RNA PROCESSING AND MODIFICATIONS

The regulation and functions of the nuclear RNA exosome complex

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Abstract | The RNA exosome complex is the most versatile RNA-degradation machine in eukaryotes. The exosome has a central role in several aspects of RNA biogenesis, including RNA maturation and surveillance. Moreover, it is emerging as an important player in regulating the expression levels of specific mRNAs in response to environmental cues and during cell differentiation and development. Although the mechanisms by which RNA is targeted to (or escapes from) the exosome are still not fully understood, general principles have begun to emerge, which we discuss in this Review. In addition, we introduce and discuss novel, previously unappreciated functions of the nuclear exosome, including in transcription regulation and in the maintenance of genome stability.

Spinal muscular atrophy
A neuromuscular disorder characterized by degeneration of specific cells within the spinal cord that leads to muscle weakness and atrophy.

Pontocerebellar hypoplasias
A group of severe neurodegenerative disorders that affect growth and function of the brain, resulting in delayed development and, frequently, early death.

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The RNA exosome complex was initially identified as a central factor in the processing of stable RNA species produced by RNA polymerases I, II, and III (Pol I, II and III), such as rRNAs, small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs) and tRNAs[1–4]. Since then, targets of the exosome complex have been catalogued genome-wide in yeast, flies, mice, humans and plants[5–11], revealing a wide variety of substrates. In all eukaryotic cells, the exosome has a central role in RNA quality control.

An important function of the exosome is the removal of RNAs that arise as a result of cryptic transcription[10,12–19]. 'Cryptic' is the name given to any RNA species that are highly unstable in wild-type cells and therefore can only be detected when nuclear RNA surveillance is compromised[14,15,20,21]. In large part, these cryptic unstable transcripts (or CUTs, as they are known in yeast) are derived from transcription at bidirectional promoters, at which antisense transcripts — termed promoter upstream transcripts (PROMPTs) in humans or upstream antisense RNA (uaRNA) in mice — are rapidly degraded by the nuclear exosome[12,13,20,22]. Similarly, mammalian enhancers give rise to unstable bidirectional transcripts, which are commonly referred to as enhancer RNAs (eRNAs) and are also targeted by the exosome[10,18,19]. Heterochromatin-forming repetitive elements, such as ribosomal DNA (rDNA) repeats and centromeres, are another source of unstable transcripts[21,23–25]. Although cryptic transcripts are commonly short, many intergenic or antisense long non-coding RNAs (lncRNAs) are also rapidly degraded by the exosome. Their degradation frequently depends on the same factors that are involved in the destabilization of short cryptic transcripts[17], which are described below.

In addition to cryptic transcripts, the exosome removes various aberrant transcripts, including stable RNA species that are incorrectly processed and mRNAs that fail to undergo proper splicing or 3′ end formation[7,8,10,26–28]. Accumulation of aberrant RNA species is harmful, because they can compete with properly processed RNAs for factors. For example, accumulation of unprocessed snRNAs has been associated with defective splicing[29]. Moreover, having 'too much' RNA poses a threat in itself: accumulation of exosome substrates in the cytoplasm activates antiviral defence mechanisms and can trigger autoimmunity[30]. In the nucleus, excess RNA can hybridize with homologous genomic loci, forming RNA–DNA hybrids[31]. These can induce DNA double-strand breaks (DSBs) and are associated with increased genomic instability.

In addition, recent studies have revealed that the exosome regulates the levels of specific transcripts in response to environmental cues and is required to orchestrate genetic programmes during development[32–36]. Given all of these functions, it is not surprising that the exosome complex is essential. Deregulation of the exosome leads to severe neurological diseases such as spinal muscular atrophy and pontocerebellar hypoplasias[37,38].

In this Review, we summarize the roles of the exosome complex in the nucleus. First, we give a short description of the structure of the complex, which has been covered in detail in excellent recent reviews[1,39]. We then introduce important cofactors and delineate known mechanisms of exosome recruitment to RNA. We outline the nuclear pathways of mRNA quality control and describe how the exosome is harnessed to regulate mRNA levels. Finally, we discuss recent work describing potential new roles for the RNA exosome

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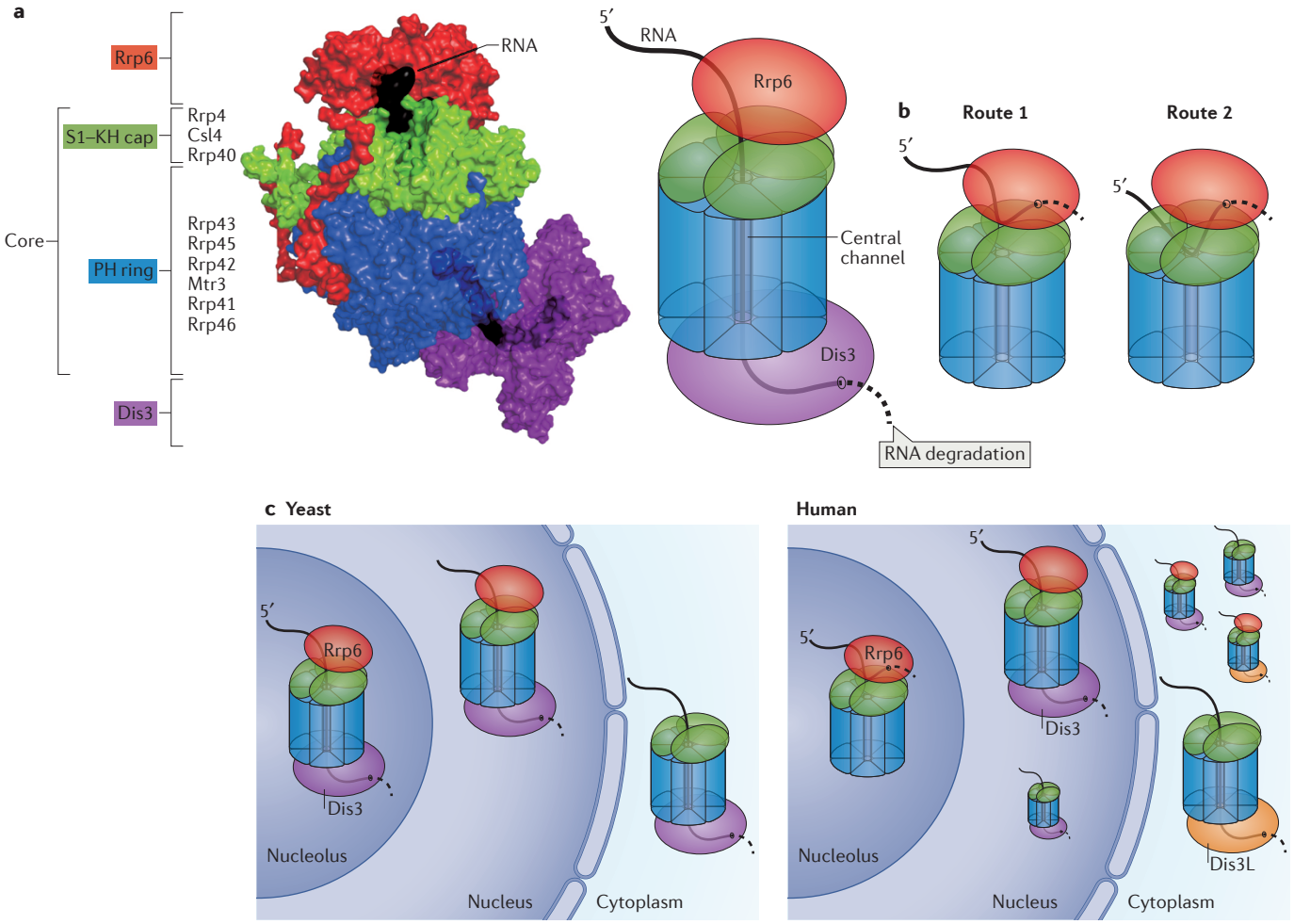

