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Rrp6: Integrated roles in nuclear RNA metabolism and transcription termination

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Abstract

The yeast RNA exosome is a eukaryotic ribonuclease complex essential for RNA processing, surveillance and turnover. It is comprised of a barrel-shaped core and cap as well as a 3'–5' ribonuclease known as Dis3 which contains both endo- and exonuclease domains. A second exonuclease, Rrp6, is added in the nucleus. Dis3 and Rrp6 have both shared and distinct roles in RNA metabolism, and this review will focus primarily on Rrp6 and the roles of the RNA exosome in the nucleus. The functions of the nuclear exosome are modulated by cofactors and interacting partners specific to each type of substrate. Generally, the cofactor TRAMP (Trf4/5-Air2/1-Mtr4 polyadenylation) complex helps unwind unstable RNAs, RNAs requiring processing such as rRNAs, tRNAs, or snRNAs or improperly processed RNAs and direct it toward the exosome. In yeast, Rrp6 interacts with Nrd1, the cap binding complex (CBC), and RNA Polymerase II to aid in nascent RNA processing, termination, and polyA tail length regulation. Recent studies have shown that proper termination and processing of short, non-coding RNAs by Rrp6 is particularly important for transcription regulation across the genome and has important implications for regulation of diverse processes at the cellular level. Loss of proper Rrp6 and exosome activity may contribute to various pathologies such as autoimmune disease, neurological disorders, and cancer.

Rrp6 is the nuclear specific catalytic subunit of the RNA exosome, a 3'–5' ribonuclease complex involved in termination of short RNAs, RNA quality control surveillance, and RNA degradation (first described in ¹). Rrp6 functions in the nucleus in both yeast and humans (also known as Exosc10 and the PM/Sc1100kDa auto-antigen in humans) but is also present in the cytoplasm in human cells ^{2, 3}. Rrp6 has been shown to be recruited to the site of RNAPII transcription in multiple organisms ^{4–6}. However, much of our knowledge of the functions of the RNA exosome has come from studies in *Saccharomyces cerevisiae*. Therefore, findings described here are from studies in yeast unless otherwise noted. The core exosome in yeast is comprised of nine subunits arranged as a barrel with a central core and is proposed to channel the RNA substrate to the catalytic subunit Dis3 (also known as Rrp44). In yeast, there are two forms of the RNA exosome: the cytoplasmic exosome consisting of the core complex only, and the nuclear exosome consisting of the core exosome with the addition of Rrp6.

Although Rrp6 is not essential for viability in yeast, it is required for proper 3' end trimming of primary small nucleolar/nuclear RNAs (sn/snoRNAs) ^{7, 8}, degradation of short-lived,

non-coding cryptic unstable transcripts (CUTs)⁹ and improperly terminated RNAs^{7, 10}, as well as regulation of polyA tail length^{11, 12} and termination of specific short transcripts. Recent studies using high-resolution tiling arrays found that Dis3 and Rrp6 have both shared and distinct roles in the degradation and processing of various RNAs^{13, 14}. Rrp6 has also been implicated in the termination and 3' end processing of a variety of noncoding RNAs, most that are not terminated by traditional polyA-dependent termination mechanisms^{15, 16}.

Rrp6 and other exosome subunits are required for proper meiosis¹⁷ and cellular differentiation¹⁸. Additionally, inhibition of exosomal proteins, either by mutation or production of autoantigens, can lead to a variety of human diseases¹⁹. Here we will discuss the latest research on Rrp6 focusing on specific substrates of the nuclear exosome and its known and proposed mechanisms of action. It is clear that the RNA exosome is essential for transcriptome-wide regulation of gene expression and RNA surveillance leading to regulation of diverse cellular processes across eukaryotes.

