Book Section:
Butler, J. Scott and Mitchell, Phil (2010) Rrp6, Rrp47 and co-factors of the nuclear exosome. In: Jensen, Torben Heick, (ed.) RNA Exosome. Advances in Experimental Medicine and Biology, 702. Landes Bioscience, Austin, TX, USA, pp. 91-104. ISBN: 978-1-4419-7840-0.

Rrp6, Rrp47 and co-factors of the nuclear exosome

J. Scott Butler[1] and Phil Mitchell[2]

[1]Departments 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

[2]Department of Molecular Biology and Biotechnology,
The University of Sheffield,
Firth Court,
Western Bank,
Sheffield S10 2TN
UK
p.j.mitchell@shef.ac.uk

Keywords:
exosome, Rrp6, PM-Scl100, Rrp47, Lrp1, C1D, Mpp6, TRAMP, Nrd1, RNA, RNP particle, RNA processing, RNA surveillance,

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 it 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 Ngl2.[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 psuedouridine 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 programmes[76] 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 Rrp6[91] 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 1μM (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 3μm,[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 substrates[97] 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-Scl100 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 interaction[106] 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 study[86] 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-Scl100 and hMtr4,[14] that it is associated with the exosome in the absence of Rrp44[105] 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.

Acknowledgements

S.B. was supported by a grant from the National Science Foundation (MCB-0817324) and P.M. was supported by a research grant from the Wellcome Trust (088306/Z/09/Z).

References

1. Haile S, Estevez A, Clayton C. A role for the exosome in the in vivo degradation of unstable mRNAs. RNA 2003; 9: 1491-1501.

2. Lange H, Holec S, Cognat V et al. Degradation of a polyadenylated rRNA maturation by-product involves one of the three RRP6-like proteins in Arabidopsis thaliana. Mol Cell Biol 2008; 28: 3038-3044.

3. Lejeune F, Li X, Maquat L. Nonsense-mediated mRNA decay in mammalian cells involves decapping, deadenylating, and exonucleolytic activities. Mol Cell 2003; 12: 675-687.

4. Briggs M, Burkard K, Butler J. Rrp6p, the yeast homologue of the human PM-Scl 100-kDa autoantigen, is essential for efficient 5.8 S rRNA 3' end formation. J Biol Chem 1998; 273: 13255-13263.

5. Zuo Y, Deutscher M. Exoribonuclease superfamilies: structural analysis and phylogenetic distribution. Nucleic Acids Res 2001; 29: 1017-1026.

6. Phillips S, Butler J. Contribution of domain structure to the RNA 3' end processing and degradation functions of the nuclear exosome subunit Rrp6p. RNA 2003; 9: 1098-1107.

7. Midtgaard S, Assenholt J, Jonstrup A et al. Structure of the nuclear exosome component Rrp6p reveals an interplay between the active site and the HRDC domain. Proc Natl Acad Sci U S A 2006; 103: 11898-11903.

8. Liu Q, Greimann J, Lima C. Reconstitution, activities, and structure of the eukaryotic RNA exosome. Cell 2006; 127: 1223-1237.

9. Zuo Y, Wang Y, Malhotra A. Crystal structure of Escherichia coli RNase D, an exoribonuclease involved in structured RNA processing. Structure 2005; 13: 973-984.

10. Callahan K, Butler J. Evidence for core exosome independent function of the nuclear exoribonuclease Rrp6p. Nucleic Acids Res 2008; 36: 6645-6655.

11. Stead J, Costello J, Livingstone M et al. The PMC2NT domain of the catalytic exosome subunit Rrp6p provides the interface for binding with its cofactor Rrp47p, a nucleic acid-binding protein. Nucleic Acids Res 2007; 35: 5556-5567.

12. Thomson E, Tollervey D. The final step in 5.8S rRNA processing is cytoplasmic in Saccharomyces cerevisiae. Mol Cell Biol 2010; 30: 976-984.

13. Mitchell P, Petfalski E, Houalla R et al. Rrp47p is an exosome-associated protein

required for the 3' processing of stable RNAs. Mol Cell Biol 2003; 23: 6982-6992.

