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Rrp6, Rrp47 and co-factors of the nuclear exosome

J. Scott Butler¹ and Phil Mitchell²

¹Departments of Microbiology and Immunology, and Biochemistry and Biophysics,
and Center for RNA Biology: from Genome to Medicine

University of Rochester Medical Center,

Rochester,

New York 14642

USA

scott_butler@urmc.rochester.edu

²Department of Molecular Biology and Biotechnology,

The University of Sheffield,

Firth Court,

Western Bank,

Sheffield S10 2TN

UK

p.j.mitchell@shef.ac.uk

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Abstract

This chapter reviews the present state of knowledge on the activity of enzymes that function with the RNA exosome in the nucleus. In this compartment, the exosome interacts physically and functionally with the exoribonuclease Rrp6 and several co-factors, most prominently Rrp47 and the TRAMP complex. These interactions decide the fate of RNA precursors from transcription through the formation of mature ribonucleoprotein particles (RNPs) and the export of the RNPs to the cytoplasm. The nuclear exosome catalyzes the formation of the mature 3' ends of many of these RNAs, but in other cases degrades the RNAs to mononucleotides. Co-factors such as Mpp6, TRAMP and the Nrd1/Nab3 complex play important roles in determining the outcome of the interaction of RNPs with the nuclear exosome. The details that govern the specificity of these decisions remain a rich source for future investigation.

Introduction

The RNA exosome plays an essential role in the processing and degradation of RNAs in eukaryotic organisms. In the nucleus and the cytoplasm the nine-subunit exosome core, Exo9, and the ribonuclease Dis3/Rrp44 function as a unit, designated Exo10. In *Saccharomyces cerevisiae* this complex interacts physically and functionally with a nucleus specific enzyme, Rrp6, to form the nuclear exosome, Exo11. While the majority of Rrp6 resides in the nucleus in *S. cerevisiae*, evidence suggests its presence in the cytoplasm in humans, *T. brucei* and *A. thaliana*.¹⁻³ In *S. cerevisiae*, where Rrp6 has been studied most extensively, deletion of the sole copy of its gene (*RRP6*) causes a slow growth phenotype at 30°C and extremely poor growth at 37°C.⁴ Nevertheless, the fact that deletion of any of the other exosome genes causes lethality has made the use of *rrp6*- Δ strains a valuable tool for the study of exosome defects in nuclear RNA processing. These studies revealed a critical role for Rrp6 in maturation and degradation pathways that include all known classes of RNA. The targeting of Rrp6 and Exo10 to these different RNA processing pathways is specified by interactions with protein co-factors such as Rrp47, Mpp6 and the TRAMP complex. Moreover, studies in *rrp6* mutants, or cells depleted of Rrp6, uncovered the existence of RNA polymerase II transcripts from virtually every part of the genomes of organisms as divergent as yeast, plants and humans. These revelations along with evidence that Rrp6 regulates the levels of specific mRNAs indicate that the nuclear exosome and its co-factors may have key functions in the control of gene expression and organismal development.

Structure and activity of Rrp6

Rrp6 belongs to the RNaseD family of the DEDD superfamily of exoribonucleases, which use a two-metal ion mechanism for RNA hydrolysis (Figure 1).⁵ Structure-function studies of Rrp6 proteins with point mutations in the exonuclease domain confirmed the two-metal ion mechanism and suggested that, like the exonuclease domains of DNA polymerases, Rrp6 utilizes a phenylalanine to stabilize the hydroxyl anion intermediate activated for phosphodiester bond cleavage.^{6,7} Unlike Dis3/Rrp44, whose activity is attenuated by interaction with Exo9, Rrp6 retains its characteristic properties in the Exo11 complex.⁸ Rrp6 contains two HRDC (Helicase RNaseD C-terminal) domains, only one of which was predicated by sequence homology. The Rrp6 HRDC1 domain folds into a characteristic 5-helix structure nearly identical to the homologous portion of *E. coli* RNaseD (Figure 1).^{7,9} Surprisingly, a second HRDC domain appears directly after this in the RNaseD structure, despite a paucity of sequence similarity to HRDC1 or other HRDC domains. Although the polypeptide used for crystal structure analysis of *S. cerevisiae* Rrp6 did not carry HRDC2, it seems reasonable to believe that this region folds to create a similar structure in eukaryotes. Comparison of the activities of *S. cerevisiae* Rrp6 derivatives deleted for either HRDC1 or HRDC2 indicated that HRDC2 plays a critical role in the ability of the protein to interact with Exo10 in vivo.¹⁰ Rrp6 deleted for HRDC2, but not HRDC1, carries out RNA 3' end processing of pre-snoRNAs and pre-5.8S rRNA (see below), but fails to degrade certain rRNA intermediates that require co-operation between the activities of Rrp6 and Dis3/Rrp44. Thus, the HRDC2 domain appears to facilitate protein-protein interaction between Rrp6 and Exo10. Rrp6 and its eukaryotic homologues carry an N-terminal

