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A Yeast Exosome Cofactor, Mpp6, Functions in RNA Surveillance and in the Degradation of Noncoding RNA Transcripts

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A genome-wide screen for synthetic lethal (SL) interactions with loss of the nuclear exosome cofactors Rrp47/Lrp1 or Air1 identified 3′→5′ exonucleases, the THO complex required for mRNP assembly, and Ynr024w (Mpp6). SL interactions with mpp6Δ were confirmed for rrp47Δ and nuclear exosome component Rrp6. The results of bioinformatic analyses revealed homology between Mpp6 and a human exosome cofactor, underlining the conservation of the RNA surveillance system. Mpp6 is an RNA binding protein that physically associates with the exosome and was localized throughout the nucleus. The results of functional analyses demonstrated roles for Mpp6 in the surveillance of both pre-rRNA and pre-mRNAs and in the degradation of "cryptic" noncoding RNAs (ncRNAs) derived from intergenic regions and the ribosomal DNA spacer heterochromatin. Strikingly, these ncRNAs are also targeted by other exosome cofactors, including Rrp47, the TRAMP complex (which includes Air1), and the Nrd1/Nab3 complex, and are degraded by both Rrp6 and the core exosome. Heterochromatic transcripts and other ncRNAs are characterized by very rapid degradation, and we predict that functional redundancy is an important feature of ncRNA metabolism.

The results of genetic and biochemical analyses indicate that the exosome 3′-to-5′ exonuclease complex has potent in vivo RNA degradation activity, even on highly structured RNAs and large RNA-protein (RNP) complexes. However, the purified eukaryotic exosome shows limited activity in vitro, and this has been interpreted as showing its dependence on cofactors for in vivo activity (reviewed in reference 21). Consistent with this idea, several exosome cofactors have been identified. The Skl2/3/8 complex functions with the cytoplasmic exosome and Ski7 during mRNA turnover and surveillance (3, 53). Known nuclear cofactors include the RNA binding proteins Rrp47 (Lrp1) (31, 34), the Nrd1/Nab3 heterodimer (54), and the TRAMP polyadenylation complexes (16, 23, 27, 51, 60). However, analyses of mutations in the exosome complex components have revealed nuclear RNA degradation phenotypes that are not shown by mutations in the TRAMP complexes, Rrp47, or Nrd1/Nab3, strongly suggesting that additional exosome cofactors remained to be identified.

Rrp47 is believed to act as a specific cofactor for the nuclear 3′ exonuclease Rrp6 (31, 44). Although Rrp6 is associated with the nuclear exosome, strains lacking the activity of Rrp6 show RNA processing defects that are distinctly different from those seen following the loss or inactivation of any other exosome component. In contrast, very similar phenotypes are seen in strains with mutations in any of the “core” exosome components that are common to the nuclear and cytoplasmic forms of the complex (reviewed in reference 21). This suggests that the activity of the core exosome requires the intact structure of the complex and is functionally distinct from the activity of Rrp6. The TRAMP complexes appear to function together with both Rrp6 and the core exosome. Saccharomyces cerevisiae has two forms of the TRAMP complex; both contain the essential DEVH box, putative helicase Mtr4, but differ in the presence of the poly(A) polymerase Trf4 in the TRAMP4 complex or the homologous Trf5 in TRAMP5 (23). In addition, each TRAMP complex contains a zinc knuckle protein, either Air1 or Air2; these proteins are ~60% identical.

Here we have made use of a recently developed screen for the identification of synthetic lethal (SL) genetic interactions termed genetic interaction mapping (GIM) (13) to identify genes that become essential for cell growth in the absence of Rrp47 or Air1. These screens identified several potential SL interactions, including a novel yeast exosome cofactor that we designate Mpp6.

MATERIALS AND METHODS

Strains and microbiological techniques. Standard procedures were used for the propagation and maintenance of yeast. A full list of strains used in this study can be found in Table 1. All deletion and GAL1-regulated strains were constructed by using a one-step PCR strategy. Transformants were selected for resistance to G418 or nourseothricin (NAT) and screened by PCR and immunoblotting. For depletion of Mpp6 and Rrp47, cells were pregrown on permissive medium containing 2% galactose and harvested immediately prior to transfer (zero-hour samples) and 6 h after transfer to nonpermissive medium containing 2% glucose.