REGULATION OF RRP6 ACTIVITY

Rrp6 Can Function Apart From the Core Exosome

The TRAMP complex and core exosome barrel help to target specific RNA degradation substrates to Rrp6 and Dis3, but there is evidence that Rrp6 may be capable of performing some of its functions apart from the core exosome. Callahan *et al* studied the effects of disrupting Rrp6, Dis3, and Rrp43 of the exosome individually, and Rrp6 and Dis3 together²⁰. They found that the individual perturbations resulted in accumulation of distinct classes of transcripts. Disruption of both Rrp6 and Dis3 resulted in an increase of the core exosome-specific transcripts and those that require both Rrp6 and Dis3. However there were no additional increases in the Rrp6-targeted transcripts following depletion of Dis3, suggesting that Rrp6 may have exosome-independent functions. In support of this hypothesis, a C-terminal domain Rrp6 mutant that disrupts the interaction between Rrp6 and the core exosome is capable of 3' end processing of 5.8S rRNA and some snRNAs. However, this mutant is no longer capable of degrading transcripts shown to require both Dis3 and Rrp6 function. Additionally, Rrp6 has been shown to preferentially bind some transcripts relative to Dis3 using RNA-protein crosslinking³. These Rrp6-bound transcripts include intron-containing pre-mRNAs and ribosomal protein coding RNAs. RNA-Seq analysis of RRP6 mutants revealed that ribosomal protein coding RNAs are significantly decreased relative to WT yeast¹⁵. The mechanism(s) by which Rrp6 acts independently of the core exosome remains unknown; however association of Rrp6 with various interacting partners may regulate its autonomous function.

Rrp6 Interacting Partners

The TRAMP Complex—In the nucleus, the exosome interacts with an RNA helicase, Mtr4, and its associated complex known as TRAMP (the Trf4/5, Air1/2, Mtr4 Polyadenylation complex) (Figure 2). Mtr4 can work alone to unwind RNA substrates or it can act as part of TRAMP^{21, 22}. It contains a 'ratchet helix' and arch domain required for RNA processing, although the specific roles for each of these domains remain unclear^{23–25}. The TRAMP complex aids in substrate specificity²⁶, and prepares some target RNAs for

processing by Exo11 by adding a short (3 – 50 nucleotide) poly(A) tail that may provide an unstructured starting point to use to thread the RNA through the core barrel of the exosome^{27–30}. This poly(A) tail is added by one of two poly(A) polymerases in the TRAMP complex, Trf4 or Trf5. These poly(A) polymerases exist in separate complexes, called TRAMP4 and TRAMP5, respectively. The TRAMP complex has been shown to be important in degradation of pre-tRNAⁱMet^{31, 32} as well as degradation of rRNA and small nuclear/nucleolar RNAs^{27–29}.

Mpp6—M-phase Phosphoprotein 6, or Mpp6, is important for the proper processing of the 3' ends of 5.8S rRNAs³³. The yeast homolog of Mpp6 was found to be an RNA-binding protein required for proper surveillance of pre-mRNAs, pre-rRNAs, and the degradation of cryptic non-coding RNAs transcribed from ribosomal DNA spacer heterochromatin³⁴. It has been suggested that Mpp6, and probably other cofactors like it, play a role in specifying the function to be carried out by the exosome regarding specific substrates³⁵.