Figure 1 | **Structure of exosome complex isoforms. a** | Left: a model of the exosome $EXO11^{Dis3+Rrp6}$ complex. The surface structure of $EXO11^{Dis3+Rrp6}$ was generated by superimposing the structures of $EXO10^{Dis3+Rrp6Cterm}$ (rRNA-processing protein 6 (Rrp6) in red and the cap complex in green; Protein Data Bank (PDB) ID: 4IFD) and $EXO10^{Rrp6}$ (PH ring complex in blue, chromosome disjunction 3 (Dis3) in purple and RNA in black; PDB ID: 4OO1) from *Saccharomyces cerevisiae*. Right: schematics of an RNA molecule threading 3′ to 5′ through the central channel of the cap and PH ring to the Dis3 exonucleolytic centre, where it is degraded. **b** | There are two possible paths for RNA substrates to the Rrp6 active site when bound to the exosome complex. In route 1, the RNA enters Rrp6 as in part **a**, but instead of going into the central channel, it turns as it exits Rrp6 and reaches the active site with its 3′ nucleotide. In this case, the RNA does not pass through the exosome core. Alternatively, in route 2, the RNA enters the complex from the side, between the PH ring and S1–KH cap, and then bends upwards to reach the active site of Rrp6. Here, the RNA traverses the channel of the S1–KH cap with its 3′ end first from bottom to top and therefore has the opposite orientation to that shown in part **a**, where the RNA enters from the top. **c** | The composition of exosome complexes in different compartments differs between yeast and human. In *S. cerevisiae* (left), exosome complexes with the Rrp6 subunit are exclusively localized in the nucleus, whereas exosomes with Dis3 can be found throughout the cell. In human cells (right), however, Dis3 is excluded from the nucleolus. The Dis3 homologue Dis3L also associates with the exosome core and is restricted to the cytoplasm. In addition, small amounts of exosomes with different exonuclease subunits are present in the cytoplasm (indicated by relative sizes). Csl4, Cep1 synthetic lethal 4; Mtr3, mRNA transport 3.

— for example, in transcription regulation, in the removal of RNA–DNA hybrids and in the DNA damage response (DDR).

The exosome complex and its regulation

The exosome acts on all classes of cellular RNAs at various stages of their existence, from synthesis to maturation and turnover. One of the long-standing questions in the field is how the exosome complex can be targeted to such diverse substrates. This is now known to involve a variety of cofactors that recognize the RNA substrate and help to initiate its degradation by the exosome complex.

The exosome complex. The eukaryotic RNA exosome is an evolutionarily conserved ribonucleolytic complex that consists of 10 or 11 subunits (FIG. 1a). The barrel-shaped nine-subunit core (known as EXO9) is catalytically inactive and comprises the 'cap' structure, which is formed of the three proteins rRNA-processing

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protein 4 (Rrp4), Rrp40 and Cep1 synthetic lethal 4 (Csl4), which contain both the S1 and the KH RNA-binding domains, and the 'ring' complex, which is composed of RNase PH-like proteins Rrp41, Rrp42, Rrp43, Rrp45, Rrp46 and mRNA transport 3 (Mtr3). The core associates with the processive 3′ to 5′ exo- and endoribonuclease chromosome disjunction 3 (Dis3; also known as Rrp44) at the bottom of the PH-protein barrel and/or with the distributive 3′ to 5′ exonuclease Rrp6 (also known as exosome component 10 or PM/Scl-100 in humans) at the cap-side, forming the exosome complexes EXO10^{Dis3}, EXO10^{Rrp6} or EXO11$^{Dis3+Rrp6}$, respectively[1,39,40]. Although some RNAs can be targeted directly to Dis3 or Rrp6, the majority of substrates enter the barrel-like structure of the exosome through a pore at the centre of the S1–KH cap and are threaded through the central channel in a 3′ to 5′ orientation to access the active centre of Dis3 (REFS 41–44). The endonuclease activity of Dis3 facilitates the degradation process[7,45,46]. Although Dis3 is active in isolation, all subunits of EXO10^{Dis3} are essential for viability in *Saccharomyces cerevisiae*[3], suggesting that the requirement for substrates to traverse the channel is important in controlling the activity of the catalytic subunit. In the case of EXO11$^{Dis3+Rrp6}$, the RNA can either be threaded through the channel to reach Dis3, or it can be degraded by Rrp6. The path the RNA takes to Rrp6 is currently not clear. On the basis of a recent crystal structure, it was proposed that the RNA reaches the active site of Rrp6 directly, without making contacts with any of the other exosome subunits[47] (FIG. 1b). However, a different route has also been suggested: crystallographic evidence, together with UV crosslinking experiments, indicated that the 3′ end of the RNA could enter from the side — between the cap and the ring — and then traverse the cap into the Rrp6 active site[44,48]. Which model is correct, or indeed whether both routes are utilized *in vivo*, has yet to be determined. In the case of very structured, bulky RNAs, Rrp6 was shown to detach from the core and swing away from the channel, which allows the substrate to enter the channel directly[47]. It was also demonstrated that Rrp6 can allosterically stimulate the nucleolytic activity of Dis3 in budding yeast[48], probably by direct RNA binding as well as through widening of the channel[44,47]. Which exonuclease is selected *in vitro* seems to be stochastic, and the substrate is repeatedly bound and released until the processive Dis3 is reached, which then degrades the RNA to completion. The path taken may be influenced by the structure of the RNA substrate, as well as by cofactors bound to it[44].

Rrp6, Dis3 or the Dis3-like exonuclease DIS3L in higher eukaryotes interact with EXO9 to form distinct complexes with discrete subcellular localizations[39,40,49] (FIG. 1c). These exosome isoforms are proposed to carry out specialized functions in different cellular compartments. In this Review, we focus mainly on the functions of the nuclear exosome.

Regulators of exosome activity. On its own, the exosome will degrade any RNA, provided it is not too structured. However, the activity of the isolated complex is comparatively weak[3,50,51]. Interestingly, *in vitro*, substrate turnover by Dis3 is significantly increased in the absence of the EXO9 core[48]. In addition, the length of unstructured sequence at the 3′ end that is required for Dis3 to be able to degrade an RNA drops from 30–35 bp to 7–9 bp in the absence of EXO9 (REFS 51,52), demonstrating that the long central channel restricts substrate access to the nuclease. Befittingly, it is becoming increasingly apparent that RNA-unwinding activities are central to the regulation of the exosome, as they facilitate threading of the RNA substrate through the narrow channel entrance. In *S. cerevisiae*, two related conserved DExH-box helicases, Mtr4 and superkiller 2 (Ski2), are required for RNA degradation in the nucleus and the cytoplasm, respectively[53,54]. Both helicases are associated with accessory factors. Mtr4, for example, interacts with a non-canonical poly(A) polymerase (Trf4 (also known as Pap2) or Trf5) and a Zn-knuckle RNA-binding protein (Arg methyltransferase-interacting RING finger 1 (Air1) or Air2) to form the Trf4/5–Air1/2–Mtr4 polyadenylation complex (TRAMP)[55]. This complex facilitates the degradation of substrates by the exosome: addition of short unstructured poly(A) tails by Trf4 or Trf5 is thought to provide 'grip' for Mtr4, which then unwinds the RNA and feeds it into the exosome complex[17,56].

The central role of Mtr4 in nuclear RNA turnover is widely conserved. However, apart from *S. cerevisiae*, most organisms seem to have developed a division of labour between a nucleolar complex, which is associated with a poly(A) polymerase, and a nucleoplasmic complex, which is not (TABLE 1). In humans, for example, MTR4 (also known as SKIV2L2) associates with two such separate complexes: in the nucleoplasm, it is part of the nuclear exosome-targeting (NEXT) complex, which mediates decay of PROMPTs and eRNAs and controls levels of snRNA precursors[18,57,58]. The second complex resembles yeast TRAMP and contains the poly(A) polymerase TRF4-2, but it is exclusively nucleolar and is predominantly involved in rRNA processing[18]. In the fission yeast *Schizosaccharomyces pombe*, Mtr4 associates with the poly(A) polymerase Cid14 and is also enriched in the nucleolus, where it is involved in the processing of rRNA intermediates and the turnover of heterochromatic RNA[21,25,59]. Fission yeast TRAMP is complemented by an additional, Mtr4-like helicase, Mtl1, for the turnover of cryptic transcripts and aberrant mRNAs, which is presumed to take place in the nucleoplasm[60–62]. Similarly, the predominantly nucleolar *Arabidopsis thaliana* MTR4 (encoded by *AT1G59760*) is aided by a nucleoplasmic helicase, HUA ENHANCER 2 (HEN2), which is plant-specific[63]. In addition, other helicases, such as the mammalian DEAD-box protein DDX5 and the DEAH-box helicase RHAU (also known as DHX36), are thought to facilitate RNA decay by the exosome complex[53].