region (PMC2NT) not found in bacteria. This region is dispensable for interaction with Exo10, but is necessary and sufficient for binding to the Rrp6 co-factor Rrp47/Lrp1 (C1D in humans; see below).¹¹ Thus, for RNA 3' end maturation reactions the interaction of Rrp6 with Rrp47 appears more important than the ability of Rrp6 to bind Exo10.

The role of Rrp6 in the maturation of non-coding RNAs

Early studies showed that Rrp6 plays a critical role in the maturation of 5.8S pre-RNA.⁴ Rrp6 catalyzes the 3' end trimming of 5.8S pre-rRNA from its 5.8S+30 form to the 6S form, whose end is trimmed to the mature 5.8S rRNA by cytoplasmic Ng12.^{4,12} Rrp6 requires Rrp47/Lrp1 in budding yeast, or its homologue C1D in humans, for efficient trimming of 3' extended 5.8S precursors.^{11, 13, 14} The 5.8S+30 pre-RNA substrate results from 3'-5' processing by Exo10 of a longer precursor, 7S pre-rRNA, generated by cleavage at the C2 site with the pre-rRNA internal transcribed spacer.¹⁵ Thus, generation of 6S pre-rRNA in the nucleus requires the concerted 3'-5' exoribonuclease activities of Dis3/Rrp44 (in the context of Exo10) and Rrp6, suggesting that the Exo11 complex converts 7S to 6S pre-rRNA. However, a mutation that disrupts the interaction between Rrp6 and Exo10 in vivo has no apparent effect on the conversion of 5.8S+30 pre-RNA to 6S pre-rRNA, indicating physical independence of Rrp6 and Exo10 in this step.¹⁰

Likewise, Rrp6 and Exo10 carry out stepwise 3' end formation of sno-RNAs and sn-RNAs precursors.^{16, 17} The independently transcribed sno-RNAs and some of the sn-RNAs are synthesized as 3' extended precursors that are trimmed in the 3'-5' direction by Exo10, followed by removal of the last few nucleotides by Rrp6. Like the processing of

7S pre-rRNA to 6S pre-rRNA, the last step, catalyzed by Rrp6, occurs efficiently in an Rrp6 mutant defective for interaction with Exo10, but requires interaction with Rrp47 via the Rrp6 PMC2NT domain.^{11, 18} Several studies suggest that some portion of mature snoRNAs results from the 3' trimming of extended, polyadenylated forms of snoRNAs precursors, as opposed to their total degradation by an exosome catalyzed surveillance mechanism.¹⁹⁻²¹ This raises a longstanding question of how Rrp6 and Exo10 recognize which RNA substrates should be destroyed and which should be trimmed to their normal 3' ends. Presumably, the answer depends on whether the RNAs have the combined RNA and protein structural information to keep the RNPs in a productive biogenesis pathway.

The role of Rrp6 in mRNA surveillance

Rrp6 was discovered by virtue of the fact that loss of function mutations suppress the temperature sensitive growth phenotype of a *S. cerevisiae* strain carrying a mutation (*pap1-1*) in the gene encoding the canonical mRNA poly(A)-polymerase Pap1.⁴ Interestingly, loss of Rrp6 activity allowed the accumulation of poly(A)+ mRNAs under conditions where partial inactivation of Pap1 otherwise resulted in the disappearance of most mRNAs.²² Localization of Rrp6 to the nucleus, demonstration of its hydrolytic exoribonuclease activity and the fact that loss of its activity allowed accumulation of poly(A)+ mRNAs in *pap1-1* strains without changing the rate limiting step in mRNA decay lead to the proposal that the enzyme plays a role in a nuclear mRNA surveillance pathway.²² Indeed, loss of Rrp6 activity reverses the disappearance of mRNAs caused by defects in other components of the mRNA 3' end formation pathway, as well as cis-acting defects that inhibit polyadenylation.²³⁻²⁵