Nrd1—Rrp6 has been found to interact with the RNA Polymerase II (RNAPII) transcription termination factor Nrd1⁷ (Figure 1). Nrd1 interacts with Nab3, Sen1, and the CBC and is required for RNAPII termination at short RNA target genes (<1000bp) including snRNAs and CUTs^{7, 16, 36}. Nrd1 binds phosphorylated serine 5 (Ser5-P) in the repeated amino acid sequence of YSPTSPS which makes up the C-terminal Domain (CTD) of Rpb1, the largest subunit of RNAPII. Nrd1 interacts with the phosphorylated CTD through a CTD-Interacting Domain (CID) and also interacts with consensus sequences in the nascent RNA through an RNA Recognition Motif (RRM). Because Nrd1 is a sequence-specific RNA binding protein, it has been suggested that Nrd1 binding may block Rrp6-dependent degradation from proceeding past specific sequences⁷. This mechanism could allow Exo11 to distinguish between RNA targets for degradation or 3' end processing. Intriguingly, misprocessed transcripts in yeast caused by ectopic expression of the bacterial RNA Polymerase termination factor Rho are degraded by Exo11³⁷, and the formation of these transcripts results in increased recruitment of Nrd1 and Rrp6 to the transcription machinery by its RNAPII CTD-Interacting Domain (CID) and its RNA recognition motif (RRM)³⁸. This, taken in context with the known interaction between Rrp6 and Nrd1, suggests that Nrd1-interaction with RNAPII and the nascent RNA may be an important step in recruitment of Exo11. In support of this model, Heo *et al* has shown that disrupting the Nrd1-RNAPII CTD interaction by replacing the Nrd1 CID with the CID of Rtt103, which recognizes Ser2-P RNAPII CTD, reduces binding of Nrd1 to Rrp6⁸.

Nab3—The Nrd1 binding partner, Nab3, can also interact with Rrp6 independently of Nrd1³⁹. Fasken *et al* recently found that overexpression of Nab3 in strains carrying mutations in the TRAMP RNA-binding subunits Air1 and Air2 can suppress the slow growth phenotype and decrease the amount of extended transcripts. This effect was not observed for Nrd1 or Sen1 and does not require the Nrd1-interacting domain of Nab3, but it does require the RNA-binding domain of Nab3 and Rrp6. Deletion of the Nrd1 binding domain of Nab3 revealed that Nab3 is capable of interacting with Rrp6 independently of Nrd1³⁹.

Exosome Interacts with Capping Complex—A number of studies report links between the nuclear exosome and the Cap Binding Complex (CBC) in yeast and humans (Figure 1) ^{7, 40, 41}. The 5' mRNA cap is involved in coordinating a number of mRNA regulating events such as splicing ⁴², 3' end formation ⁴³, and RNA turnover ⁴⁴, but the specific mechanisms underlying its regulation of these processes is unclear. Regarding RNA turnover by the nuclear exosome, it appears that specific cofactors may form a physical link between Exo11 and CBC. In yeast, Rrp6 can directly interact with Nrd1, which co-purifies with CBC proteins based on affinity purification-mass spectrometry experiments ⁷. In humans, the exosome cofactor the Nuclear Exosome Targeting complex (NEXT) has been shown to interact with the CBC ^{40, 41}. Furthermore, aberrant mRNAs that cannot be exported from the nucleus are subjected to degradation by a process requiring both Cbc1, a nuclear cap-binding protein, and Rrp6 in *S. cerevisiae* ^{45, 46}.

ROLES IN TRANSCRIPTION

Transcription Termination

Termination Overview—In yeast, Rrp6 is involved in the termination and 3' end processing of a variety of noncoding RNAs, most of which are terminated Nrd1-dependent termination and not by polyA-dependent termination mechanisms ^{15, 16}. An Nrd1-like termination pathway has not been discovered in higher eukaryotes as of yet, and therefore, our discussion of the roles of Rrp6 in termination will focus on studies in yeast. There are two characterized RNAPII transcription termination mechanisms in yeast, both of which are coupled to 3' end processing of the RNA ⁴⁷. The factors involved in these pathways are recruited by orchestrated post-translational modifications along the repetitive C-terminal domain (CTD) of Rpb1, the largest subunit of RNAPII. In general, long (>1000 bp) RNAs are terminated by a polyadenylation dependent mechanism involving the Cleavage and Polyadenylation Factor (CPF) and Cleavage Factor (CF) complexes. Short (<1000 bp) RNAPII transcripts including snRNAs, cryptic unannotated transcripts (CUTs), stable unannotated transcripts (SUTs), and some short mRNAs are terminated by an Nrd1, Nab3, and Sen1 (NNS) dependent mechanism. It has been well characterized that termination by NNS is coupled to RNA 3' end processing and/or degradation by Exo11 ¹⁶.