14. Schilders G, van Dijk E, Pruijn G. C1D and hMtr4p associate with the human exosome subunit PM/Scl-100 and are involved in pre-rRNA processing. Nucleic Acids Res 2007; 35: 2564-2572.

15. Allmang C, Petfalski E, Podtelejnikov A et al. The yeast exosome and human PM-Scl are related complexes of 3'->5' exonucleases. Genes Dev 1999; 13: 2148-2158.

16. van Hoof A, Lennertz P, Parker R. Yeast exosome mutants accumulate 3'-extended polyadenylated forms of U4 small nuclear RNA and small nucleolar RNAs. Mol Cell Biol 2000; 20: 441-452.

17. Allmang C, Kufel J, Chanfreau G et al. Functions of the exosome in rRNA, snoRNA and snRNA synthesis. EMBO J 1999; 18: 5399-5410.

18. Callahan K, Butler J. The TRAMP complex enhances RNA degradation by the nuclear exosome component Rrp6. J Biol Chem 2009; 285: 3540-3547.

19. Egecioglu D, Henras A, Chanfreau G. Contributions of Trf4p- and Trf5p-dependent polyadenylation to the processing and degradative functions of the yeast nuclear exosome. RNA 2006; 12: 26-32.

20. Grzechnik P, Kufel J. Polyadenylation linked to transcription termination directs the processing of snoRNA precursors in yeast. Mol Cell 2008; 32: 247-258.

21. Lemay J, D'Amours A, Lemieux C et al. The nuclear poly(A)-binding protein interacts with the exosome to promote synthesis of noncoding small nucleolar RNAs. Mol Cell 2010; 37: 34-45.

22. Burkard K, Butler J. A nuclear 3'-5' exonuclease involved in mRNA degradation interacts with Poly(A) polymerase and the hnRNA protein Npl3p. Mol Cell Biol 2000; 20: 604-616.

23. Torchet C, Bousquet-Antonelli C, Milligan L et al. Processing of 3'-extended read-through transcripts by the exosome can generate functional mRNAs. Mol Cell 2002; 9: 1285-1296.

24. Milligan L, Torchet C, Allmang C et al. A nuclear surveillance pathway for mRNAs with defective polyadenylation. Mol Cell Biol 2005; 25: 9996-10004.

25. Das B, Butler J, Sherman F. Degradation of normal mRNA in the nucleus of Saccharomyces cerevisiae. Mol Cell Biol 2003; 23: 5502-5515.

26. Hilleren P, McCarthy T, Rosbash M et al. Quality control of mRNA 3'-end processing is linked to the nuclear exosome. Nature 2001; 413: 538-542.

27. Libri D, Dower K, Boulay J et al. Interactions between mRNA export commitment, 3'-end quality control, and nuclear degradation. Mol Cell Biol 2002; 22: 8254-8266.

28. Rougemaille M, Gudipati R, Olesen J et al. Dissecting mechanisms of nuclear mRNA surveillance in THO/sub2 complex mutants. EMBO J 2007; 26: 2317-2326.

29. Saguez C, Schmid M, Olesen J et al. Nuclear mRNA surveillance in THO/sub2 mutants is triggered by inefficient polyadenylation. Mol Cell 2008; 31: 91-103.

30. Qu X, Lykke-Andersen S, Nasser T et al. Assembly of an export-competent mRNP is needed for efficient release of the 3'-end processing complex after polyadenylation. Mol Cell Biol 2009; 29: 5327-5338.

31. Lum P, Armour C, Stepaniants S et al. Discovering modes of action for therapeutic compounds using a genome-wide screen of yeast heterozygotes. Cell 2004; 116: 121-137.

32. Fang F, Hoskins J, Butler J. 5-fluorouracil enhances exosome-dependent accumulation of polyadenylated rRNAs. Mol Cell Biol 2004; 24: 10766-10776.

33. Kammler S, Lykke-Andersen S, Jensen T. The RNA exosome component hRrp6 is a target for 5-fluorouracil in human cells. Mol Cancer Res 2008; 6: 990-995.

34. Hoskins J, Scott Butler J. Evidence for distinct DNA- and RNA-based mechanisms of 5-fluorouracil cytotoxicity in Saccharomyces cerevisiae. Yeast 2007; 24: 861-870.