Glycerol gradient analysis. Glycerol gradient analysis was performed on a 10 to 30% gradient as previously described (31). Protein was precipitated from each fraction by using trichloroacetic acid and resolved by using standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) techniques. The sedimentation profile of Mpp6-TAP was detected by immunoblotting with

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peroxidase-conjugated rabbit immunoglobulin G (IgG) (Sigma). A polyclonal, antipeptide antibody was raised against Rrp43 (data not shown) and used to indicate the sedimentation profile of the core exosome.

**Affinity purification of Mpp6-TAP.** Affinity purification of Mpp6-TAP was performed as previously described (38) with the following modifications. Frozen yeast pellet obtained from exponentially growing cells (optical density of 0.6 at 600 nm) was ground in liquid nitrogen and thawed in extraction buffer (50 mM HEPES, pH 7.4, 50 mM KCl, 5 mM MgCl₂, 10% glycerol). Clarified extract was performed as previously described (38). Frozen yeast pellet obtained from exponentially growing cells (optical density of 0.6 at 600 nm) was ground in liquid nitrogen and thawed in extraction buffer (50 mM HEPES, pH 7.4, 50 mM KCl, 5 mM MgCl₂, 10% glycerol). Clarified extract was performed as previously described (38).

**SDS-PAGE analysis and in-gel digestion.** The entire lane of the silver-stained gel was chopped into small pieces and placed in a 96-well plate for trypsin digestion as described elsewhere (43). In brief, proteins were reduced in 20 μl dithiothreitol for 30 min at 37°C, alkylated in 50 mM iodoacetamide for 30 min at room temperature in the dark, and digested at 37°C overnight with 12.5 ng/μl trypsin (Promega; Grade; Sigma). The digestion medium was then acidified to 0.1% of trifluoroacetic acid and spun onto StageTips as described elsewhere (36). Peptides were eluted in 20 μl 80% acetonitrile, 0.5% acetic acid and were concentrated to 2 μl (concentrator 5301; Eppendorf AG, Hamburg, Germany). They were then diluted to 5 μl with 0.1% trifluoroacetic acid for liquid chromatography-tandem mass spectroscopy (LC-MS-MS) analysis.

**MS analysis.** An LTQ-Orbitrap MS (ThermoElectron, Bremen, Germany) was coupled online to an Agilent 1100 binary nanopump (Palo Alto, CA) and an HTC PAL autosampler (CTC, Zwingen, Switzerland). To prepare an analytical standard error was used throughout.

**Immunofluorescence.** For Mpp6-green fluorescent protein (GFP) localization studies, cells were fixed in 3% paraformaldehyde at room temperature, spheroplasted by using zymolase, and dehydrated in 70% ethanol overnight at −20°C. To stain nuclear DNA, 4',6'-diamidino-2-phenylindole (DAPI) was included in

### TABLE 1. Strains used in this study

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<th>Strain</th>
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<th>Reference or source</th>
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<td>D658</td>
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<td>YLM151</td>
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### TABLE 2. Oligonucleotides used in this study

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<td>CYH2</td>
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<td>GTGCCCTTCGTGCTTACCCGATAC</td>
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<td>IT52 5′B</td>
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<td>004</td>
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</tbody>
</table>

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a 3HA, triple-hemagglutinin tag; KlURA3, Kluveromyces lactis URA3.
the mounting medium (Vectashield; Vector laboratories). Images were captured using a Coolsnap CCD camera fitted to a DeltaVision RT restoration imaging system. The images captured using the DeltaVision system were subjected to real-time two-dimensional deconvolution algorithms. Single optical sections were selected following deconvolution and assembled by using Adobe Photoshop software.

RNA binding assays. Binding of [35S]Mpp6 to homopolymeric nucleotides and biotinylated, in vitro-transcribed RNA was performed as previously described (32, 37). Equivalent amounts of protein recovered from bound, unbound, and total fractions were separated by SDS-PAGE on a 4 to 12% Bis-Tris gel. Following migration, the gel was dried and protein was visualized by autoradiography.