Targeting RNA to the exosome. RNA helicases, like the exosome complex itself, are largely unspecific[53,54,64,65]. Thus, selective targeting of the exosome *in vivo* relies on the ability of primary exosome-specificity factors (ESFs)

PH-like proteins
Proteins that resemble the bacterial ribonuclease PH, which is a phosphorolytic exoribonuclease.

DExH-box
A short motif that characterizes a family of RNA- or DNA-binding, ATP-hydrolysing proteins, many of which are helicases. The DEAD-box is a similar motif.

Non-canonical poly(A) polymerase
Member of a conserved subclass of nucleotidyl transferases. Related to the canonical poly(A) polymerase, which catalyses the addition of poly(A) tails to mRNAs, but with diverse substrates and functions.

Zn-knuckle
A zinc-binding structural motif that frequently mediates interactions with nucleic acids.

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Table 1 | **Exosome-activating Mtr4-like helicase complexes in different organisms**

Organism	Complex components and their functions		Refs
	Nucleoplasm	Nucleolus	
S. cerevisiae	• Mtr4 within TRAMP • Poly(A) polymerases Trf4 and Trf5 (add short poly(A) tails) • Zn-knuckle proteins Air1 and Air2 (RNA-binding proteins) • Substrates: cryptic transcripts, misprocessed RNA, replication-dependent histone mRNAs*	• Mtr4 within TRAMP • Poly(A) polymerasesTrf4 and Trf5 • Zn-knuckle proteins Air1 and Air2 • Substrates: rRNA precursors, heterochromatic RNA*	14, 23, 55, 119
S. pombe	• Mtl1 within MTREC • Substrates: cryptic transcripts, misprocessed RNA, transposons, meiotic mRNAs	• Mtr4 within TRAMP • Poly(A) polymerase Cid14 • Zn-knuckle protein Air1 • Substrates: rRNA precursors, heterochromatic RNA	21, 25, 32, 60–62
H. sapiens	• MTR4 within NEXT • Zn-knuckle protein ZCCHC8 (RNA-binding protein) • Substrates: cryptic transcripts, misprocessed RNA, transposons	• MTR4 within TRAMP • poly(A) polymerase TRF4-2 • Zn-knuckle protein ZCCHC7 (RNA-binding protein) • Substrates: rRNA precursors	18, 57
A. thaliana	• HEN2‡ • Zn-knuckle proteins AT5G38600 (RNA-binding protein) • Substrates: cryptic transcripts, misprocessed RNA, transposons	• MTR4‡ • Substrates: rRNA precursors	63

Air, Arg methyltransferase-interacting RING finger 1; *A. thaliana*, *Arabidopsis thaliana*; HEN2, HUA ENHANCER 2; *H. sapiens*, *Homo sapiens*; Mtr4, mRNA transport 4; MTREC, Mtl1–Red1 core; NEXT, nuclear exosome targeting complex; *S. cerevisiae*, *Saccharomyces cerevisiae*; *S. pombe*, *Schizosaccharomyces pombe*; TRAMP, Trf4/5–Air1/2–Mtr4 polyadenylation complex. *The different targets of nucleoplasmic and nucleolar TRAMP are inferred by localization and homology only. ‡In *A. thaliana*, the complexes that contain Mtr4-like helicases have not been named.

to recruit the helicases to specific substrates (FIG. 2a). To date, only a handful of ESFs have been identified. The earliest-known examples were proteins that bind to AU-rich elements (AREs), which are often present in the 3′ untranslated regions (UTRs) of unstable mRNAs[66]. ARE-binding proteins (ARE-BPs) are found in all eukaryotes and regulate RNA metabolism. Importantly, they mediate decay of ARE-containing mRNAs by the exosome[66]. In humans, ARE-BPs recruit the DEAH-box helicase RHAU, which interacts with the exosome complex and enhances ARE-dependent RNA decay[67] (FIG. 2b).

The nucleolar rRNA-processing factors U3 protein 18 (Utp18) and nucleolar protein 53 (Nop53) in *S. cerevisiae* were recently shown to recruit TRAMP to different rRNA-processing intermediates[68]. Both proteins contain a conserved arch interaction motif (AIM), which directly binds to the conserved arch domain of Mtr4. Also in *S. cerevisiae*, the trimeric complex NNS, which is composed of the RNA-binding proteins nuclear pre-mRNA downregulation 1 (Nrd1) and nuclear polyadenylated RNA-binding 3 (Nab3) and the helicase splicing endonuclease 1 (Sen1), recognizes sequence elements that are enriched on unstable Pol II-transcribed ncRNAs. NNS also associates with snRNAs and snoRNAs that are known to undergo 3′ processing by the exosome complex[69–71]. Through its interaction with Pol II early in the transcription process, NNS mediates transcription termination and hands the transcripts over to Mtr4-containing TRAMP complexes, which feed the RNA into the exosome complex[72–75] (FIG. 2c). This specialized machinery for transcription termination-coupled decay seems to be unique to budding yeast. By contrast, human PROMPTs, which rely on the conventional polyadenylation and cleavage machinery for transcription termination[76,77], are targeted to the exosome by NEXT[18,57] (TABLE 1). The exact mechanism, however, remains unclear.

In *S. pombe*, the RNA-binding protein Mmi1 is co-transcriptionally recruited to sequence elements known as 'determinants of selective removal' (DSRs), which are enriched on meiotic mRNAs[32,78]. Mmi1 binding programmes transcripts for rapid exosome-dependent decay[32,79,80] and — together with other proteins — recruits the helicase Mtr4-like protein 1 (Mtl1), which is required for transcript turnover[60,62] (FIG. 2d). The Mmi1 pathway is essential to suppress the activation of the meiotic programme during mitotic growth, but it has also been found to mediate degradation of regulatory ncRNAs[32,34,35,81]. Intriguingly, Mmi1 binding to RNA involves its YTH domain[82]. Proteins of the YTH family have recently attracted interest, because they were shown to selectively bind to N^6-methylated adenosines on RNA[83]. The residues involved in methyl recognition are conserved in Mmi1 (REF. 84), and Mmi1 binding overlaps with mapped sites of N^6-methyl adenosine on snRNAs (C. Kilchert, S. Granneman and L. Vasiljeva; unpublished results), raising the possibility that nuclear RNA decay by the exosome could be regulated by RNA modifications.

Altogether, the ability of ESFs to recognize specific RNA sequence elements, secondary structures and/or modifications allows cells to distinguish between different RNA species. It has to be noted that although the known ESFs recognize sequences that are very common in the genome, actual binding often seems to be more selective[16,35]. This suggests cooperativity for RNA binding among different ESFs or different components of

YTH domain
A conserved RNA-binding domain named after the splicing factor YT521-B. YTH domains of some proteins specifically recognize methylated adenosines.

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ESF complexes. In addition, cooperative binding of multiple elements, which is exemplified by the dual affinity of Nrd1 for early elongating Pol II and RNA sequence elements[72,85] (FIG. 2c), may allow for selective targeting. Combined, substrate recognition by ESFs and the selective recruitment of RNA helicases that facilitate RNA degradation form the basis for the regulatory functions of the exosome complex.