35. Hoskins J, Butler J. RNA-based 5-fluorouracil toxicity requires the pseudouridylation activity of Cbf5p. Genetics 2008; 179: 323-330.

36. Roth K, Wolf M, Rossi M et al. The nuclear exosome contributes to autogenous control of NAB2 mRNA levels. Mol Cell Biol 2005; 25: 1577-1585.

37. Roth K, Byam J, Fang F et al. Regulation of NAB2 mRNA 3'-end formation requires the core exosome and the Trf4p component of the TRAMP complex. RNA 2009; 15: 1045-1058.

38. Ciais D, Bohnsack M, Tollervey D. The mRNA encoding the yeast ARE-binding protein Cth2 is generated by a novel 3' processing pathway. Nucleic Acids Res 2008; 36: 3075-3084.

39. Reis C, Campbell J. Contribution of Trf4/5 and the nuclear exosome to genome stability through regulation of histone mRNA levels in Saccharomyces cerevisiae. Genetics 2007; 175: 993-1010.

40. Canavan R, Bond U. Deletion of the nuclear exosome component RRP6 leads to continued accumulation of the histone mRNA HTB1 in S-phase of the cell cycle in Saccharomyces cerevisiae. Nucleic Acids Res 2007; 35: 6268-6279.

41. Graham A, Kiss D, Andrulis E. Core exosome-independent roles for Rrp6 in cell cycle progression. Mol Biol Cell 2009; 20: 2242-2253.

42. McPheeters D, Cremona N, Sunder S et al. A complex gene regulatory mechanism that operates at the nexus of multiple RNA processing decisions. Nat Struct Mol Biol 2009; 16: 255-264.

43. Harigaya Y, Tanaka H, Yamanaka S et al. Selective elimination of messenger RNA prevents an incidence of untimely meiosis. Nature 2006; 442: 45-50.

44. Kuai L, Das B, Sherman F. A nuclear degradation pathway controls the abundance of normal mRNAs in Saccharomyces cerevisiae. Proc Natl Acad Sci USA 2005; 102: 13962-13967.

45. Kuai L, Fang F, Butler J et al. Polyadenylation of rRNA in Saccharomyces cerevisiae. Proc Natl Acad Sci USA 2004; 101: 8581-8586.

46. Kadaba S, Krueger A, Trice T et al. Nuclear surveillance and degradation of hypomodified initiator tRNAMet in S. cerevisiae. Genes Dev 2004; 18: 1227-1240.

47. Vanácová S, Wolf J, Martin G et al. A new yeast poly(A) polymerase complex involved in RNA quality control. PLoS Biol 2005; 3: e189.

48. LaCava J, Houseley J, Saveanu C et al. RNA degradation by the exosome is promoted by a nuclear polyadenylation complex. Cell 2005; 121: 713-724.

49. Houseley J, Tollervey D. Yeast Trf5p is a nuclear poly(A) polymerase. EMBO Rep 2006; 7: 205-211.

50. Win T, Draper S, Read R et al. Requirement of fission yeast Cid14 in polyadenylation of rRNAs. Mol Cell Biol 2006; 26: 1710-1721.

51. Wyers F, Rougemaille M, Badis G et al. Cryptic pol II transcripts are degraded by a nuclear quality control pathway involving a new poly(A) polymerase. Cell 2005; 121: 725-737.

52. Belostotsky D. Exosome complex and pervasive transcription in eukaryotic

genomes. Curr Opin Cell Biol 2009; 21: 352-358.

53. Jacquier A. The complex eukaryotic transcriptome: unexpected pervasive transcription and novel small RNAs. Nat Rev Genet 2009; 10: 833-844.

54. Kapranov P, Willingham A, Gingeras T. Genome-wide transcription and the implications for genomic organization. Nat Rev Genet 2007; 8: 413-423.

55. Neil H, Malabat C, d'Aubenton-Carafa Y et al. Widespread bidirectional promoters are the major source of cryptic transcripts in yeast. Nature 2009; 457: 1038-1042.

56. Xu Z, Wei W, Gagneur J et al. Bidirectional promoters generate pervasive transcription in yeast. Nature 2009; 457: 1033-1037.