Nrd1 Termination—In the current working model of NNS termination (diagrammed in Figure 2 top panel), Nrd1 is recruited to the termination site by its CTD interacting domain (CID) which binds to Ser5-P, as well as RNA recognition motifs (RRM) present in both Nrd1 and its binding partner Nab3 which bind specific sequences in the nascent RNA. The helicase Sen1 unwinds RNA:DNA hybrids which result from the nascent RNA binding to the template strand and promotes RNAPII termination, possibly by directly displacing RNAPII from the DNA ⁴⁸. In this way, RNAPII transcribes beyond what will become the 3' end of the functional RNA into a “termination zone” before it is terminated by Sen1 ⁴⁸. Interaction between Nrd1 and the TRAMP subunit Trf4 release Nrd1 from the CTD where Nrd1 interacts with Ser5-P repeats (Figure 2, ⁴⁹). A short polyA tail is added to the RNA by the TRAMP complex, guiding it to the nuclear exosome and Rrp6 which trims the ends of stable transcripts (such as snRNAs and SUTs) or completely degrades the transcript in the case of CUTs ^{9, 47, 50}.

It has recently been shown that Rrp6 is required for proper Nrd1-dependent termination (Figure 2)¹⁵. To study the role of Rrp6 in Nrd1-dependent termination in yeast, transcriptome-wide sequencing was performed in *rrp6* cells, followed by ChIP-exo (chromatin IP followed by exonuclease treatment) analysis of RNAPII localization using FLAG-tagged Rpb3. The data was also compared to previously published RNA-sequencing data from cells with Nrd1 depleted from the nucleus⁵¹ and previously mapped Nrd1-RNA binding sites obtained using photoactivatable-ribonucleoside-enhanced crosslinking and immunoprecipitation⁵². Many snRNAs and cryptic unannotated transcripts (CUTs) shown to be dependent on Nrd1 for termination were extended in *rrp6* cells by RNA-sequencing (example gene snR62 in Figure 3, data obtained from¹⁵). As mentioned, Nrd1-dependent termination has been proposed to occur in a zone of termination resulting in heterogeneous 3' ends that are subsequently processed by Rrp6⁴⁸. Therefore, it is possible that these extended transcripts were properly terminated, but lacked proper processing by Exo11. However, high-resolution ChIP-exo results using Rpb3-FLAG (a core subunit of RNAPII) clearly show that RNAPII occupancy increases downstream of what is observed in WT cells, indicating that RNAPII termination is defective in the absence of Rrp6 (Figure 3). It is important to note that not all Nrd1 targets exhibited improper termination in *rrp6* cells suggesting that the role of Rrp6 in Nrd1-termination is substrate-specific.

It is unclear at this time if Rrp6 plays a direct or indirect role in the regulation of RNAPII termination (Figure 4). For instance, a potential indirect model could involve an uncharacterized lncRNA that is normally degraded by Rrp6 but, when stabilized, acts to interfere with the activity of one or more proteins required for proper Nrd1-dependent termination (Figure 4). In WT cells, this Nrd1 and/or Nab3 bound ncRNA would be targeted for degradation by the nuclear exosome. However in the absence of Rrp6 function, this ncRNA could accumulate as is observed for CUTs, leading to a decrease in the available pool of Nrd1 and/or Nab3 for NNS termination. This speculative mechanism would be similar to that of NEAT1, a lncRNA that is thought to sequester RNA-binding proteins in subnuclear bodies called paraspeckles (reviewed in⁵³). In this theoretical model, the lncRNA could be bound by the RNA Recognition Motifs (RRM domains) of Nrd1 or Nab3, or another protein important for the NNS pathway. This would not result in decreased RNAPII transcription or decreased expression of NNS components, but would nonetheless inhibit termination activity through the Nrd1 pathway. If Nrd1 or Nab3 sequestration by a ncRNA occurs in the absence of Rrp6, all Nrd1 terminated transcripts would be expected to experience read-through transcription due to a lack of NNS components available for termination. However, studies on the *RRP6* deletion mutant found that some NNS-terminated transcripts, including snR13, were terminated correctly in the absence of Rrp6¹⁵. The C/D box snoRNA snR13 has been used as a model gene for NNS termination and mutants in Nrd1, Nab3, and Sen1 have all been shown to cause RNAPII termination defects at snR13^{48, 54, 55}. Together, these findings suggest that since *RRP6* deletion only regulates a subset of NNS-targets it is unlikely that subnuclear trapping of Nrd1, Nab3, or Sen1 alone causes the RNAPII termination defects observed in *rrp6* cells.