The role of Rrp6 in nuclear mRNA surveillance includes an interesting, but poorly understood function in the accumulation of incompletely processed mRNAs at, or near, their site of transcription.²⁶ Retention of specific transcripts in single nuclear foci in budding yeast occurs as a result of defects in 3' end processing, or defects in the formation of export competent RNPs.²⁷ Strains lacking Rrp6 fail to form these foci and the transcripts exit the nucleus. These observations suggest that, in addition to its role in degrading aberrant transcripts, the presence of Rrp6 slows the transition of pre-mRNAs to export competent RNPs. Indeed, defects in components of the THO/Sub2 complex, which bridges posttranscriptional events with mRNA export, result in mRNA degradation and Rrp6-dependent retention of pre-mRNAs in nuclear foci.²⁷⁻²⁹ The mechanistic details of the connection between the THO/Sub2 complex and the nuclear exosome remain unclear, but recent evidence suggests that THO/Sub2 defects inhibit the activity of the mRNA 3' end processing machinery.^{29, 30} One interpretation of these findings is that disruption of the transition from pre-mRNA to an export competent RNP may feedback to down regulate mRNA 3' end processing, thereby exposing transcripts to degradation by Exo11.

Defects in Rrp6 and other exosome components render yeast and human cells hypersensitive to the chemotherapeutic drug 5-fluorouracil (5FU).³¹⁻³³ This effect; (i) results from incorporation of the base analogue into RNA, (ii) is abolished by a mutation in Rrp6 that inhibits its degradation, but not by one that inhibits its 3' end maturation function, (iii) correlates with the accumulation of poly(A)+ RNA degradation

intermediates and (iv) is independent of the transcription-coupled DNA repair pathway.^{32,}

³⁴ Moreover, hypersensitivity of *rrp6*- Δ strains to 5FU requires a catalytically active pseudouridine synthetase, Cbf5.³⁵ Some pseudouridine synthetases cannot convert 5FU into pseudouridine and the incomplete enzymatic reaction results in covalent adducts between the enzyme and 5FU-RNA. These findings suggest that a significant component of 5FU cytotoxicity in *S. cerevisiae* may result from the inability of Rrp6 to degrade RNA molecules trapped in complexes with Cbf5.

The role of Rrp6 in the regulation of mRNA levels

Rrp6 controls the levels of normal mRNAs as exemplified by the autoregulation of the nuclear poly(A)-binding protein Nab2 activity in budding yeast.³⁶ The interaction of Nab2 with an oligoadenylate sequence in the 3' UTR of its own mRNA destabilizes the transcript in a manner dependent on Rrp6 activity. Interestingly, NAB2 mRNA 3' end formation occurs by a non-canonical mechanism that requires Exo10 and the Trf4 component of the TRAMP complex.³⁷ A similar mechanism forms the mature end of the CTH2 mRNA and, possibly other mRNAs.³⁸ Rrp6 and the TRAMP complex regulate the levels of histone mRNAs by degrading the transcripts and contributing to their removal at the end of the S-phase of the cell cycle. The requirement for Rrp6 suggests that the negative effect of histone synthesis beyond the end of S-phase requires the rapid destruction of their mRNAs in the nucleus as well as in the cytoplasm.^{39,40} In *Drosophila melanogaster*, depletion of Rrp6 leads to mitotic defects that may reflect altered accumulation of mitotic mRNAs.⁴¹ Similarly, loss of Rrp6 activity causes meiotic defects in *Schizosaccharomyces pombe*, and evidence suggests that the Mmi protein

targets meiosis-specific pre-mRNAs for degradation by Rrp6.^{42, 43} Rrp6 also participates in a pathway that degrades mRNAs that exit the nucleus slowly; either due to defects in nuclear export, or naturally slow, mRNA-specific export rates.⁴⁴ In this pathway, called DRN (Degradation of RNA in the Nucleus), Rrp6 acts in concert with the nuclear RNA cap-binding complex to accelerate the degradation of slowly exported mRNAs.²⁵ These findings indicate that cells utilize the nuclear mRNA surveillance function of Rrp6 and perhaps Exo11 to regulate the concentration of a number of mRNAs for the purposes of simple feedback control and more complex regulation of cell cycle events. It seems likely that the list of mRNAs whose levels are subject to regulation by Rrp6 and the exosome will continue to grow.