57. Uhler J, Hertel C, Svejstrup J. A role for noncoding transcription in activation of the yeast PHO5 gene. Proc Natl Acad Sci U S A 2007; 104: 8011-8016.

58. Houseley J, Rubbi L, Grunstein M et al. A ncRNA modulates histone modification and mRNA induction in the yeast GAL gene cluster. Mol Cell 2008; 32: 685-695.

59. Camblong J, Iglesias N, Fickentscher C et al. Antisense RNA stabilization induces transcriptional gene silencing via histone deacetylation in S. cerevisiae. Cell 2007; 131: 706-717.

60. Chekanova J, Gregory B, Reverdatto S et al. Genome-wide high-resolution mapping of exosome substrates reveals hidden features in the Arabidopsis transcriptome. Cell 2007; 131: 1340-1353.

61. Bühler M, Spies N, Bartel D et al. TRAMP-mediated RNA surveillance prevents spurious entry of RNAs into the Schizosaccharomyces pombe siRNA pathway. Nat Struct Mol Biol 2008; 15: 1015-1023.

62. Wang S, Stevenson A, Kearsey S et al. Global role for polyadenylation-assisted nuclear RNA degradation in posttranscriptional gene silencing. Mol Cell Biol 2008; 28: 656-665.

63. Bühler M, Haas W, Gygi S et al. RNAi-dependent and -independent RNA turnover mechanisms contribute to heterochromatic gene silencing. Cell 2007; 129: 707-721.

64. Nehls P, Keck T, Greferath R et al. cDNA cloning, recombinant expression and characterization of polypetides with exceptional DNA affinity. Nucleic Acids Res 1998; 26: 1160-1166.

65. Kumar A, Agarwal S, Heyman J et al. Subcellular localization of the yeast proteome. Genes Dev 2002; 16: 707-719.

66. Gavin A, Bösche M, Krause R et al. Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature 2002; 415: 141-147.

67. Peng W, Robinson M, Mnaimneh S et al. A panoramic view of yeast noncoding RNA processing. Cell 2003; 113: 919-933.

68. Vanacova S, Stefl R. The exosome and RNA quality control in the nucleus. EMBO Rep 2007; 8: 651-657.

69. Lebreton A, Séraphin B. Exosome-mediated quality control: substrate recruitment and molecular activity. Biochim Biophys Acta 2008; 1779: 558-565.

70. Schmid M, Jensen T. The exosome: a multipurpose RNA-decay machine. Trends Biochem Sci 2008; 33: 501-510.

71. Lykke-Andersen S, Brodersen D, Jensen T. Origins and activities of the eukaryotic exosome. J Cell Sci 2009; 122: 1487-1494.

72. Zamir I, Dawson J, Lavinsky R et al. Cloning and characterization of a corepressor and potential component of the nuclear hormone receptor repression complex. Proc Natl Acad Sci USA 1997; 94: 14400-14405.

73. Yavuzer U, Smith G, Bliss T et al. DNA end-independent activation of DNA-PK mediated via association with the DNA-binding protein C1D. Genes Dev 1998; 12: 2188-2199.

74. Erdemir T, Bilican B, Cagatay T et al. Saccharomyces cerevisiae C1D is implicated in both non-homologous DNA end joining and homologous recombination. Mol Microbiol 2002; 46: 947-957.

75. Hieronymus H, Yu M, Silver P. Genome-wide mRNA surveillance is coupled to mRNA export. Genes Dev 2004; 18: 2652-2662.

76. Kelley L, Sternberg M. Protein structure prediction on the Web: a case study using the Phyre server. Nat Protoc 2009; 4: 363-371.

77. Spriggs R, Murakami Y, Nakamura H et al. Protein function annotation from sequence: prediction of residues interacting with RNA. Bioinformatics 2009; 25: 1492-1497.

78. Ptacek J, Devgan G, Michaud G et al. Global analysis of protein phosphorylation in yeast. Nature 2005; 438: 679-684.

79. Synowsky S, van den Heuvel R, Mohammed S et al. Probing genuine strong interactions and post-translational modifications in the heterogeneous yeast exosome protein complex. Mol Cell Proteomics 2006; 5: 1581-1592.