Exosome Aids in Backtracking RNA Polymerase—Although most studies have implied that the exosome targets RNA substrates after they are released from polymerase,

the core exosome can associate with polymerase on DNA. Indeed, the exosome has been found in association with RNAPII target genes in *Drosophila melanogaster*, the dipteran *Chironomus tentans*, and fission yeast ^{4–6}. As mentioned, both catalytic subunits in the yeast nuclear exosome are 3' – 5' exonucleases. As such, the nascent RNA being actively elongated by RNAPII would not be accessible to the exosome. However, recent work has shown that the core exosome can aid in terminating backtracked RNAPII at protein coding genes in *S. pombe* ⁵. By backtracking, the 3' end of the RNA can become solvent exposed possibly allowing access and degradation by the exosome ⁵⁶. Termination of backtracked RNAPII required Dis3 with no detectable effect from an *RRP6* knockout for protein coding mRNAs ⁵. However, Rrp6 may play a role in rescuing backtracked RNAPII at noncoding RNAs, as it has been shown to play a role in termination of specific snRNAs ¹⁵ and cryptic transcripts originating from within gene bodies (model shown in Figure 5) ⁵⁷.

Gene Regulation by Early Termination—Studies in human cells suggest that Rrp6 and Senataxin (Setx, human homolog of Sen1) may cooperate with Microprocessor to regulate RNAPII premature termination via RNAi at some genes ⁵⁸. The Microprocessor complex is required for microRNA (miRNA) processing and includes the RNase II Drosha and the dsRNA-binding protein Dgcr8 ⁵⁹. Typically, miRNAs regulate protein expression post-transcriptionally by binding to the target RNA and inducing degradation or inhibition of translation. However, Wagschal *et al* reported that cooperation between Microprocessor, Rrp6, and Setx regulate *HIV-1* transcription by inducing premature termination ⁵⁸. In the current model, Microprocessor is recruited to the nascent stem-loop TAR RNA located in the most 5' portion of the *HIV-1* gene, causes RNAPII pausing and subsequent cleavage of the RNA by Rrp6. Studies in yeast suggest that this mechanism of promoter-proximal pausing followed by premature termination induced by cooperation with Rrp6 and Sen1 and/or other Nrd1-dependent termination factors may occur at a number of genes (reviewed in ⁹).

Regulation of PolyA Tail Length—The length of the polyA tail added to pre-mRNAs during transcription termination affects the stability and translation efficiency of the mRNA (reviewed in ⁶⁰). Briefly, the normal tail length (about 70 – 80 nucleotides) is carefully regulated by interactions between PolyA Binding Proteins (PABPs) and polyA polymerases. Two PABPs have been identified in yeast: Pab1 which is primarily in the cytoplasm but can be shuttled into the nucleus, and Nab2 which is primarily in the nucleus but is exported with the mRNP into the cytoplasm where it is exchanged for Pab1. Nab2 undergoes post-transcriptional autoregulation in a proposed model in which Nab2 binding in the 3'UTR of a *NAB2* mRNA results in recruitment of Rrp6 and mRNA degradation ⁶¹. It has been reported that Rrp6 can physically interact with Nab2, displacing Nab2 from polyA tails and subsequently degrading the mRNA ¹¹.

