual disability.

Mammals. The marked similarity of human exosome cofactors with *S. pombe* MTREC components indicates that functions may be conserved. Indeed, depletion of MTREC homologues PABPN1 or ZFC3H1 leads to misexpression of several poly(A)$^+$ RNAs, including mRNAs[12,68]. An interesting example concerns the auto-regulation of PABPN1 itself. Here, PABPN1 can bind to an A-rich region in the 3′-UTR of its own pre-mRNA, which prevents splicing of the terminal intron and thereby triggers nuclear decay of the unspliced RNA[85]. Although regulation of other targets is presently less well understood, it is likely that nuclear decay has much broader roles than currently appreciated.

Global RNA homeostasis

The ability of nuclear RNA decay systems to impact all RNA types, including mRNA, suggests their utility in regulating global transcript homeostasis. For example, in *S. cerevisiae*, it has been shown that faithful maintenance of total cellular mRNA loads requires a tight balancing of global transcript production and decay. That is, *S. cerevisiae* mutants with impaired RNA decay mechanisms tend to exhibit a compensatory decrease in RNA production, whereas mutants with decreased transcription rates compensate by dampening RNA decay[86–88]. The underlying crosstalk between transcription and RNA decay machineries was implicated to involve the cytoplasmic 5′–3′ exonuclease Xrn1 (REFS[86,87]), which was suggested to regulate gene transcription levels either directly[86] or through post-transcriptional control of the global transcriptional repressor Nrg1 (REF.[87]). However, it is not clear whether such regulation is sufficient to explain balancing in all situations in which global feedback occurs.

Instead, or in addition, nuclear RNA decay systems would be well positioned to contribute to such global balancing by restricting the amount of mRNA released into the cytoplasm (FIG. 3). As discussed above, Nab2 protein levels are controlled by strict auto-regulation via the nuclear exosome[76], indicating that Nab2 availability is tightly coordinated. At the same time, Nab2 is strictly required for protection of nascent poly(A) tails[52], demonstrating that its nuclear concentration can be controlled to restrict mRNA output. Moreover, Nab2 shuttles between the nucleus and cytoplasm, where nuclear export occurs while the protein is bound to mRNA. Hence, Nab2 is perfectly placed to directly sense any changes in nuclear production or cytoplasmic RNA decay, as Nab2 sequestered in the cytoplasm upon global decreases in cytoplasmic decay would rapidly limit transcriptional output (FIG. 3b). Conversely, surplus transcription would not cause RNA overcrowding in the cytoplasm as limiting the concentration of Nab2, or other nuclear RNA-protecting proteins, restricts the cytoplasmic appearance of RNAs (FIG. 3c). This may be a general mode of action for nuclear RNP factors, as all nuclear-enriched poly(A)-binding proteins and cap-binding proteins and numerous other RNP factors are nuclear–cytoplasmic shuttle proteins.

Nuclear RNA decay and disease

Given the diverse roles and global cellular impact of nuclear decay activities, it may come as no surprise that defects in several nuclear RNA decay factors are associated with disease states (TABLE 1). Congenital mutations of RNA exosome core subunits have, for example, been linked to neurological syndromes[89]. Although presently unsettled, such defects might be due to defective nuclear functions, as a similar disease state is caused by mutation of the human NEXT component RBM7[90]. Moreover, a rare case of inherited intellectual disability is linked to defects in the nuclear poly(A)-binding protein ZCCHC14, the homologue of *S. cerevisiae* Nab2[91]. Another well-established case concerns triplet repeat expansion in PABPN1, which causes oculopharyngeal muscular dystrophy (OPMD)[92]. Finally, mutation and aberrant expression of the nucleus-specific exosome component DIS3 has been found in a range of different cancers[93]. Thus, normally functioning nuclear decay systems are essential for organismal health. However, the molecular consequences of these defects that might cause pathology are presently unknown. Given the pronounced activity in bulk removal of spurious transcripts, accumulation of such unwanted RNAs might well contribute to disease states. However, as nuclear decay also contributes to gene expression regulation, it is possible that misexpression of specific mRNAs might be causal.

Apart from their disease-causing mutations, nuclear RNA turnover pathways may also be targeted by cellular pathogens. A recent study demonstrated a requirement for the normally functioning nuclear RNA exosome in influenza virus infectivity[94]. It has long been known that influenza virus employs host RNAs to prime transcription of the viral genome. It now appears that the nuclear exosome is contacted by the influenza virus polymerase B protein and induced to deliver RNA decay fragments that can act as primers for transcription by the viral polymerase[94]. In another study linking the exosome to viral infection, it was suggested that the *Drosophila melanogaster* nuclear exosome cofactors Zcchc7 and Mtr4 (see BOX 3 for a description of mammalian homologues) partake in the cellular defence against several RNA viruses. Curiously, this was suggested to involve export of Zcchc7 and Mtr4 to the cytoplasm and their participation in the decay of viral RNA by the cytoplasmic exosome[95].

It is anticipated that reports on direct roles of nuclear decay pathways, and their decay substrates, in pathological conditions will keep accumulating and yield insight into roles of these complexes in both health and disease.

Conclusions

The cell nucleus is a place of both promiscuous RNA production and destruction. Indeed, our emerging understanding paints a picture of a highly degradative place in which RNA survival requires either swift escape or the presence of specific protective features. Although this might at first glance appear wasteful, it provides an efficient way of controlling pervasive and aberrant transcripts, which either lack such protective features or have impaired export competence. In doing so, it also provides means for more global regulation of mRNA metabolism. Shuttling RNP components such as PABPs — which impact RNA stability while also partaking in nuclear export — is likely to be at the core of such regulation. Altogether, this challenges the classical view that regulation of mRNA turnover is primarily cytoplasmic.

Despite the many new insights, an understanding of the full impact of nuclear RNA decay systems, especially in mammalian cells, is still in its infancy. For example, little is known about how nuclear decay factors and their targets are regulated during dynamic cellular processes. Such regulation might include post-translational modification as exemplified by the stress-induced phosphorylation of the NEXT component RBM7 and the resulting stabilization of lncRNAs[96,97]. Although its physiological impact is unclear, this post-translational modification provides evidence that such regulation can be used by cells to control RNA expression. Given the connectedness of RNA metabolism described here, these regulatory mechanisms are therefore likely to also affect protein-coding genes. Similar regulatory principles will possibly also have relevance for understanding various disease states and, as reported, be implicated in the evolutionary arms races between pathogens and hosts. We are therefore confident that research on nuclear RNA decay systems will continue to reveal unexpected observations of relevance far beyond the RNA research community.

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