80. Synowsky S, van Wijk M, Raijmakers R et al. Comparative Multiplexed Mass Spectrometric Analyses of Endogenously Expressed Yeast Nuclear and Cytoplasmic Exosomes. J Mol Biol 2008; 385: 1300-1313.

81. Finn R, Mistry J, Tate J et al. The Pfam protein families database. Nucleic Acids Res 2010; 38: D211-D222.

82. Wiederkehr T, Prétôt R, Minvielle-Sebastia L. Synthetic lethal interactions with conditional poly(A) polymerase alleles identify LCP5, a gene involved in 18S rRNA maturation. RNA 1998; 4: 1357-1372.

83. Uetz P, Giot L, Cagney G et al. A comprehensive analysis of protein-protein interactions in Saccharomyces cerevisiae. Nature 2000; 403: 623-627.

84. Dragon F, Gallagher J, Compagnone-Post P et al. A large nucleolar U3 ribonucleoprotein required for 18S ribosomal RNA biogenesis. Nature 2002; 417: 967-970.

85. Hoang T, Peng W, Vanrobays E et al. Esf2p, a U3-associated factor required for small-subunit processome assembly and compaction. Mol Cell Biol 2005; 25: 5523-5534.

86. Krogan N, Cagney G, Yu H et al. Global landscape of protein complexes in the yeast Saccharomyces cerevisiae. Nature 2006; 440: 637-643.

87. Kamakaka R, Rine J. Sir- and silencer-independent disruption of silencing in Saccharomyces by Sas10p. Genetics 1998; 149: 903-914.

88. Vasiljeva L, Kim M, Terzi N et al. Transcription termination and RNA degradation contribute to silencing of RNA polymerase II transcription within heterochromatin. Mol Cell 2008; 29: 313-323.

89. Houseley J, Kotovic K, El Hage A et al. Trf4 targets ncRNAs from telomeric and rDNA spacer regions and functions in rDNA copy number control. EMBO J 2007; 26: 4996-5006.

90. Jung M, Lorenz L, Richter J. Translational control by neuroguidin, a eukaryotic initiation factor 4E and CPEB binding protein. Mol Cell Biol 2006; 26: 4277-4287.

91. Staub E, Fiziev P, Rosenthal A et al. Insights into the evolution of the nucleolus

by an analysis of its protein domain repertoire. Bioessays 2004; 26: 567-581.

92. Ghaemmaghami S, Huh W, Bower K et al. Global analysis of protein expression in yeast. Nature 2003; 425: 737-741.

93. Yeh L, Lee J. Structural analysis of the internal transcribed spacer 2 of the precursor ribosomal RNA from Saccharomyces cerevisiae. J Mol Biol 1990; 211: 699-712.

94. Jorgensen P, Edgington N, Schneider B et al. The size of the nucleus increases as yeast cells grow. Mol Biol Cell 2007; 18: 3523-3532.

95. Rothbarth K, Spiess E, Juodka B et al. Induction of apoptosis by overexpression of the DNA-binding and DNA-PK-activating protein C1D. J Cell Sci 1999; 112: 2223-2232.

96. Houalla R, Devaux F, Fatica A et al. Microarray detection of novel nuclear RNA substrates for the exosome. Yeast 2006; 23: 439-454.

97. Vasiljeva L, Buratowski S. Nrd1 interacts with the nuclear exosome for 3' processing of RNA polymerase II transcripts. Mol Cell 2006; 21: 239-248.

98. Milligan L, Decourty L, Saveanu C et al. A yeast exosome cofactor, Mpp6, functions in RNA surveillance and in the degradation of noncoding RNA transcripts. Mol Cell Biol 2008; 28: 5446-5457.

99. Carroll K, Ghirlando R, Ames J et al. Interaction of yeast RNA-binding proteins Nrd1 and Nab3 with RNA polymerase II terminator elements. RNA 2007; 13: 361-373.

100. Arigo J, Eyler D, Carroll K et al. Termination of cryptic unstable transcripts is directed by yeast RNA-binding proteins Nrd1 and Nab3. Mol Cell 2006; 23: 841-851.

101. Houseley J, Tollervey D. The nuclear RNA surveillance machinery: The link between ncRNAs and genome structure in budding yeast? Biochim Biophys Acta 2008; 1779: 239-246.