RESULTS

Mpp6 is a novel exosome-associated protein. To identify novel factors that functionally interact with the nuclear exosome cofactor Rrp47 or the TRAMP complex, we performed genetic screens with strains lacking Rrp47 or the Air1 component of the TRAMP complex by using the GIM strategy (13). Briefly, query strains were constructed by replacing the KANR marker in the *rrp47Δ* and *air1Δ* MATa strains from the systematic gene knock-out collection (19) with a haploid-specific NAT resistance cassette (prMFo2-NAT). These strains were each crossed by being mated en masse to a pool of 4,800 MATa strains from the knockout collection, each of which has a single open reading frame (ORF) replaced by a KANR cassette containing unique “barcode” sequences. The diploids obtained were sporulated en masse, and the resulting haploid double-mutant spores were selected in rich medium containing Geneticin and NAT and grown in competition for about 18 generations. The change in the relative level of the cells bearing both the query mutation and a given deletion in the final population was estimated with comparative barcode-specific microarrays. Ratios between query results and the results for a reference ORF (obtained in experiments using the deletion of a dubious ORF; considered neutral, instead of the deletion of *RRP47* or *AIR1*) were around 1 for no effect and less than 1 for synthetic growth defects (negative values when the logarithms of the ratios were used). The change in the relative level of the cells bearing both the query mutation and a given deletion in the final population was estimated with comparative barcode-specific microarrays. Ratios between query results and the results for a reference ORF (obtained in experiments using the deletion of a dubious ORF; considered neutral, instead of the deletion of *RRP47* or *AIR1*) were around 1 for no effect and less than 1 for synthetic growth defects (negative values when the logarithms of the ratios were used).

To confirm the predicted genetic interaction, the *rrp47Δ*:KAN strain was crossed individually to an *mpp6Δ*:NAT strain. Among 52 haploid progeny tested, no NATR KANR strains were recovered, indicating that the mutations are indeed SL. Rrp47 functions as a cofactor for the exonuclease Rrp6, and the *rrp47Δ* mutation conferred improved relative growth in the presence of the *rrp47Δ* mutation. In other GIM screens, such improved relative growth was also seen for other pairs of mutations affecting the same process, and this was attributed to epistasis by Decourty et al. (13). Two gene deletions were identified in both screens and appeared to be highly specific to the *rrp47Δ* and *airlΔ* mutations (Fig. 1C) as shown by the absence of the NAT R KANR strains identified in multiple different screens and may, therefore, be nonspecific (Fig. 1A and B).

The GIM screens identified several mutant combinations that showed putative SL or synergistic growth inhibition and appeared to be biologically relevant. For example, gene deletions that are underrepresented in the progeny recovered with the *rrp47Δ* mutation (Fig. 1A) encode two known 3‘-to-5‘ exonucleases, Rex1 (Rnh70) and Rex3 (52). Other genes identified also participate in RNA metabolism, including factors implicated in RNA polymerase II transcription elongation and mRNA export; the THO complex (11) components Mfl1, Thp2, and Rlr1 (represented by the overlapping hypothetical ORF YNL140C); and the ribosome synthesis factors Nop13, Ssf2, and Sqs1 (Fig. 1B). Rrp47 functions as a cofactor for the nuclear exonuclease Rrp6, and the *rrp4Δ* mutation conferred improved relative growth in the presence of the *rrp47Δ* mutation. In other GIM screens, such improved relative growth was also seen for other pairs of mutations affecting the same process, and this was attributed to epistasis by Decourty et al. (13). Two gene deletions were identified in both screens and appeared to be highly specific to the *rrp47Δ* and *airlΔ* mutations (Fig. 1C) as shown by the absence of the NAT R KANR strains identified in multiple different screens and may, therefore, be nonspecific (Fig. 1A and B).

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terminal GFP fusion was constructed in the genome under the control of the endogenous \textit{MPP6} promoter (Fig. 2C). Comparison with the results of DAPI staining of the DNA showed the Mpp6-GFP fusion to be restricted to the nucleus, but no clear subnuclear localization was evident. The nuclear localization of Mpp6 is consistent with the SL interactions with Rrp47 and Rrp6, which are also nuclear specific in yeast.