Rrp6 and the TRAMP complex

The surprising discovery of polyadenylated non-coding RNAs and the existence of non-canonical poly(A)-polymerases revealed a role for Rrp6 and Exo10 in a polyadenylation dependent pathway for the degradation of aberrant RNA processing intermediates and transcripts arising from pervasive transcription of the genome. The first clues to the existence of this pathway came from experiments that demonstrated the accumulation of polyadenylated sn- and snoRNAs, and pre-rRNAs in budding yeast strains lacking Rrp6.^{16, 17, 45} A key experiment also revealed that the degradation of hypomodified pre-tRNA_i^{Met} requires the activity of Dis3/Rrp44 and Rrp6.⁴⁶ Importantly, these studies identified a requirement for the activity of the non-canonical poly(A)-polymerase Trf4 in destruction of the aberrant pre-tRNA_i^{Met}. Later studies showed that Trf4 and another non-canonical poly(A)-polymerase, Trf5, exist in complexes with putative RNA-binding

proteins Air1 and Air2, as well as with the RNA helicase Mtr4.^{47, 48} These complexes, called TRAMP4 and TRAMP5 respectively, play an essential role in the degradation of non-coding RNAs in eukaryotes.⁴⁹⁻⁵¹ In some cases, these transcripts may arise from pervasive transcription of the genome that produces a surprising array of sense, antisense and intergenic transcripts, in addition to gene-encoding RNAs.⁵²⁻⁵⁴ Many of these RNAs, as well as intermediates in the biogenesis pathways producing sn-/snRNAs and rRNAs, are polyadenylated by the TRAMP complexes, which facilitates their hydrolysis by Rrp6 and Exo10.

This surveillance mechanism ensures the destruction of aberrant RNA processing intermediates and disposes of transcripts that result from pervasive bi-directional transcription initiation by RNA polymerase II.^{55, 56} While some of the RNAs generated in this manner appear to lack any function, evidence from budding yeast and *Arabidopsis thaliana* suggests that some influence gene regulation, development and gene silencing.⁵⁷⁻⁶⁰ Experiments in *S. pombe* revealed a requirement for TRAMP and Rrp6 in the degradation of transcripts arising from silenced heterochromatin and in posttranscriptional control of RNAi-dependent gene silencing.⁶¹⁻⁶³ TRAMP complexes likely play a critical role in determining the levels of these regulatory transcripts, since polyadenylation by TRAMP enhances RNA degradation by the nuclear exosome. However, how TRAMP distinguishes between RNAs destined for rapid turnover and other stable RNAs remains a major unanswered question.

The exosome cofactor Rrp47

The yeast protein Rrp47 (also known as Lrp1 or yC1D) and its mammalian homologue C1D are eukaryotic, nuclear proteins that bind both RNA and DNA.^{11, 14, 64, 65} Research on Rrp47 has focussed on its physical association with the exosome nuclease complex.^{13, 66, 67} and the role of this complex in RNA processing and degradation pathways (for recent reviews of exosome structure and function, see^{52, 68-71}), while C1D was initially characterised as a DNA-binding protein that functions in transcription and DNA repair.^{64, 72, 73} Nevertheless, there is strong evidence of functional conservation of Rrp47/C1D; both proteins interact directly with the catalytic exosome component Rrp6 (known as PM-Scl100 in humans),^{11, 14} yeast *rrp47-Δ* mutants show defects in DNA repair^{74, 75} and similar defects on 5.8S rRNA maturation are observed in yeast *rrp47-Δ* and *rrp6-Δ* mutants and upon depletion of C1D in mammalian cells.^{4, 13, 14}

Structure of Rrp47

Rrp47 and C1D are small, basic proteins of 21kDa and 16kDa, respectively. There is no detailed structural data currently available for these proteins but sequence homology and secondary structure prediction programmes⁷⁶ suggest that the first ~120 amino acid residues of Rrp47 comprise a conserved N-terminal α -helical domain, while the C-terminal region is less structured and more variable. The C-terminus of Rrp47 and homologous proteins is rich in basic residues and presumably contributes to RNA binding.^{11, 77} Rrp47 and C1D can both be phosphorylated *in vitro*^{73, 78} but the functional relevance of this is not clear. In contrast to other exosome proteins including Rrp6, Rrp47

has not been observed as a phosphoprotein in mass spectrometric analyses of exosome complexes^{79, 80} or in orthophosphate in vivo labeling experiments (our unpublished data).