Rrp6 and the exosome activity are important for cellular differentiation—In yeast, the nuclear exosome is involved in facilitating meiosis. Budding yeast have been shown to degrade Rrp6 as they enter meiosis, leading to the accumulation of noncoding transcripts termed meiotic unstable transcripts (MUTs) ¹⁷. Degradation of Rrp6 occurs in the first hours of meiosis and corresponds to the timing of RNA replication and induced double

strand breaks¹⁷. However, cells lacking Rrp6 rarely enter meiosis. In what may be a related process, fission yeast express meiotic genes that are degraded by the exosome to inhibit meiosis⁶². Furthermore, it has recently been found that Exo11 facilitates proper meiotic recombination¹⁸. The mechanisms regulating the expression of Rrp6 during early meiosis is not clear.

The exosome is also involved in regulating blood cell maturation⁶³. EXOSC8, the human homolog of the exosome core protein Rrp43, is repressed by erythropoiesis regulator proteins GATA-1 and Foxo3. The loss of EXOSC8 or other exosome components can induce erythroid cell maturation. Expression analysis revealed that exosome activity can repress the expression of genes activated by GATA-1 and Foxo3⁶³. The mechanism by which the exosome represses expression of GATA-1/Foxo3-regulated genes has not been explored, and it is unclear what specific roles Rrp6 may play. It is possible that the RNA exosome plays an important regulatory role in many maturation and developmental processes.

INSIGHTS INTO RNA SUBSTRATE RECOGNITION

Nuclear RNA processing and surveillance

With recent advances in deep sequencing technology, it has become obvious that the majority of the genome is transcribed into RNA. The exosome must identify and properly respond to signals that determine which transcripts are to be completely degraded, which should have their 3' ends partially degraded, and which are properly processed and should avoid the activities of the exosome entirely. In the case of the 5.8S rRNA and sn/snoRNAs, Rrp6 and the exosome is required to process heterogeneous 5' ends to the final mature form⁶⁴. Furthermore, certain transcripts are transcribed and immediately degraded by the exosome, possibly to regulate the transcription of nearby regions. The nuclear exosome appears to recognize these unstable transcripts and targets them for degradation and recycles the ribonucleotides contained within. We provide a brief summary of some of known RNA processing and degradation activities of Exo11 in Figure 6.

Structural Insights

X-ray crystallography analysis has revealed structures of the nuclear exosome containing Rrp6, Dis3, and Rrp47 in complex with different RNAs⁶⁵. Makino *et al.* observed the nuclear exosome bound to an 18mer RNA (A/U)₁₈. This RNA substrate was bound directly to Dis3, and the exosome channel was empty. This structure may represent a previously proposed core-independent path to Dis3⁶⁶. Six ribonucleotides from the (A/U)₁₈ substrate also bind Rrp6 in this structure, but the ribonucleotides do not reach the Rrp6 active site and were impaired by a “flipped out” conformation of Tyr361. The authors termed this the “RNA-binding mode” of Rrp6. It appears that the RNA is bound to Rrp6 in an orientation that guides it towards the central channel where it could be threaded towards Dis3. Interestingly, when the exosome was incubated with a longer 15 nucleotide substrate with a different sequence (U₁₅), they found that the RNA binds to the same Rrp6 residues but bends to the Rrp6 active site. In the U₁₅ structure, Tyr361 resides in a “flipped in” conformation and interacts with the phosphate of the 3' - nucleotide. This conformation is

proposed to represent an “RNA-degradation mode.” The authors concluded that RNA can bind Rrp6 and then be directed toward Rrp6 or Dis3 depending on the sequence and secondary structure of the substrate ⁶⁵.