To assess the physical association between Mpp6 and the exosome, a C-terminal TAP fusion was constructed in the genome under the control of the endogenous \textit{MPP6} promoter. The results of glycerol gradient analysis followed by Western blotting showed good cosedimentation of Mpp6-TAP with the core exosome component Rrp43, and both proteins sedimented with the velocity expected for the exosome from the

FIG. 1. Identification of \textit{YNR024w} by genetic screens using the \textit{rrp47}\Delta and \textit{air1}\Delta mutations. (A) The values obtained in two independent GIM screens for the relative growth rate of double-deletion strains using as query the deletion of \textit{rrp47} were represented as a scatter plot to assess the reproducibility of the assay and to identify strong interactions. Each point represents log2(Q/R) values (the logarithm in base 2 of the ratio between the average signal measured for a given double mutant $\times$ \textit{rrp47}\Delta [query (Q) mutation] and the signal measured for the same deletion when combined with a reference mutation considered to be neutral, $\times$ \textit{rrp47}\Delta [reference (R) mutation]). The genes whose deletion was SL or caused synthetic growth inhibition when combined with \textit{rrp47}\Delta are found in the lower-left quadrant and are indicated and shaded differently based on the presumed specificity of the observed genetic interaction, as estimated by the synthetic growth defect scores after performing 41 screens (13). Deletions that appeared to be specific to the screen are indicated in bold. Deletions identified in multiple different GIM screens, which may be nonspecific despite high negative values, are in gray. (B) As described for panel A, but with \textit{air1}\Delta as the query mutation. (C) The genetic interaction profiles for selected genes that showed synergistic growth defects with \textit{rrp47}\Delta and/or \textit{air1}\Delta were retrieved from the published GIM data (13) to assess the specificity of the effects. Query gene deletions are indicated above. The genes showing the largest number of synthetic growth defects, Mft1, Thp2, and Ynl140c, were all components of the THO complex (an ORF that overlaps Rlr1 and is indicated as Rlr1*).
results of previous analyses (Fig. 3A). To confirm this association, we compared the proteins that copurified with the TAP-tagged core exosome component Csl4-TAP and with Mpp6-TAP (Fig. 3B). A similar band pattern was visible in each case, but the amount of protein recovered with Mpp6-TAP was substantially lower. Comparisons of total protein preparations revealed that Mpp6-TAP is approximately threefold less abundant than Csl4-TAP (Fig. 3C). The results of previous analyses indicated that approximately one-third of the total exosome population is associated with Rrp6, and this is assumed to represent the nuclear exosome fraction (2). To test whether the association of Mpp6 with the exosome was mediated by substrate RNAs, Mpp6-TAP bound to IgG was treated with RNase A prior to elution by TEV cleavage (Fig. 3D). RNase A treatment did not affect the association of Mpp6 with the exosome component Rrp43. To assess the stability of the Mpp6-exosome interaction, Mpp6-TAP bound to IgG was washed with increasing salt concentrations (Fig. 3E). The association of the core exosome components Rrp43 and Rrp44 with Mpp6-TAP was largely resistant to 0.1 M NaCl and progressively lost at salt concentrations of from 0.2 M to 0.6 M NaCl. The retention of a significant fraction of the association...
after washing with 0.4 M NaCl indicates that the Mpp6-exosome interaction is relatively robust.

Proteins coprecipitated with Mpp6-TAP were identified by LC-MS (Fig. 3F). All 11 components of the nuclear exosome complex were identified (Rrp4, Rrp40 to Rrp46, Mtr3, Csl4, and Rrp6), as were its characterized cofactors, including both TRAMP complexes (Trf4, Trf5, Air1, Air2, and Mtr4), Nrd1, and Rrp47. A number of other nuclear RNA binding proteins were also identified, including Pab1 and Ded1.

The data are consistent with the association of most or all of the nuclear exosome population with Mpp6 and with quantitative association of Mpp6 with the nuclear exosome. The potential RNA binding activity of Mpp6 was tested in vitro (Fig. 4).