The conserved N-terminal region of Rrp47/C1D spans the bioinformatically defined Sas10/C1D domain (residues 10-89 of Rrp47)⁸¹ also present in Sas10 (also known as Utp3) and Lcp5, two U3 small nucleolar RNA (snoRNA)-associated proteins that are components of the small subunit processosome or pre-90S subunit.⁸²⁻⁸⁶ Overexpression of Sas10 partially suppressed silencing of transcriptionally repressed chromatin in a Sir-independent manner,⁸⁷ an effect that has also been reported for mutants in the exosome and TRAMP complexes.^{88, 89} One possibility is that overexpression of Sas10 may (partially) titrate out the complex between Rrp47 and Rrp6. A Sas10/C1D domain is also found in the protein neuroguidin, an eIF4E-binding protein that is required for cytoplasmic polyadenylation-dependent translational control in neuronal cells.⁹⁰ Sas10, Lcp5 and neuroguidin also have basic regions at their C-termini that may contribute to RNA binding but whether the Sas10/C1D domain represents an RNA-binding domain requires further experimentation.

Biochemical activities of Rrp47

Rrp47 and C1D are associated with Rrp6-containing exosome complexes^{13, 67} and interact directly with Rrp6 in vitro.^{11, 14} Binding occurs via the N-terminal PMC2NT domain of Rrp6⁹¹ and the N-terminal α -helical region of Rrp47 (our unpublished observations). Westerns of epitope-tagged proteins show that Rrp47 and Rrp6 are expressed at comparable levels in yeast,¹¹ Rrp6 being present at approximately 2,000 molecules per

cell.⁹² Normal expression levels of Rrp47 in yeast are dependent upon this interaction with Rrp6.¹¹ Therefore, the interpretation of data from experiments using *rrp6-Δ* null alleles should take into account that Rrp47 expression levels are also significantly affected.

Rrp47 and C1D show specificity for structured RNA *in vitro*.^{11, 14} Consistent with an important role for Rrp47 RNA binding activity *in vivo*, RNAs that accumulate in *rrp47-Δ* mutants, such as the 3' extended 5.8S rRNA precursor, are predicted to have double stranded regions at their 3' termini.⁹³ The dissociation constant of yeast Rrp47 for RNA and DNA is approximately 1 μM (calculating the protein concentration based on the predicted molecular weight of the monomeric protein).¹¹ C1D is reported to have “exceptional DNA affinity”⁶⁴ but the dissociation constant for DNA or RNA has not been reported and it is not clear whether the native protein has a preference for DNA or RNA substrates. Assuming there are approximately 2,000 molecules of Rrp47 per yeast cell and the protein is distributed evenly throughout the nucleus, which has a volume of approximately 3 μm,⁹⁴ the intracellular concentration of Rrp47 is close to its dissociation constant. Therefore, small regulatory changes in the effective concentration of Rrp47 would impact strongly on the efficiency with which this protein would bind its target substrates. Notably, overexpression of C1D is toxic.⁹⁵

The Role of Rrp47 in RNA Processing and Degradation

Yeast strains lacking Rrp47 or Rrp6 accumulate a common set of cellular RNAs that, in wild-type cells, are normally efficiently processed to mature RNAs or rapidly degraded.