102. Mirkin N, Fonseca D, Mohammed S et al. The 3' processing factor CstF functions in the DNA repair response. Nucleic Acids Res 2008; 36: 1792-1804.

103. Schilders G, Raijmakers R, Raats J et al. MPP6 is an exosome-associated RNA-binding protein involved in 5.8S rRNA maturation. Nucleic Acids Res 2005; 33: 6795-6804.

104. Matsumoto-Taniura N, Pirollet F, Monroe R et al. Identification of novel M phase

phosphoproteins by expression cloning. Mol Biol Cell 1996; 7: 1455-1469.

105. Chen C, Gherzi R, Ong S et al. AU binding proteins recruit the exosome to degrade ARE-containing mRNAs. Cell 2001; 107: 451-464.

106. Lehner B, Sanderson C. A protein interaction framework for human mRNA degradation. Genome Res 2004; 14: 1315-1323.

107. Fleischer T, Weaver C, McAfee K et al. Systematic identification and functional screens of uncharacterized proteins associated with eukaryotic ribosomal complexes. Genes Dev 2006; 20: 1294-1307.

108. Bousquet-Antonelli C, Presutti C, Tollervey D. Identification of a regulated pathway for nuclear pre-mRNA turnover. Cell 2000; 102: 765-775.

109. Wilmes G, Bergkessel M, Bandyopadhyay S et al. A genetic interaction map of RNA-processing factors reveals links between Sem1/Dss1-containing complexes and mRNA export and splicing. Mol Cell 2008; 32: 735-746.

110. Preker P, Nielsen J, Kammler S et al. RNA exosome depletion reveals transcription upstream of active human promoters. Science 2008; 322: 1851-1854.

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.

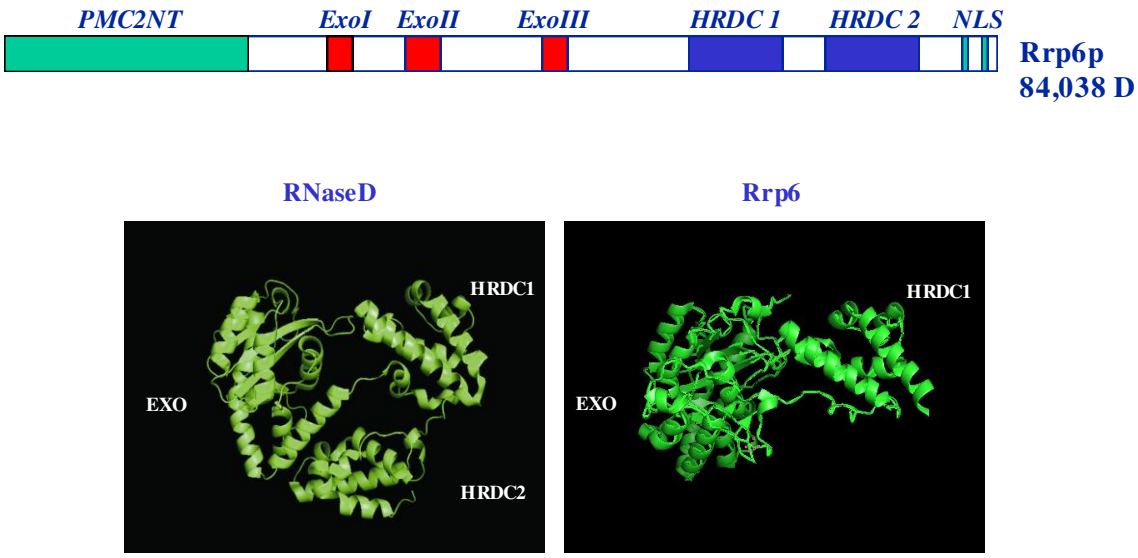
PMC2NT
ExoI
ExoII
ExoIII
HRDC 1
HRDC 2
NLS
Rrp6p
84,038 D
RNaseD
HRDC1
EXO
HRDC2
Rrp6
HRDC1
EXO