MPP6 was transcribed in vitro, and Mpp6 was then 35S labeled during translation. The binding substrates were poly(U) Sepharose and biotinylated poly(dT), poly(A), and poly(A/U) bound to streptavidin agarose. In addition, RNAs corresponding to the D-A2 region of the pre-rRNA internal transcribed spacer 1 (ITS1) or tRNA\text{\text{\textit{met}}} were biotinylated during in vitro transcription. Mpp6 bound efficiently to poly(U) with substantially weaker binding to oligo(dT) and was not recovered above the level of the background control (protein A Sepharose or streptavidin agarose alone). We conclude that Mpp6 has robust in vitro binding activity on a single-stranded poly(U) RNA. However, it seems likely that additional factors influence binding-site selection in vivo.

Mpp6 functions together with the nuclear exosome. To assess the potential role of Mpp6 in nuclear RNA processing and degradation, RNA was extracted from the mpp6Δ GAL::rrp47 and rrp47Δ GAL::mpp6 strains and the corresponding single mutants during growth on permissive galactose medium and following transfer to glucose medium for 6 h. Strains lacking Rrp47 have a pronounced defect in 3′ maturation of the 5.8S rRNA (31), with very strong accumulation of a species with a 30-nt extension, 5.8S\text{\text{\textit{30}}}, and very strong accumulation of 5.8S\text{\text{\textit{30}}}, which can readily be seen by ethidium staining of total RNA. Most yeast mutants with defects in ribosome synthesis show low levels of pre-rRNA accumulation, consistent with rapid surveillance (reviewed in reference 56). The double-mutant strains with rrp47 were also not more impaired in 5.8S...
maturation than the corresponding rrp47Δ and GAL::rrp47 strains (Fig. 5A). The loss of Mpp6 had little effect on the levels of other pre-rRNA species examined.

However, the aberrant 23S RNA, which is a characterized substrate for RNA surveillance by the TRAMP and exosome complexes, accumulated mildly (Fig. 5D, lanes 9 and 11). In addition, a set of bands accumulated markedly in both the mpp6Δ GAL::rrp47 and the rrp47Δ GAL::mpp6 double mutant on glucose medium (Fig. 5B and C, lanes 9 and 11). These do not correspond to known pre-rRNA species and likely represent intermediates in pre-rRNA degradation which have been stabilized by the loss of both Mpp6 and Rrp47. We propose that the efficient degradation by the exosome of pre-rRNAs that have been targeted by surveillance pathways requires the partially redundant activities of Mpp6 and Rrp47.

The requirement for Mpp6 in surveillance of nuclear pre-mRNAs was also tested. Strains carrying the rna14-1 mutation are defective in 3′ cleavage and polyadenylation of mRNA precursors, leading to rapid loss of mRNA synthesis following transfer to 37°C (Fig. 6A, lanes 13 to 18) (30). The absence of the nuclear exosome component Rrp6 partially restores mRNA synthesis in rna14-1 strains, probably by suppressing pre-mRNA surveillance activity (Fig. 6A, lanes 25 to 30) (50). In the rna14-1 mpp6Δ strain, mRNA levels were elevated relative to the level in the rna14-1 single mutant following transfer to 37°C (Fig. 6A, compare lanes 13 to 18 with lanes 19 to 24). The data are quantified for MFA2 in Fig. 6B; quantification of the data for RPS26 also showed statistically significant stabilization in the rna14-1 mpp6Δ strain (data not shown).

The exosome was also implicated in the degradation of unspliced pre-mRNAs that have been targeted for splicing but have failed to complete the process (8). At 37°C the prp2-1 mutation allows commitment complex formation but blocks splicing prior to the first catalytic step (48). In the prp2-1 single-mutant strain, the spliced mRNA product is lost following transfer to 37°C but the unspliced pre-mRNA does not accumulate to a corresponding degree, due to its rapid degradation (Fig. 6C, lanes 7 to 12). In prp2-1 strains lacking Rrp6 (Fig. 6C, lanes 25 to 30) or Mpp6 (Fig. 6C, lanes 19 to 24), unspliced pre-CYH2 RNA was accumulated relative to the level in the prp2-1 single mutant and, although modest, the effects were statistically significant (quantification shown in Fig. 6D). These effects were, however, much less marked than those previously observed in prp2-1 strains with defects in the core exosome (8).

We conclude that Mpp6 participates in surveillance of defective nuclear pre-mRNAs, presumably helping to target the exosome to these RNAs.