Negative regulators of exosome function. There are two different ways in which the activity of the exosome can be negatively regulated. First, factors that have a general RNA-stabilizing capacity can bind to RNA to prevent its targeting to the exosome. Such a role was described for the nuclear poly(A)-binding protein Nab2 in *S. cerevisiae*, which is recruited early during mRNA biogenesis and prevents decay by the exosome[86,87]. Other substrates

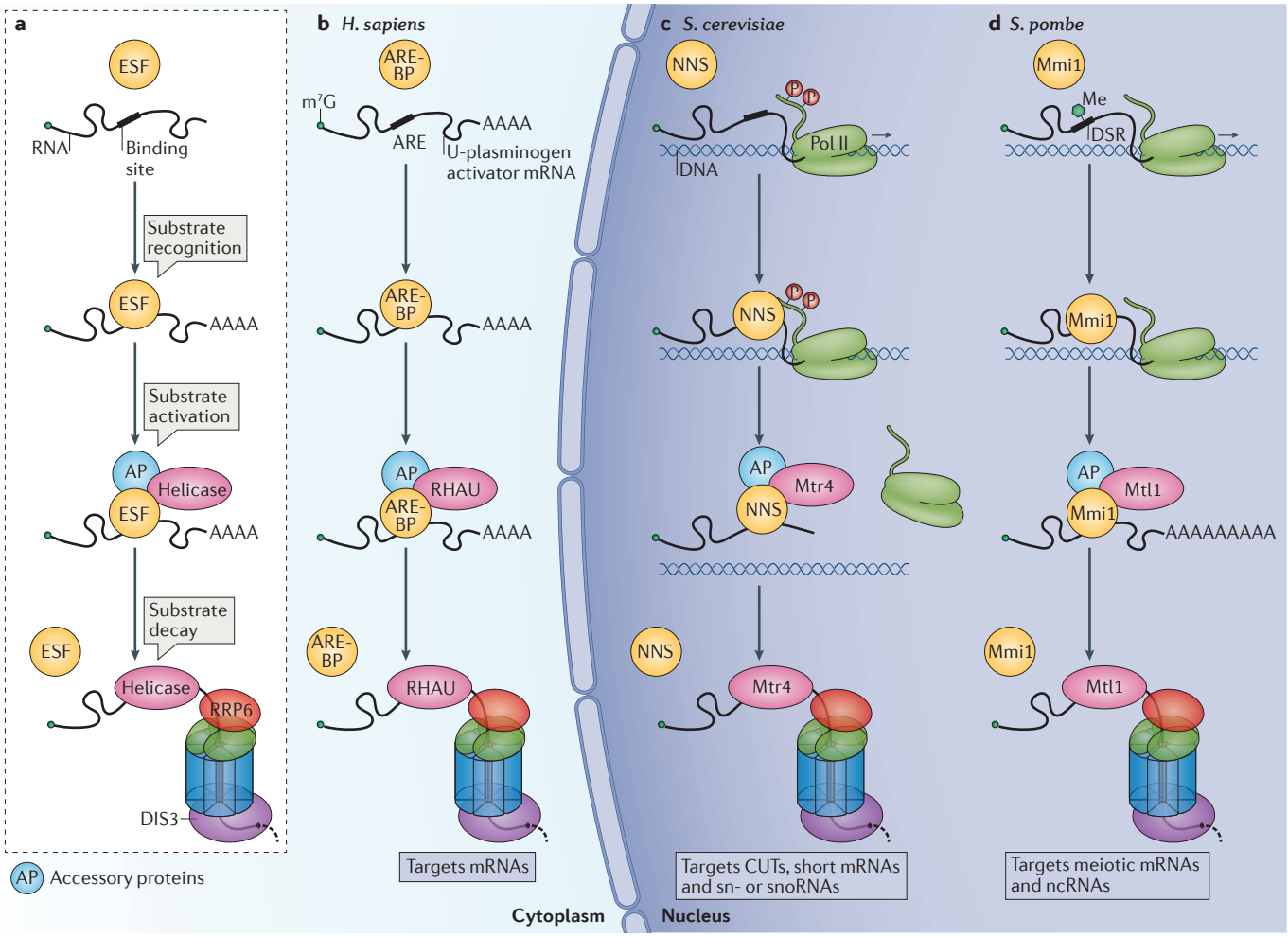

Figure 2 | **Conserved features of RNA targeting to the exosome.** **a** | Schematic model of the steps that lead to degradation of a generic exosome substrate. First, a binding site on the RNA is recognized by an exosome specificity factor (ESF). Substrate recognition may depend on RNA sequence, secondary structure and/or RNA modifications. The ESF then recruits an exosome-activating complex that contains a conserved RNA helicase (substrate activation). Recruitment of the helicase can either occur directly or be mediated by accessory proteins (APs) contained within the complex. RNA unwinding renders the substrate susceptible to degradation by the exosome complex. **b** | Tightly regulated, short-lived human mRNAs frequently contain AU-rich elements (AREs), for example, urokinase-type (U) plasminogen activator mRNA or cytokine-encoding mRNAs. AREs are recognized by ARE-binding proteins (ARE-BPs), which can recruit RNA helicases such as RHAU to enable subsequent degradation of the RNA by the exosome. **c** | Cryptic transcripts in *Saccharomyces cerevisiae* are rapidly degraded by the exosome complex. The trimeric complex of Nrd1–Nab3–Sen1 (NNS) binds to nascent RNA that is enriched with specific sequence motifs. Nrd1 (nuclear pre-mRNA downregulation) simultaneously interacts with phosphorylated Ser5 in the heptad repeats of the carboxy-terminal domain of RNA polymerase II (Pol II). This modification is a hallmark of early elongating Pol II. Through an unknown mechanism, Nrd1 induces termination of Pol II transcription. Nrd1 is then free to bind to Trf4 and to thus recruit the Mtr4-containing TRAMP (Trf4/5–Air1/2–Mtr4 polyadenylation) complex to the RNA to unwind it for degradation by the exosome complex. The NNS pathway is mostly restricted to short transcripts, because it depends on the recognition of early elongating Pol II. **d** | During mitotic growth in *Schizosaccharomyces pombe*, meiotic mRNAs that are enriched with sequence motifs termed determinants of selective removal (DSRs) are rapidly degraded by the exosome complex. DSRs can be bound by the YTH protein Mmi1. Work on other YTH proteins suggests that DSR recognition could potentially involve N^6-methylation of adenosines within the motif. Mmi1 recruits various factors, which are involved in regulating the stability of the transcript, including RNA elimination defective protein 1 (Red1), a constituent of the MTREC (Mtl1–Red1 core) complex that contains the helicase Mtr4-like protein 1 (Mtl1) (TABLE 1). RNA unwinding renders the substrate susceptible to degradation by the exosome complex. Mmi1 also targets several regulatory non-coding RNAs (ncRNAs) and precursor mRNAs (pre-mRNAs). *H. sapiens*, *Homo sapiens*.

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are only partially degraded by the exosome complex, for example, during 3′ end processing of stable rRNAs, snRNAs and snoRNAs[4,88]. Here, it is generally believed that the presence of protective sequence features shields stable transcripts from complete decay, although direct experimental evidence is scarce. For example, the Sm ring binds at the 3′ ends of mature snRNAs and of yeast telomerase RNA and is thought to physically impede RNA degradation by the exosome. Insertion of an Sm binding site was shown to stabilize the unstable non-coding transcript NTS[29]. By contrast, human telomerase RNA is protected by a snoRNA-like domain at its 3′ end[89]. snoRNA-like structures also stabilize several human sno-lncRNAs[90]. This and other mechanisms that allow RNAs to escape from degradation by the exosome are the focus of a recent review[91].

Exosome roles in mRNA quality control

To yield functional transcripts, mRNAs need to be extensively processed. Like many complex biological processes, RNA processing is intrinsically error prone and can result in the production of dysfunctional and potentially toxic molecules. The exosome complex has a central role in nuclear mRNA quality control, and aberrant mRNA species accumulate massively following its depletion. However, the mechanisms that underlie exosome targeting to defective mRNAs are not well understood. In particular, the timing of quality control relative to RNA synthesis is uncertain. In contrast to the cytoplasm, in which exosome-dependent degradation takes place in the context of translation, nuclear RNA decay is intimately linked to transcription. There is a general notion that susceptibility to degradation by the nuclear exosome is a fate that is set co-transcriptionally[71,92,93]. It is currently unclear whether the triggering of decay following the recognition of an error is also limited to this narrow temporal window, or whether nuclear mRNA quality control can function post-transcriptionally.