Rrp47 is not required for normal Rrp6 expression levels or the association of Rrp6 with exosome complexes.¹³ Since a trimeric complex can be formed between Rrp47, Rrp6 and RNA, it has been suggested that Rrp47 promotes substrate binding to Rrp6.¹¹ Purified Rrp6 degrades unstructured RNA efficiently but is blocked by stem loop structures,^{8, 22} suggesting that Rrp47 may facilitate degradation of structured RNA. Rrp47/C1D does not have any sequence homology to RNA helicases. Rrp47 might simply increase the retention time of Rrp6 on structured substrates or it may function sterically by positioning the 3' end of structured RNA close to the catalytic centre of Rrp6, as has been proposed for the C'-terminal HRDC domain of RNase D.⁹ Notably, while the pattern of stable RNA precursors observed in *rrp47-Δ* and *rrp6-Δ* strains by northern blot analyses are largely indistinguishable,¹³ distinct effects are seen in these mutants upon analysis of global mRNA profiles.^{75, 96} This suggests that Rrp6 can function independently of Rrp47 on some substrates.

Rrp47 might also function in substrate recognition, either through interaction with RNA or other processing factors (Figure 2). Interactions between the Nrd1 termination complex and the exosome and TRAMP complexes are thought to recruit the degradation/processing machinery to some of its RNA substrates⁹⁷ and Rrp47 is present in such large RNA processing/degradation complexes.⁹⁸ Nrd1 and the associated protein Nab3 are RNA-binding proteins that bind cooperatively to multiple copies of tetrameric recognition sequences present in the terminator regions of target transcripts.⁹⁹ The *rrp47-Δ* mutation is synthetic lethal with the RNA-binding defective *nrd1-102* mutation,¹⁰⁰ suggesting that the RNA-binding activity of Rrp47 may play an important role in the

recruitment of the exosome to the termination complex. It has also been suggested that the interaction between Rrp47 and DNA may help position exosome complexes at transcriptionally active sites.¹⁰¹

Rrp47/C1D is well annotated as a DNA repair factor. Yeast strains lacking Rrp47 or Rrp6 are defective in the degradation of specific mRNA transcripts upon exposure to UV irradiation and in the repair of UV irradiation-induced cyclobutane dimers, and *rrp6-Δ* mutants are synergistically sick in combination with *rad26-Δ* mutants.⁷⁵ The sensitivity of *rrp6-Δ* and *rrp47-Δ* mutants to UV irradiation may reflect a quality control function of the exosome to eliminate aberrant transcripts that arise as a result of DNA damage in transcription-coupled DNA repair.¹⁰²

The exosome cofactor Mpp6

Mpp6 (MPP6 in humans) shares a number of similarities with Rrp47, both being exosome-associated RNA-binding proteins that are small and basic in nature. The proteins have distinct substrate specificities, however, Rrp47 recognising structured RNA while Mpp6 binds pyrimidine-rich sequences.^{11, 14, 98, 103} While Rrp47 is functionally linked to the activity of Rrp6, the role of Mpp6 in exosome function is less clear.

MPP6 was first identified as a nuclear protein that is phosphorylated and distributed throughout the cell during mitosis.¹⁰⁴ MPP6 copurifies with exosome complexes that contain the Rrp6 homologue PM-Sc1100 and hMtr4 but which lack the Rrp44/Dis3 homologue.¹⁰⁵ Thus, the interaction between MPP6 and the exosome is independent of

Rrp44. Interactions between MPP6 and PM-Scl100 or hMtr4 were first suggested by two-hybrid interaction¹⁰⁶ and subsequently shown to be direct by pull-down experiments using recombinant and in vitro translated proteins.¹⁴ The binding sites within Rrp6 for MPP6 and C1D apparently do not overlap, since a stable trimeric complex could be assembled.

Depletion of MPP6 caused an accumulation of 3' extended forms of 5.8S rRNA similar to that seen upon loss of exosome function.¹⁰³ Notably, the 3' extended 5.8S pre-rRNA that accumulates upon depletion of MPP6 contains a pyrimidine-rich sequence at its 3' end, suggesting that MPP6 might target the exosome to this substrate. Consistent with a role in pre-rRNA processing, both the mammalian and yeast proteins are found associated with large ribosome-containing complexes.^{103, 107}

The yeast protein Mpp6 was first demonstrated to be associated with the exosome complex in a global proteomics study⁸⁶ and subsequently shown to be required for viability of *rrp47-Δ* mutants.⁹⁸ In common with other exosome mutants, *mpp6-Δ* strains showed defects in 5.8S rRNA maturation, accumulated the IGS1-R rDNA intergenic transcript and suppressed the loss of mRNA observed in *rna14-1* and *prp2-1* strains.^{23, 88, 89, 98, 108} Notably, the accumulation of the NEL025C CUT upon loss of both Mpp6 and Rrp47 was significantly greater than each single mutant,⁹⁸ suggesting that an exacerbated accumulation of regulatory RNA transcripts might contribute to the synthetic lethality of *rrp47-Δ mpp6-Δ* strains.