Rrp47 is implicated in the degradation of a class of non-protein-coding RNAs (ncRNAs), termed cryptic unstable tran-
scripts (CUTs), which are generated from intergenic spacer regions at many sites in the yeast genome (4, 60). To assess whether Mpp6 also participates in CUT degradation, we examined the well-characterized species NEL025C (Fig. 7A). The NEL025C CUT shows substantial 3′ heterogeneity and therefore appears as a smear in Northern hybridization. NEL025C is almost undetectable in wild-type cells but was accumulated in the rrp47Δ strain (Fig. 7A, lane 3) and in the GAL::rrp47 strain on glucose medium (Fig. 7A, lane 7). Similar increases in NEL025C abundance were seen in the mpp6Δ strain and in the GAL::mpp6 strain on glucose medium, although the effect was milder in this strain, probably due to residual expression. Both the mpp6Δ GAL::rrp47 and the rrp47Δ GAL::mpp6 double-mutant strain showed higher NEL025C accumulation on glucose medium than any single mutant, indicating that Mpp6 and Rrp47 each participate in NEL025C degradation. Notably, when grown on galactose medium, the mpp6Δ GAL::rrp47 strain did not show the NEL025C accumulation seen in the mpp6Δ single mutant (Fig. 7A, lane 8). This suppression of the phenotype for mpp6Δ for NEL025C degradation probably results from overexpression of Rrp47, since GAL-regulated constructs are generally overexpressed on galactose medium.

Another class of ncRNA is transcribed from the telomeres and ribosomal DNA (rDNA) spacer regions in S. cerevisiae, which are generally described as heterochromatic (20, 22, 55). The most-studied rDNA spacer transcript is designated IGS1-R and is very rapidly degraded following transcription. Like NEL025C, the IGS1-R transcripts show great 3′ heterogeneity (20). The levels of IGS1-R transcripts were elevated in the mpp6Δ and rrp47Δ single-mutant strains and in the GAL::mpp6 and GAL::rrp47 strains on glucose medium (Fig. 7A). In the mpp6Δ GAL::rrp47 and the rrp47Δ GAL::mpp6 double mutant, accumulation on glucose medium was not clearly higher than in the single mutants. However, as for NEL025C, growth of the mpp6Δ GAL::rrp47 strain on galactose medium suppressed the mpp6Δ phenotype.

We conclude that Mpp6 and Rrp47 both participate in the rapid degradation of ncRNAs. The apparent suppression of the mpp6Δ phenotype by overexpression of Rrp47 is consistent with direct interactions or closely related functions.

FIG. 7. Exosome cofactors involved in surveillance of ncRNAs. Northern analyses of RNA species resolved on a 6% polyacrylamide–8.3 M urea gel probed for the CUT NEL025C, the rDNA intergenic transcript IG51-R, or cytoplasmic scR1 RNA. (A) Total RNA was extracted from the wild-type strain (lane 1); strains carrying either the mpp6Δ (lane 2) or rrp47Δ (lane 3) mutation during growth on glucose medium; and strains carrying the single or double mutations Pgal::MPP6 (lanes 4 and 5), Pgal::RRP47 (lanes 6 and 7), mpp6Δ Pgal::RRP47 (lanes 8 and 9), and rrp47Δ Pgal::MPP6 (lanes 10 and 11) during growth on galactose medium (GAL) and 6 h after transfer to glucose medium (GLU). (B) Total RNA was extracted from the NRD1 mutant strain (lanes 1 and 2); from mrd1-102 (lanes 3 and 4), nab3-11 (lanes 5 and 6), and mtr3-1 (lanes 7 and 8) mutant strains during growth at 23°C or 1 h after transfer to 37°C, from a Pgal::RRP47 mutant strain (lanes 9 and 10) during growth in galactose medium (GAL) or after transfer to medium containing glucose for 20 h (GLU); from an isogenic wild-type strain (lane 11); and from strains carrying either the air1Δ air2Δ (lane 12) or trf4Δ (lane 13) mutation. WT, wild type.
Both ncRNAs, NEL025C and IGS1-R, were stabilized by the loss of components of the TRAMP complex, either the poly(A) polymerase Trf4 or Air1 plus Air2 (Fig. 7B) (20, 55, 60). Stabilization of the ncRNAs was also seen in strains depleted of the core exosome component Rrp41 or carrying a temperature-sensitive-lethal point mutation in the core exosome component Mtr3 (Fig. 7B), and these RNAs are also stabilized in strains lacking the nuclear-specific exonuclease Rrp6 (data not shown) (20, 60). The NEL025C RNA accumulated in the exosome appeared to be longer and more heterogeneous than in the TRAMP mutants, probably due to hyperadenylation by TRAMP in the absence of exosome activity. In strains with temperature-sensitive-lethal point mutations in the Nrd1/Nab3 complex, ndr1-102 and nab3-11, stabilization of longer forms of NEL025C was seen (Fig. 7B), consistent with the reported dual roles of Nrd1/Nab3 in transcription termination and subsequent degradation. The IGS1-R transcripts were strongly stabilized by ndr1-102 but, as previously observed (20), there was little stabilization in the nab3-11 strain, probably reflecting allele specificity.