Quality control of 3′ end processing. One type of transcripts that are co-transcriptionally targeted to the exosome are those in which transcription fails to terminate at the polyadenylation site (PAS). In *S. cerevisiae*, such read-through transcription frequently terminates through non-conventional mechanisms, for example, at transcription roadblocks constituted by DNA-binding proteins such as RNA polymerase I enhancer binding protein 1 (Reb1); at sites that are recognized by the NNS complex; or following cleavage of the nascent RNA by the RNase III enzyme Rnt1, which acts on double-stranded RNA. Such non-conventional termination events are coupled to rapid RNA decay by the exosome complex[94–97]. Sites of non-conventional termination are enriched downstream of genes and are thought to function as 'fail-safes' that protect neighbouring genes from transcriptional interference[98].

Splicing-coupled quality control. An important class of substrates for the nuclear exosome are precursor mRNAs (pre-mRNAs) with retained introns[7,8,28,99]. Accumulation of pre-mRNAs in exosome mutants is frequently accompanied by an increase in levels of the spliced transcript, suggesting that at least some of the pre-mRNAs targeted by the exosome have the potential to complete splicing[8,28]. Hence, it has been proposed that splicing and the quality control pathways that lead to transcript degradation by the exosome kinetically compete for their pre-mRNA substrates. Slow splicing leads to the degradation of a large proportion of pre-mRNAs and, as a result, to less mRNA production, whereas faster splicing converts more pre-mRNA into mRNAs. However, how decay is initiated on pre-mRNAs remains obscure. Across organisms, nuclear poly(A)-binding proteins have been implicated in pre-mRNA turnover and can, in some cases, also affect splicing efficiency[86,92,99–102]. In addition, at least in human cells, susceptibility to nuclear poly(A)-binding protein-mediated decay seems to correlate with inefficient nuclear export and prolonged nuclear retention[103]. In *S. cerevisiae*, unspliced pre-mRNAs are bound by the SR proteins G-strand binding protein 2 (Gbp2) and hypothetical RNA-binding protein 1 (Hrb1), which prevent their nuclear export[92,104]. In the absence of these factors, pre-mRNAs with retained introns evade degradation by the exosome and escape into the cytoplasm[104]. Splicing, or at least commitment to splicing, is now thought to occur co-transcriptionally in most organisms, and cross-talk with quality control factors has been observed[105,106]. In budding yeast, for example, co-transcriptional recruitment of the TRAMP complex to introns was found to stimulate splicing[106]. In *S. pombe* and humans, there is evidence for direct interactions of exosome cofactors with the spliceosome, and disruption of these interactions leads to an increase in pre-mRNA levels[60,61,107]. Together, this suggests that splicing and mRNA quality control form an integrated process. However, a clear picture of the regulation of this process has yet to emerge.

Quality control of RNA packaging. The exosome also degrades mRNAs with defects in their co-transcriptional packaging into ribonucleoprotein particles (mRNPs), for example, in *S. cerevisiae* cells with mutations in factors of the THO complex[108,109]. The THO complex is recruited to nascent transcripts and is an important RNA-packaging factor that links mRNA transcription with nuclear export. How prevalent packaging defects are in physiological contexts remains unclear, but the genetic links between mRNP biogenesis factors and Rrp6 are strong: as well as transcript destabilization, mutations in THO can result in premature transcription termination and RNA retention at the site of transcription (see below), in a manner dependent on Rrp6 (but not on the exosome in general)[109,110].

Retention of aberrant transcripts on the chromatin. Decay of faulty transcripts is only one aspect of nuclear quality control. Across eukaryotes, release of aberrant transcripts from the chromatin is inhibited[109,111–114]. Retention at the site of transcription seems to be an almost universal response to RNA-processing failure and was observed following defects in diverse maturation processes such as mRNA splicing, 3′ end processing, and mRNP formation and export. In all cases, RNA

Sm ring
Heptameric protein complex that associates with Sm sites located at the 3′ end of small nuclear RNAs (snRNAs) and telomerase RNA and stabilizes them.

sno-lncRNAs
A class of long non-coding RNAs (lncRNAs) that is protected from degradation by the presence of a small nucleolar RNA (snoRNA) fold. Their biological function is unknown.

SR proteins
A conserved family of proteins involved in RNA splicing, characterized by a domain with Ser and Arg repeats.

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retention on the chromatin was found to depend on Rrp6 (REFS 109,111–113). However, the molecular mechanism remains unclear. Defects were frequently associated with either hyper- or hypo 3′ polyadenylation, and the retained mRNAs presumably lack translation competence. This has been confirmed for mRNAs that accumulate in mutants of the mRNA export factor *mex67* in *S. cerevisiae*[115]: when release of the retained mRNAs was forced by overexpression of export factors, no protein was produced. By contrast, retained mRNAs which were released from the chromatin over time were translationally active, indicating that they had the potential to mature into functional mRNPs[115]. Thus, retention of aberrant mRNA at the chromatin may have a dual purpose: it prevents release of faulty transcripts into the cytoplasm, while possibly also providing transcripts with compromised processing additional time to complete their maturation. Susceptibility to decay by the exosome and retention at the chromatin are both features that are more typically associated with ncRNAs[116], and it is striking that mRNAs with processing defects seem to acquire a ncRNA fate. For some ncRNAs, Rrp6-dependent tethering to the chromatin has been convincingly linked to their function. One notable example is from recent work in *A. thaliana*, in which RRP6L1 — one of three Rrp6-like proteins in plants — was found to be involved in tethering a Pol V-transcribed scaffold RNA to the chromatin, where it functions as a guide for RNA-directed DNA methylation and gene silencing[117].

Regulation of gene expression

Orchestrated changes in mRNA stability play an important part in enabling transitions between different metabolic states and in quick adaptation to external cues[118]. Previously, it has been assumed that modulation of mRNA stability is primarily coordinated in the cytoplasm. However, it has now become clear that the nuclear exosome also targets and represses the expression of specific genes or complete genetic programmes.

Regulation of decay by mRNA processing. A striking case of regulated mRNA decay is the Mmi1-dependent degradation of meiotic mRNAs during mitotic growth in *S. pombe*, which prevents the untimely induction of meiosis (FIG. 2d). When cells enter meiosis, Mmi1 is sequestered in nuclear foci. This abrogates Mmi1-dependent decay and results in the stabilization of meiotic transcripts[32]. Another example comes from *S. cerevisiae*, in which Rrp6p is known to rapidly degrade mRNAs that encode replication-dependent histones as cells leave the S phase. The process is TRAMP-dependent (TABLE 1), but the mechanism of TRAMP targeting remains unknown[119,120]. Interestingly, decreased RNA stability is frequently a consequence of changes in mRNA processing (FIG. 3). *S. cerevisiae NRD1* mRNA, for example, contains multiple NNS-binding sites[121]. If levels of Nrd1 are low, transcription of the mRNA terminates normally, and it is stably expressed. However, if Nrd1 levels are high, recruitment of the NNS complex to *NRD1* mRNA induces premature termination, thus reducing gene expression in an auto-regulatory feedback loop[121] (FIG. 3a). Other mRNAs were also suggested to be repressed by premature termination[122]. The mRNAs that contain cryptic introns are another example. These are generally spliced at low rates, but splicing can be activated in response to environmental cues. Splicing of cryptic introns can be coupled to RNA decay by the exosome (FIG. 3a), which is then referred to as spliceosome-mediated decay (SMD)[123]. For example, the bromodomain protein Bdf1 in *S. cerevisiae* facilitates recruitment of the spliceosome to a cryptic splice site in the *BDF2* mRNA. In striking contrast to the conventional scenario, in which splicing generates functional mRNA, splicing products in this case are rapidly degraded by the exosome complex, thereby reducing the levels of functional (unspliced) *BDF2* transcript[123]. For Bdf2, this mechanism is particularly active during the DDR[124]. SMD was shown to repress several genes in *S. cerevisiae*[123]. In addition, recent studies in *S. pombe* identified cryptic introns in many exosome targets, suggesting that the mechanism may be conserved[27,60].