The *mpp6-Δ* mutation is also synthetic lethal with the *rrp6-Δ* mutation.⁹⁸ This implies that Mpp6 and Rrp47 do not function in a functionally redundant manner to target substrates to Rrp6. It has been proposed that Mpp6 might promote the activity of the other exosome catalytic subunit, Rrp44.⁹⁸ Given that human MPP6 protein physically interacts with PM-Sc1100 and hMtr4,¹⁴ that it is associated with the exosome in the absence of Rrp44¹⁰⁵ and that the yeast *mpp6-Δ* mutation shows strong genetic interactions with *air1-Δ* mutations,^{98, 109} another possibility is that Mpp6 promotes the functional coupling between Rrp6 and the TRAMP complex.

Conclusion

The recent discovery of diverse noncoding RNAs that are stabilised in the absence of Rrp6^{55, 56, 110} has dramatically increased the number of substrates known to be processed or degraded by the nuclear exosome. The molecular mechanisms by which the exosome is targeted to its substrate molecules and how RNA processing substrates are distinguished from those targeted to complete degradation, however, remain largely unresolved. These outstanding questions address fundamental issues concerning the links between transcription termination, RNA processing and RNP particle assembly, and the distinction between RNA processing events and RNA surveillance. A general point appears to be the redundancy seen for nucleolytic, polymerase and RNA-binding activities in exosome-mediated RNA 3' processing that presumably facilitate efficient processing and provide a system that is well buffered against genetic modulation.

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Legends to Figures

Figure 1. Structure of Rrp6.

The top diagram illustrates the polypeptide structure of Rrp6. The bottom panels compare the crystal structures of *E. coli* RNaseD and a N-terminal and C-terminal truncated version of Rrp6 from *S. cerevisiae*. The molecules in the panels were derived from the Protein Data Base using PyMol.

Figure 2. Substrate recognition and degradation by the nuclear exosome.

The schematic depicts the general pathway of exosome recruitment and substrate digestion/processing by the nuclear exosome complex. The exosome is recruited to its RNA substrates (either polymerase-engaged transcription termination complexes or RNP particles with accessible 3' ends) through a poorly understood set of interactions that, in most cases, will involve both RNA binding with undetermined sequences or structures and protein recognition with poorly characterised partner proteins (labelled "X"). The DNA binding activity of Rrp47 may also promote recruitment of the exosome to termination regions. Degradation/processing of the RNA can be promoted by Trf4- or Trf5-mediated oligoadenylation and by the RNA helicase activity of Mtr4, all of which are components of the TRAMP complex, while Rrp47, Mpp6 and the Air proteins contribute to RNA binding. RNA hydrolysis by the Rrp6 and Rrp44 exonucleases progresses until enzyme activity is blocked by the preassembled RNP particle, allowing 3' end maturation. In the absence of correct RNP particle assembly, the RNA is degraded completely by the exosome and TRAMP complexes. Other substrates are generated by endonucleolytic cleavage or arise through delayed transcription/processing events. For

some substrates, Rrp6 can function together with Rrp47 and TRAMP, independently of the core exosome complex.