Together these observations reveal a remarkable degree of apparent redundancy in ncRNA degradation, with at least four cofactor complexes and two nucleases participating in their degradation.

**DISCUSSION**

Here we report the use of genome-wide screens for genetic interactions to identify a new nuclear cofactor for the yeast exosome.

Gene deletions underrepresented in the collection of double mutants with the rrp47Δ mutation included the 3′ to 5′ exonucleases, Rex1 (Rnh70) and Rex3. Rrp47 is believed to function as a cofactor for the nuclear exosome component Rrp6, and deletion of RRP6 was previously shown to be SL with rex1Δ (52). The identification of these apparently biologically relevant putative SL interactions provided a strong indication that the screen had identified bona fide interacting genes. These interactions also suggested that common substrates are shared by the exosome and other, individually nonessential, 3′ exonucleases present in yeast cells.

The rrp4Δ screen also identified genes encoding the ribosome synthesis factors Ssf2, Nop13, and Alb1. Since the exosome and its cofactors are involved in surveillance of defective preribosomes (1, 14, 15), the identification of synergistic growth inhibition resulting from the combination of defects in ribosome synthesis and degradation was not entirely unexpected, but it is less clear why this particular set of mutations were identified. By definition, only the few ribosome synthesis factors that are not essential for viability could have been identified in the screen, which used deletion mutations. One possibility is that the encoded proteins have important functions during preribosome surveillance, conceivably signaling defects in ribosome synthesis to the degradation machinery.

GIM screens with both rrp4Δ and air1Δ identified strong negative interactions with components of the THO complex, which is probably required for packaging of newly synthesized mRNPs and also interacts genetically with Rrp6 (11, 29, 33, 40, 61). These interactions suggest roles for both Rrp47 and Air1 in the surveillance of pre-mRNAs. In addition to their roles in the TRAMP complexes, Air1 and Air2 were reported to bind the arginine methyltransferase Hmt1 and inhibit its activity. Hmt1 methylates the glycine-arginine repeat tracts on several RNA binding proteins, including the mRNP proteins Npl3 and Hrp1. Whether this is related to the SL interactions of air1Δ with THO complex mRNP proteins remains to be determined.

There are no previously reported functional distinctions between Air1 and Air2; the single mutants show no detectable growth inhibition and no clear defects in RNA metabolism, whereas double mutants are severely impaired in growth and show strong defects in TRAMP activity. However, the GIM screens indicated that mpp6Δ is SL with air1Δ, but not with air2Δ (Fig. 1C), apparently showing that there are indeed clear functional distinctions.

Strong negative interactions with the mpp6Δ mutation were identified for both the rrp47Δ and air1Δ mutation, and the SL interaction between the rrp47Δ and mpp6Δ mutations was subsequently confirmed by crossing. The mpp6Δ mutation was also SL in combination with the rrp6Δ mutation, making it unlikely that Mpp6 functions in parallel with Rrp47 in targeting RNA substrates to Rrp6. Rather, we predict that Mpp6 targets RNAs to the core exosome, possibly to the active 3′ exonuclease Rrp44. Mpp6 bound RNA in vitro, showing substantially higher binding to poly(U) than to poly(dT), with only background binding to poly(A), poly(A/U), or natural RNAs with more structure. The lack of binding to poly(A) may be significant, since many exosome substrates are polyadenylated and a high affinity of Mpp6 for poly(A) might well interfere with their degradation. Yeast Rrp47, in contrast, specifically binds structured RNAs (44), indicating that Mpp6 and Rrp47 will have distinctly different preferred binding sites in vivo.