In both activation of premature termination and SMD, the unprocessed transcripts are functional protein-coding mRNAs, whereas RNA processing induces RNA decay (FIG. 3a). This novel role for RNA processing as a negative regulator of gene expression is opposed to its classical positive contribution to gene expression by generating functional mRNAs. Conversely, transcript decay by the exosome can also be induced by suppression of mRNA processing. This exploits the stringent nature of nuclear quality control, which ensures that unprocessed mRNAs are rapidly degraded (FIG. 3b). Negative regulation of mRNA processing can take place at several stages during mRNA maturation, but regulated intron retention and inhibition of 3′ end processing have been most frequently reported[33,96,99,100,125,126]. It is striking that suppression of mRNA processing is particularly prevalent among the transcripts of RNA-binding proteins, which frequently destabilize their own transcripts in auto-regulatory feedback loops. For example, recruitment of ribosomal 60S subunit Rpl9 to a structural element in the *RPL9B* mRNA prevents efficient transcription termination in *S. cerevisiae*[96]. The read-through product is terminated at a downstream NNS-dependent site and is rapidly degraded by the exosome. The poly(A)-binding protein Nab2 is also involved in the 3′ end processing of its own mRNA[127].

Coordination of intron retention and pre-mRNA turnover. Auto-regulation of other RNA surveillance factors, such as the human nuclear poly(A)-binding protein PABN1, or the RNA helicase DEAD-box protein 2 (Dbp2) and the mRNA export factor yeast RNA annealing protein 1 (Yra1) in *S. cerevisiae*, involves intron retention[100,125,126]. In yeast, multiple genes are duplicated, and expression of paralogous genes is frequently controlled by negative feedback regulation. For example, splicing of mRNAs that encode ribosomal proteins was shown to be repressed in the presence of the paralogous protein, which presumably binds to the pre-mRNA to interfere with RNA processing[99,128–130]. In several cases, paralogue-mediated inhibition of splicing was shown to be coupled to RNA decay by the exosome[35,99] (FIG. 3b).

Cryptic introns
Introns that are usually spliced at low frequency. Their splicing can be activated in response to external cues and does not necessarily generate a functional transcript.

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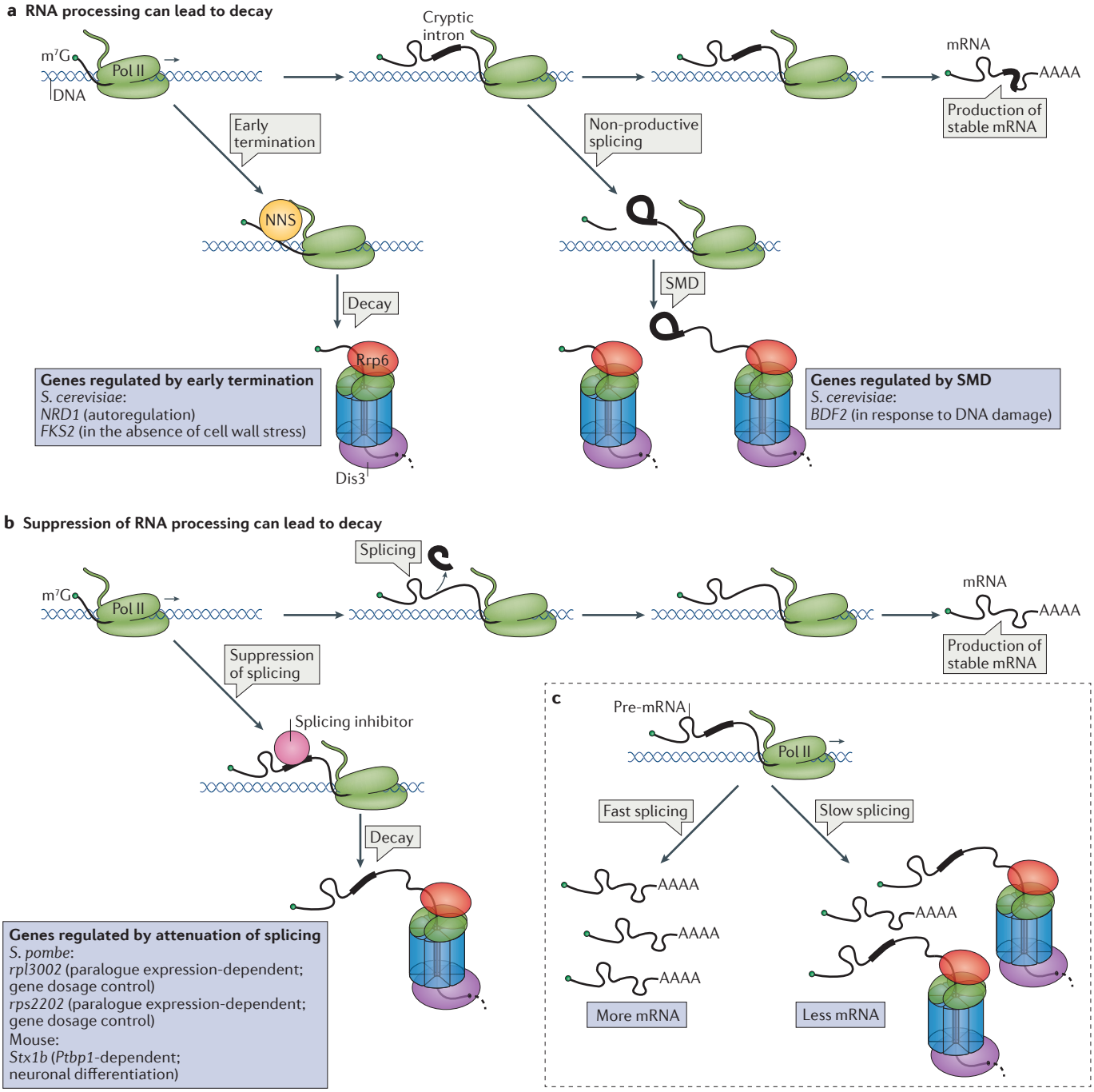

During cellular differentiation, coordinated intron retention in multiple transcripts has been observed in human cells, resulting in pre-mRNA decay in the cytoplasm or in the nucleus[33,131–133]. Here, negative regulation of RNA processing is central to the efficient execution of genetic programmes.

The fraction of pre-mRNAs that is degraded by the nuclear exosome under normal growth conditions is substantial[7,8]. In *S. cerevisiae dis3* mutants, 49% of intronic sequences were found to be upregulated. In 46% of cases in which the pre-mRNA was stabilized, levels of the spliced transcript also increased[8]. Why intron-containing mRNAs are subject to such high levels of turnover has been a matter of debate. Deletion of introns in yeast genes frequently resulted in reduced fitness under stress[134], indicating that their presence confers an advantage during cellular adaptation. We suggest that splicing efficiency at many genes could be fine-tuned to render introns 'sensitive to quality control' (FIG. 3c). Futile cycles of RNA synthesis and decay could provide the cell with great regulatory flexibility, as slowing down splicing compared to its normal rate could, through subjection of the RNA to decay, considerably reduce the expression levels of an intron-containing gene.