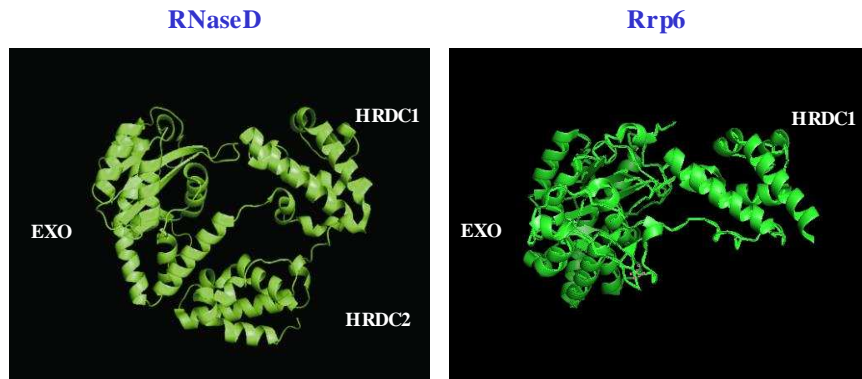


Figure 1

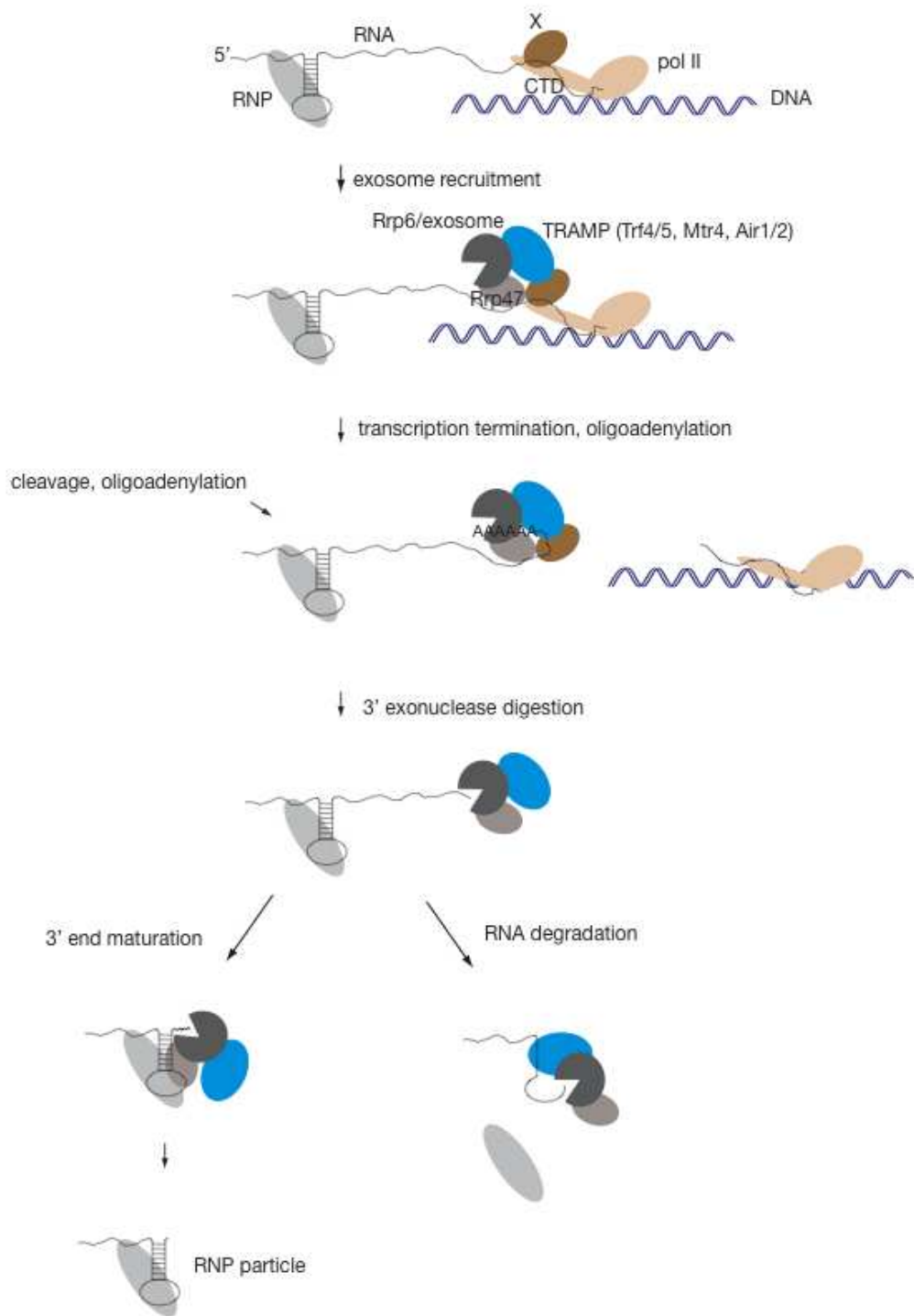


Figure 2