Additionally, the authors addressed the effects of bulky RNA on the structure of the nuclear exosome. For these studies, the authors used a 31mer with 5' duplex (DuplexU₃₁). It has previously been reported that the 5.8S rRNA requires both Dis3 and Rrp6 for processing and that a Dis3 to Rrp6 handoff occurs when the exosome encounters the ribosome particle ⁶⁷. The DuplexU₃₁ RNA structure revealed that, when the bulky region of the RNA reaches Rrp6, Rrp6 and Rrp47 swing away from the core exosome via a hinge formed by their disordered N-terminal regions ⁶⁵. The authors propose that Rrp6 and Rrp47 may move of the way so that Mtr4 and Mpp6 can pull the RNA from the central channel and redirect the RNA from Dis3 to Rrp6. It is interesting to consider, when discussing the role of Rrp6 and the exosome in NNS-dependent termination, that a similar mechanism may allow for the precise 3' end processing of sn/snoRNAs. It is possible that the exosome encounters Nrd1 bound to the pre-sn/snoRNA substrate and it is sterically hindered by the bulk of Nrd1, causing Rrp6 and Rrp47 to swing away from the core. Similar to the 5.8S rRNA model, this would allow Rrp6 to process the sn/snoRNA up to a precise location, likely determined by the Nrd1 and/or Nab3 binding site on the RNA.

Conclusions

The exonuclease Rrp6, in coordination with the core exosome and cofactors, is important for regulation of a variety of cellular processes. Rrp6 and the exosome primarily regulate their genomic targets through transcription termination, RNA processing, and degradation of short, non-coding RNA substrates. Studies on the exosome highlight the importance of RNA surveillance as a regulatory mechanism for diverse cell processes. Continuing work is needed to further characterize the exact mechanisms through which Rrp6 regulates such diverse processes as RNAPII transcription termination and RNA interference pathways. Thus far, many Rrp6 studies in yeast have been carried out in *RRP6* knockout strains. Understanding the specific functions of Rrp6 is complicated by its interaction with Rrp47 that is required for the stability of Rrp47 and recruitment of Mtr4 ^{68, 69}. It is currently unclear which effects are specific to the loss of Rrp6 and which are dependent on Rrp47 and/or TRAMP recruitment. Additionally, the differential regulation of Rrp6 function in various cellular states, such as meiosis, warrants further investigation.

The exosome has been implicated in various disease states. Antibodies against many of the subunits of the human exosome, including Rrp6, have been characterized in autoimmune disorders affecting the connective tissue, specifically: PM (polymyositis), Scl (scleroderma) and PM/Scl overlap syndrome ^{19, 70}. Two of the core subunits of the human exosome, EXOSC3 (homolog to the yeast cap protein Rrp40) and EXOSC8 (homolog to the yeast barrel protein Rrp43), have been implicated in pontocerebellar hypoplasia type 1 (PCH1) and spinal motor neuron degeneration in several families ^{71, 72}. In fact, other studies involving mRNA processing factors indicate that proper regulation of RNA processing is critical in neuromuscular development and maintenance ^{73, 74}. Furthermore, in a genome-wide drug-target screen, Rrp6 was found to be a potential target of the cell growth inhibitor

5-fluorouracil used in chemotherapy for the treatment of solid tumors. The role of Rrp6 in rRNA processing is proposed as a pathway by which 5-fluorouracil inhibits growth⁷⁵. These findings highlight the importance of study of Rrp6 through analysis of its catalytic mechanisms and its role in human disease.

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Figure 1. Schematic representation of the *S. cerevisiae* nuclear exosome and its known cofactors

The core barrel structure (also known as Exo9), in green, consists of nine subunits: Rrp41, Rrp42, Rrp43, Rrp45, Rrp46, and Mtr4 (the six RNase PH-like proteins), and Rrp4, Rrp40, and Csl4 (the three S1/KH cap proteins). The nuclease Dis3 associates with the core barrel in both the cytoplasm and the nucleus. This complex is also called Exo10. Rrp6 only joins the core exosome in the nucleus, in a complex known as Exo11. The cofactor Rrp47 (dark red) binds to Rrp6 and structured RNA and improves the stability of Rrp6. The TRAMP complex (orange) binds to the surface formed by Rrp6/Rrp47 to thread RNA substrates through the exosome. Nrd1 and its binding partner Nab3 (gray) interact with Rrp6, both together and independently of one another to coordinate termination and processing of short