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Figure 3 | **RNA surveillance by the exosome cooperates with RNA processing to regulate mRNA levels.** **a** | RNA processing can lead to decay at different stages of mRNA biogenesis. Early during transcription, binding of the *Saccharomyces cerevisiae* Nrd1–Nab3–Sen1 complex (NNS) to nascent mRNA can induce transcription termination coupled to decay by the exosome, and thus suppression of gene expression (left). For example, the *NRD1* (nuclear pre-mRNA downregulation) mRNA contains several Nrd1-binding sites, which are bound by the NNS complex when Nrd1 protein levels are high, thereby inducing premature transcription termination. When Nrd1 levels are low, full-length *NRD1* mRNA can be produced. Similarly, mRNA transcribed from the stress-responsive gene *FKS2* (FK506 sensitivity 2) contains Nab3-binding sites and is terminated prematurely under normal growth conditions. Under conditions of cell wall stress, the physical association of a MAP kinase with the gene locus prevents the recruitment of NNS and suppresses premature termination. Hence, more *FKS2* mRNA is generated. Other mRNAs contain cryptic introns that need to be retained to produce functional transcript (right). During transcription elongation, these introns are generally spliced at low frequency. However, in response to environmental cues, splicing of cryptic introns can be activated but remains non-productive. These splicing products are then rapidly degraded by the nuclear exosome in a process termed spliceosome-mediated decay (SMD). When levels of SMD are high, mRNA production is low. For example, splicing of a cryptic intron in the *BDF2* (bromodomain factor 2) mRNA is activated following DNA damage. **b** |Suppression of RNA processing can lead to decay at various stages of mRNA biogenesis. For example, recruitment of a splicing inhibitor can lead to intron retention. Precursor mRNAs (pre-mRNAs) with retained introns are rapidly degraded by the exosome complex. Known cases include the ribosomal protein genes *rpl3002* and *rps2202* in *Schizosaccharomyces pombe*. Here, it was proposed that the protein products of the paralogous genes, *rpl3001* and *rps2201*, respectively, may bind to the retained introns and suppress splicing, thus reducing mRNA levels and controlling ribosomal protein homeostasis. During mouse neuronal development, expression of polypyrimidine tract-binding protein 1 (PTBP1) is decreased. Reduction of PTBP1 levels alleviates the suppression of splicing and degradation by the exosome of several mRNAs that encode neuronal proteins, including syntaxin-binding protein 1 (STXB1), which is a neuron-specific presynaptic protein. **c** | Schematic depicting the interplay between regulated RNA processing, exosomal degradation and mRNA levels for the example of intron retention. Under conditions in which splicing occurs rapidly, all nascent transcripts mature productively, and a large amount of mRNA is generated. When splicing is slow, a large proportion of transcripts are degraded, and little mRNA is produced. Through this mechanism, negative regulation of RNA processing can repress gene expression very efficiently.

Interestingly, introns sometimes contain features that render them particularly sensitive to quality control. The introns of *RPL18A* and *RPL22B* in *S. cerevisiae*, for example, contain an RNase III cleavage site that renders the pre-mRNAs unstable and restricts the expression of both genes[135]. In *S. pombe*, Mmi1 is recruited to several intron-containing genes, including *rps2202*, which is the homologue of *RPL22B*. Mmi1 binding induces rapid exosome-mediated decay of the pre-mRNA and limits the level of spliced transcript. Under conditions where splicing occurs rapidly, Mmi1 is no longer recruited, and gene repression is relieved[35]. We refer to such quality control-hypersensitive introns as 'decay-promoting' and suggest that they are associated with genes that require particularly tight regulation.

Box 1 | **R-loops**

R-loops are RNA–DNA-hybrid structures that are by-products of transcription[158,159]. Sporadically, nascent RNA will invade the transcription bubble and hybridize with the template DNA strand, which leaves the non-template strand exposed as a single-stranded loop and can lead to polymerase stalling (see FIG. 4). For various reasons, R-loops frequently lead to DNA double-strand breaks (DSBs), to such an extent that they contribute significantly to genomic instability[158,159]. Accordingly, cells employ various means to restrict R-loop formation. This includes both preventive measures, such as coating the nascent RNA with splicing or packaging factors such as the THO complex, and salvage pathways, such as hybrid unwinding by helicases such as splicing endonuclease 1 (Sen1; SETX in humans)[163,164] or the degradation of RNAs caught in R-loops by RNase H[158,159]. Recently, it was suggested that RNA–DNA hybrids can also form in *trans*, when RNA hybridizes with distant, homologous genomic sequences. RNA–DNA-hybrid formation in *trans* is mediated by the homologous recombination machinery and is integral to RNA template-mediated DSB repair[31,157].

Additional functions of the exosome

Mutation in exosome components can result in very complex phenotypes. Recent studies have identified several functions for the exosome complex that go beyond its well-known role in the post-transcriptional regulation of RNA stability; for example, it has been implicated in the regulation of transcription and in the maintenance of genome integrity.

Transcription. Recent studies suggest that the exosome complex may play a part in transcription termination. In *S. cerevisiae*, Rrp6 is required for the recruitment of Nrd1 to the phosphate metabolism 84 (*PHO84*) locus and for early, NNS-dependent transcription termination of a regulatory antisense transcript produced from the locus[136]. A recent genome-wide study identified additional transcripts in budding yeast that depend on Rrp6 for transcription termination[137]. In *S. pombe*, Rrp6 is required for proper termination of a regulatory ncRNA, but not of the overlapping *pho1* mRNA that shares the same PAS[34]. Although this observation is intriguing, the mechanism of transcription termination of the ncRNA remains unclear. Also in fission yeast, depletion of Dis3 led to the accumulation of mRNAs with extended 3′ ends and increased association of Pol II with chromatin downstream of PASs[138]. Here, it was proposed that Pol II might stall, backtrack, and thus expose the 3′ OH end of the nascent transcript, which would be susceptible to decay by the exosome. This could enable the exosome to displace Pol II from the nascent transcript, causing both transcription termination and transcript degradation. This 'reverse torpedo' model is somewhat analogous to the 'torpedo' model of transcription termination after PAS cleavage, which posits that degradation of the nascent RNA by a 5′ to 3′ exonuclease (ribonucleic acid trafficking 1 (Rat1) in *S. cerevisiae*; 5′–3′ exoribonuclease 2 (XRN2) in humans) helps to disassociate Pol II from the DNA[139,140]. Exosome depletion had no effect on Pol II levels upstream of the PAS[138], suggesting that exosome-dependent Pol II displacement functions as a fail-safe rather than as a constitutive mechanism of transcription termination. In mammalian cells at least, termination regions of many genes are prone to form RNA–DNA hybrids ('R-loops', see BOX 1), which induce Pol II pausing and could favour its backtracking[141].

It should be noted, however, that transcriptional changes in exosome mutants can often be indirect. In *S. pombe*, heterochromatin is transcriptionally silenced through an RNA interference (RNAi)-dependent mechanism that results in the deposition of the repressive chromatin mark histone 3 Lys9 trimethylation (H3K9me3). In *rrp6* mutants, H3K9me3 deposition is deregulated, which leads to reduced silencing of constitutive

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Ataxia with oculomotor apraxia 2
(AOA2). A neurodegenerative disorder characterized by juvenile onset of progressive cerebellar ataxia and peripheral neuropathy.

heterochromatic regions such as centromeres and induces the formation of novel heterochromatin domains that are not present in wild-type cells. The altered pattern of heterochromatinization correlates with changes in the expression levels of small RNAs in the exosome mutant[25,142–145]. Equally, the exosome in *A. thaliana* was found to control the expression of several small interfering RNAs (siRNAs). Following the mutation of the exosome core subunit RRP45B, but not of its paralogue RRP45A, specific siRNA species accumulated, leading to increased repression of their target genes[146]. The opposite scenario was described in humans: RRP6 mediates transcriptional silencing of the HIV-1 promoter in the absence of a viral transactivator by processing the viral TAR transcript into small RNAs, which were proposed to hybridize with the promoter and inhibit its activity. When *RRP6* is mutated, the inhibition of the HIV-1 promoter is lost[147]. Other examples in which the exosome modulates small-RNA production have been reported, and, overall, the scope of indirect regulation of mRNA levels by the exosome is potentially vast.