Figure 1

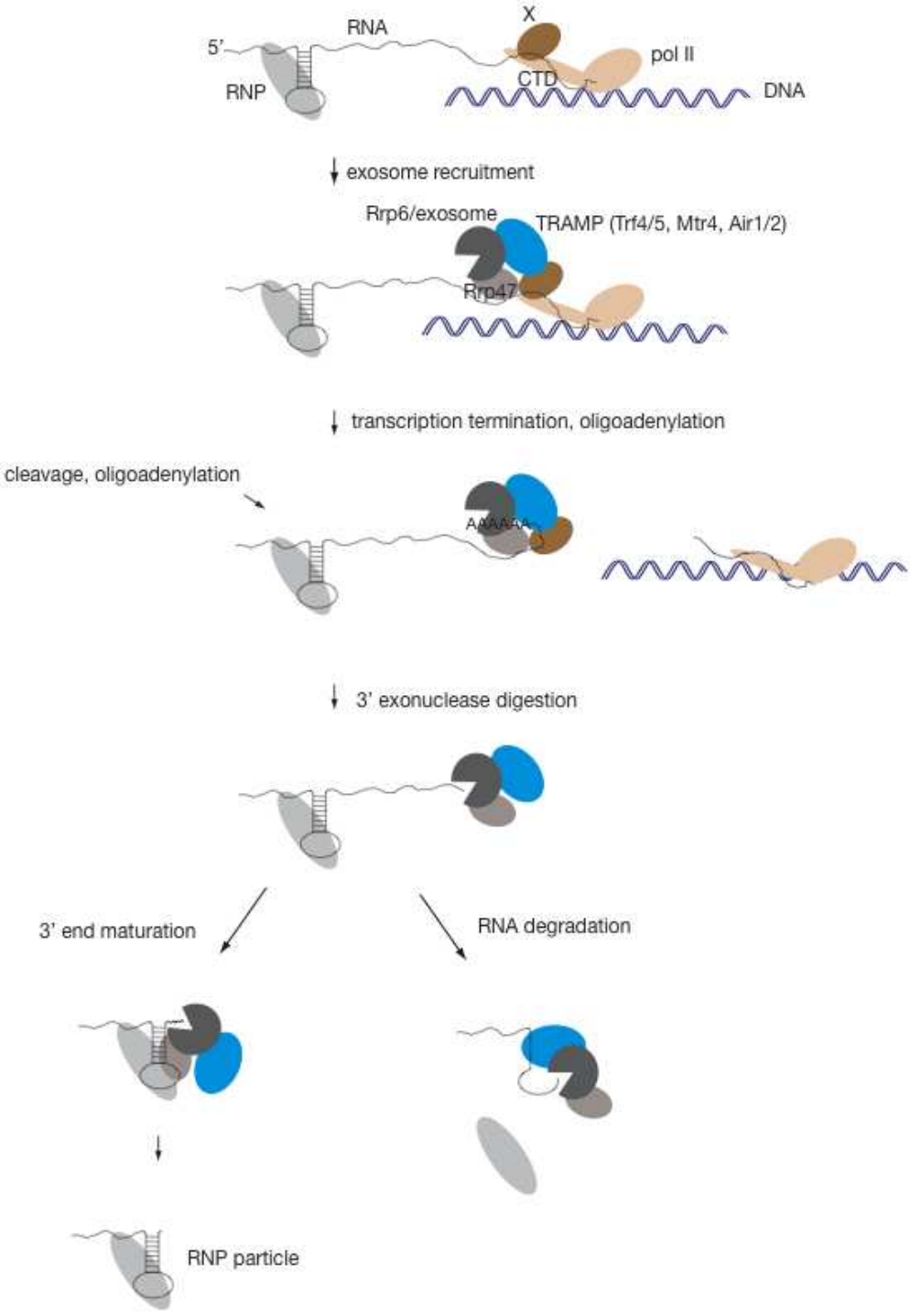
5'
RNA
X
pol II
CTD
RNP
DNA
exosome recruitment
Rrp6/exosome
TRAMP (Trf4/5, Mtr4, Air1/2)
Rrp47
transcription termination, oligoadenylation
cleavage, oligoadenylation
AAAAAA
3' exonuclease digestion
3' end maturation
RNA degradation
RNP particle

Figure 2