(<1000 nt) RNAs. The cap binding complex (purple) interacts with Rrp6 during co-transcriptional processes in humans. In yeast, the cap binding complex copurifies with Nrd1 and Nab3, which may lead to an indirect interaction with Rrp6. Mpp6 (dark green) is a general nuclear exosome cofactor involved in a number of exosome-dependent mechanisms such as processing and degradation of a RNA arising from rDNA arrays.

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Figure 3. Rrp6 is required for proper Nrd1-dependent termination at some short RNAs
 RNA sequencing and Rpb3 ChIP-exo data at snR62 in wild type and *rrp6Δ*. Data previously published in ¹⁵. **(A)** Graphical representation of strand-specific RNA-seq reads mapped to snR62 region. Only reads mapped to the snR62 strand are shown because reads mapped to the other strand are minimal. The location and direction of transcription for all analyzed annotations on the strand of interest are diagrammed below the graphs to scale. Processed length of snRNAs and mRNAs are in black, snRNA-extended transcripts, including pre-snRNAs and termination read-through products, are in green (labeled “ETs”), NUTs are in aqua, and arrows indicate direction of transcription. **(B)** Rpb3-FLAG localization as determined by ChIP-exo sequencing reads mapped to the same region and

aligned to (A). Wild-type normalized read counts are in black, and *rrp6* are in orange. The region of increased RNAPII localization in *rrp6* is indicated by the black arrows.

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RNA Dependent Model (Indirect Role in termination) **Figure 4. Rrp6 could play an indirect role in the regulation of Nrd1-dependent termination**

In the speculative RNA-dependent model, a theoretical cryptic lncRNA that is usually degraded by Rrp6 may be stabilized in its absence. This trans-acting RNA may then sequester one or more proteins required for Nrd1-dependent termination. This subnuclear trapping may be similar to the mechanism by which NEAT1 sequesters RNA-binding proteins in paraspeckles, effectively inhibiting their activity without decreasing their overall abundance. Nrd1 and Nab3 would be reasonable candidates for such a mechanism considering their RRM domains, or another currently unidentified protein could also be affected.

in response to an obstacle in the DNA or improperly incorporated nucleotide, the 3' end of the RNA emerges through a 'backtrack site' in RNAPII and can be accessed by Rrp6⁵⁶.

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Figure 6. Multiple mechanisms by which Rrp6 processes or degrades its many RNA substrates (A) The 18S, 5.8S, and 25S rRNAs are transcribed as one molecule and cleaved and processed by several nucleases. Specifically, Rrp6 is required for processing of the 3' end of the pre-5.8S product. (B) Improperly processed tRNAs in the nucleolus are targeted by the TRAMP complex which adds a short polyA tail and directs the substrate to the exosome for complete degradation by Rrp6. (C, left) Exo11 interact directly with the spliceosome to degrade introns co-transcriptionally. (C, right) Rrp6 also degrades mRNAs that cannot be exported and accumulate at the transcription site when the accumulation is due to

improperly processed 3' ends or inhibition of nuclear export machinery. **(D, left)** Rrp6 is required for proper termination by Nrd1. The mechanism by which Nrd1 causes termination is not known. It may sometimes require an interaction with Rrp6 to properly terminate, including release of DNA and RNA from RNAPII and the termination factors. **(D, right)** Rrp6 processing the 3' end of snRNAs terminated in a heterogeneous "zone." The extended 3' ends of the pre-snRNA are trimmed back by Rrp6. (E) Cryptic Unstable Transcripts (CUTs) and unstable nuclear lncRNAs are both targeted to the Exo11 by the TRAMP complex.

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