The results of detailed bioinformatics analyses starting from the human exosome-binding protein Mhosp6 show that Mpp6 is the only budding yeast (S. cerevisiae) protein with the two signature motifs for Mhosph6 homologues and that the occurrence of both motifs in a protein is statistically significant (\( P < 0.00015 \)). Human MPHOSPH6 was reported to associate with the human exosome and also bound single-stranded RNA (42).

Strains lacking Mpp6 showed weak but detectable stabilization of unspliced pre-mRNAs, and stronger stabilization was seen for mRNAs with defects in 3′ cleavage and polyadenylation. These data show that Mpp6 participates in the surveillance of defective mRNA precursors. We predict that the effects of loss of Mpp6 on the degradation of these exosome substrates are relatively mild because of functional redundancy with other exosome cofactors and, possibly, other nucleases.

The role of redundancy in RNA degradation. The results of recent analyses for yeast and humans suggest that ncRNAs are very wide-spread and may, indeed, dominate total Pol II transcription (7, 46, 47). Moreover, regions of the genome that are embedded in heterochromatin, including the rDNA spacers, were long considered to be transcriptionally inert since transcripts derived from these regions could not be detected, whereas it is now clear that, at least in yeast, heterochromatic regions are actively transcribed (10, 20, 55, 58). This raises the obvious question of why the apparently almost ubiquitous ncRNA transcripts largely escaped detection in previous analyses.

At least part of the answer is that many ncRNAs, including...
those derived from the rDNA heterochromatin described here, are subject to very rapid degradation, keeping their steady-state levels very low. This, in turn, raises the question of how such rapid degradation can be achieved. One feature that likely contributes to the rapid degradation of ncRNAs is the cotranscriptional binding of at least some exosome cofactors (12, 20), potentially allowing “pretargeting” of the nascent transcript and rapid degradation as soon as transcription termination makes the 3′ end available (Fig. 8) (reviewed in reference 22).

A second important feature is likely to be the functional redundancy that is manifested among the components of the degradation machinery (Fig. 8). The ncRNAs analyzed here appear to be targeted by at least four exosome cofactors: Mpp6, Rrp47, the Nrd1-Nab3 heterodimer, and the TRAMP4 polyadenylation complex. Moreover, inhibition of the activity of either the nuclear-specific exonuclease Rtp6 or the core exosome also stabilized the ncRNAs. This indicates that multiple cofactors can target the ncRNAs to different exonucleases for degradation. Such competition would presumably avoid any bottlenecks during degradation—if any factor fails to bind, another will do the job. The suppression of the mpp6Δ phenotype on the CUT ncRNA observed in strains overexpressing Rrp47 supports the idea of competition for the substrate.

The results of recent analyses indicate that at least some ncRNAs exert their influence on gene expression at the level of chromatin structure and act in trans (6, 39). Since there can only be a very small number of target genes per cell for each ncRNA, maintaining accurate, and very low, levels of the ncRNAs may be crucial for the regulation of gene expression. More generally, such “genetic buffering” may protect the cell against the failure of any specific component.

Functional redundancy may also be important for the efficient degradation of “difficult” substrates, a notable example of which may be the preribosomes. The predominant phenotype of most ribosome synthesis mutants in yeast is not the accumulation of defective preribosomes but the rapid and complete degradation of the pre-rRNAs (reviewed in reference 56). These RNAs are kilobases in length, highly structured, and packaged with many different proteins but can be completely degraded without the appearance of clear intermediates. Consistent with the importance of redundancy in pre-rRNA surveillance, strains lacking both Mpp6 and Rrp47 accumulated heterogeneous pre-rRNA fragments, which we predict represent degradation intermediates.

Redundancy is believed to be important for robustness in many biological systems, and this probably includes nuclear RNA surveillance.

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REFERENCES


60. Wyers, F., M. Rougemaille, G. Badis, J.-C. Rousselle, M.-E. Dufour, J.