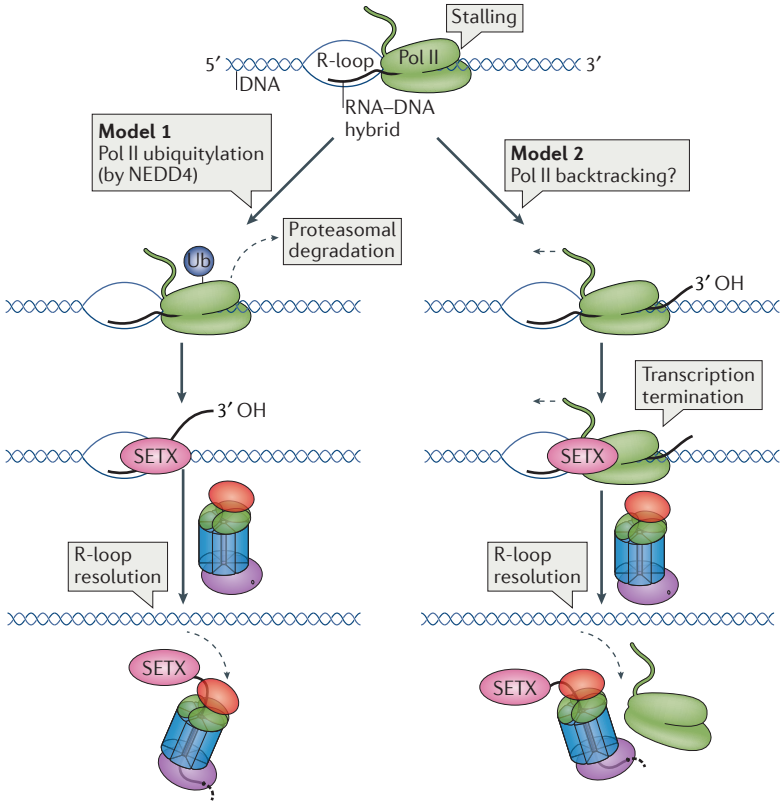

Figure 4 | **Potential mechanisms of R-loop resolution by the exosome complex.** During transcription, nascent RNA can invade the transcription bubble and hybridize with the template DNA strand. This leaves the non-template strand exposed as a single-stranded loop and can lead to RNA polymerase II (Pol II) stalling. These R-loop structures can lead to DNA double-strand breaks and indeed have been associated with genomic instability. The exosome has been suggested to play a part in the resolution of R-loops by degrading the RNA strand that forms the RNA–DNA hybrid. Two models of how the exosome could gain access to the RNA substrate have been proposed. First, stalled Pol II can be ubiquitylated by the E3 ligase NEDD4, which triggers its degradation by the proteasome, thus exposing a free 3′ OH end. This could serve as a substrate for the exosome complex, which would then degrade the RNA and resolve the R-loop (left). R-loop resolution could be facilitated by additional factors, such as the helicase senataxin (SETX; Sen1 in *Saccharomyces cerevisiae*). Alternatively, the stalled polymerase is known to have a tendency to backtrack on the template strand, causing the 3′ OH of the nascent transcript to protrude from the transcription complex (right). It has been suggested that this could also be an exosome substrate. The exosome could then degrade the RNA and displace Pol II to resolve the R-loop. Again, R-loop unwinding could be facilitated by other factors, for example, SETX.

DNA damage repair and genomic integrity. The DDR is one of many cellular processes to which changes in post-transcriptional regulation of gene expression are known to contribute[148]. Thus, it may not be too surprising that the activity of the nuclear exosome is regulated in response to DNA damage. RNA-binding motif protein 7 (RBM7), for example, which is a component of the human NEXT complex, is phosphorylated following UV-induced DNA damage. This leads to its binding by the adaptor protein 14-3-3 and reduces its association with exosome substrates such as PROMPTs, possibly freeing it to pursue DNA damage-related tasks[149]. Furthermore, a direct involvement of exosome factors in the repair of DNA lesions has also been suggested. The nuclear matrix protein Rrp47 (C1D in humans), which forms a stable heterodimer with Rrp6 (REFS 150,151), is required for the repair of UV-induced lesions in *S. cerevisiae*[152,153]. Human C1D was found to bind to translin-associated factor (TRAX) and to activate the DNA-dependent protein kinase (DNA-PK); these proteins are both known to play a part in DSB repair[154,155]. In *Drosophila melanogaster*, DSB repair is also compromised in the absence of *Rrp6* (REF. 156). Intriguingly, it was recently reported that DSBs can be repaired by an RNA-template-based mechanism that involves the formation of RNA–DNA hybrids at the break points[157], and it is conceivable that the exosome is required to remove the RNA template to allow DNA repair to proceed.

RNA–DNA hybrids also occur as a by-product of transcription. Unless it is removed, the resulting R-loop structure can lead to DSBs[158,159] (BOX 1). It has been suggested repeatedly that the exosome could have a role in the resolution of R-loops and thereby contribute to genome integrity[9,19,160,161]. Strikingly, the increased incidence of genomic rearrangements in *S. cerevisiae* exosome mutants can be rescued by overexpression of RNase H, which removes RNA–DNA hybrids[162]. In mice, loci that produce exosome-targeted transcripts were found to accumulate R-loops following exosome knockdown[9,19]. Moreover, in human cells, the exosome is recruited to R-loops that form at sites of replication–transcription collision[160]. In this case, exosome recruitment depended on the induction of a SUMO-dependent interaction between the R-loop-associated helicase senataxin (SETX)[163,164] and the exosome subunit RRP45. Recessive *SETX* mutations that prevent its association with RRP45 cause ataxia with oculomotor apraxia 2 (AOA2), which is one of several human Mendelian diseases that have been functionally linked to the exosome complex[160].

Antibody class switch recombination in B cells. The nuclear exosome has been implicated in another biological process that is intimately linked with R-loops: the diversification of immunoglobulins by somatic hypermutation and antibody class switch recombination (CSR) in B lymphocytes[165]. CSR is initiated by activation-induced cytidine

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deaminase (AID), which induces DSBs by modifying both DNA strands at the class switch region. The ensuing repair by homologous recombination results in rearrangements of the antibody locus and the production of different antibody isotypes. In mice, it was proposed that recruitment of the exosome to the class switch region, which is G-rich and very prone to R-loop formation[166], is required for optimal CSR[161]. If the exosome is mutated, the DNA strand involved in RNA–DNA hybrid formation across the class switch region is protected from deamination, again suggesting that the exosome may help to resolve R-loops[161]. Interestingly, AID frequently associates with other loci that produce unstable RNAs targeted by the exosome[9,165]. In exosome mutants, these loci accumulate R-loops. Mistargeting of AID to other loci is dangerous and can cause genomic translocations that are associated with cancer[165]. A model of how the RNA molecule in the RNA–DNA hybrid could be rendered susceptible to the exosome has also been suggested (FIG. 4): in human B cells, Pol II bound by AID was found to be ubiquitylated by the E3 ubiquitin ligase NEDD4 (REF. 167), the same enzyme that targets Pol II in response to UV damage, thereby inducing degradation of Pol II by the proteasome[168]. In the absence of NEDD4, exosome RNA substrates at AID target genes were stabilized, and it was suggested that ubiquitin-dependent destabilization of Pol II stalled in an R-loop is required to expose RNA 3′ OH ends, which are substrates for the exosome[167] (FIG. 4). It has also been hypothesized that, alternatively, backtracking of an R-loop-producing Pol II could provide a means of exposing the required 3′ OH end[165]. As was suggested for *S. pombe*[138], this could allow the exosome to disassociate stalled Pol II and resolve the associated R-loop[165].

Concluding remarks

Recent years have seen a considerable advance in our understanding of how the nuclear exosome is regulated. Nevertheless, central aspects of substrate recognition still remain obscure, particularly in organisms other than *S. cerevisiae*, and the quest for factors that determine RNA fates — stable expression or rapid degradation — is still in progress. We have begun to understand that changes in RNA processing coupled to exosome-dependent decay constitute an important means of global gene regulation, and we expect to see the identification of many more genetic programmes regulated by the RNA exosome. Until now, research has focused on the regulation of splicing as the primary mechanism of exosome-mediated gene regulation; however, other RNA maturation processes, such as 3′ end formation, may turn out to be as heavily exploited for regulatory purposes. RNA–DNA hybrids seem to be universal by-products of transcription and are increasingly linked to disease[158,159,169,170]. Thus, the links between the exosome and R-loop dynamics identified by recent work are likely to have important implications, because they directly connect RNA surveillance with transcription regulation and may represent a general mechanism of exosome targeting. The data on R-loops discussed in this Review is promising, and we are looking forward to future work that will corroborate it.

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Competing interests statement
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