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Review

# Bacterial Rho helicase: a new tool to dissect mRNP biogenesis and quality control in yeast

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## ABSTRACT

In eukaryotic cells, the co-transcriptional mRNA processing and packaging reactions that lead to the formation of export competent messenger ribonucleoprotein particles (mRNPs) are under the surveillance of quality control (QC) steps. Aberrant mRNPs resulting from faulty events are detected by the QC apparatus and retained in the nucleus with ensuing elimination of their mRNA component by the RNA degradation machinery. A decade of biochemical and genetic experiments in yeast allowed the identification of the nuclear degradation machinery including the core exosome and its two associated catalytic subunits Rrp6p and Rrp44p, its cofactors Rrp47p and Mpp6p as well as the activator complex TRAMP. Similarly, studies of the THO-Sub2 complex of the mRNP assembly and export apparatus have provided valuable information on the nuclear retention and degradation of a particular class of aberrant mRNPs. However, a unifying mechanism of action underlying the QC process remains elusive. Here, we review the implementation of a new experimental approach whereby the production of aberrant mRNPs is massively increased upon heterologous expression of the bacterial Rho helicase in yeast. Using this methodology, we have shown that the QC process is coordinated by Nrd1p (a component of the early termination complex) whose increased co-transcriptional recruitment promotes the attachment of the 3'-5' exonuclease Rrp6p along with the co-factors Rrp47p and Mpp6p. Interestingly, we established that Rrp6p functions independently from the core exosome, yet is stimulated by two forms of the TRAMP complex that include Trf4p or Trf5p and Air2p but not Air1p. The results suggest that specific substrates could be primed for decay via various QC pathways owing to the versatility of the mRNA degradation apparatus. In this context, the bacterial Rho helicase provides a valuable tool to decipher the QC molecular process in yeast and possibly the homologous process in mammalian cells.

**KEYWORDS:** bacterial Rho factor, yeast, mRNP biogenesis, mRNP quality control

## INTRODUCTION

In eukaryotic cells, nascent pre-mRNA molecules are sequentially coated with a large set of processing and binding proteins that mediate their transformation into export-competent ribonucleo-protein particles (mRNPs) ready for translation in the cytoplasm [1, 2]. Genetic and biochemical studies, using in particular the yeast *Saccharomyces cerevisiae* as a model organism, suggested that the various mRNA processing and packaging reactions known as mRNP biogenesis are physically and functionally coupled to transcription elongation by RNA polymerase II (RNAP II). The co-transcriptional maturation and assembly of export-competent mRNPs is facilitated by the C-terminal

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domain (CTD) of the largest subunit of RNAP II that serves as a platform for sequential recruitment of the various acting factors. The production of mature export-competent mRNPs is also under the surveillance of quality control (QC) steps that are interconnected with transcription elongation and mRNP biogenesis. Aberrant mRNPs resulting from suboptimal processing and packaging reactions are targeted by the surveillance system leading to their retention in the nucleus with ensuing degradation of their mRNA component by ribonuclease activities associated with the RNA exosome [3-7].

The interdependence and coordination among transcription elongation, mRNP biogenesis, quality control and export has been the subject of extensive investigations in the last decade. Most studies relied on the use of yeast strains with defects in the THO-Sub2p complex which mediates the assembly and export of a subset of mRNPs. It was shown that deletion or mutation of any component of the complex leads to a decrease in the steady-state level of some THO-Sub2p-dependent transcripts. The most prominent example of such a decrease was obtained with the heat shock inducible HSP104 mRNA. The wild-type level of the transcript could be recovered when the nuclear RNA degradation machinery was compromised by the removal or inactivation of some components of the RNA exosome and associated factors. However, the precise nature of the defects inflicted to the mRNPs in this particular experimental system and how the defective mRNPs are detected by the surveillance system and directed to the RNA degradation machinery remained unclear. Moreover, most of the phenotypes associated with the targeting of the HSP104 transcripts by the surveillance system (nuclear retention and degradation) were obtained under heat shock conditions which open the question whether the defects in the THO-Sub2p complex are actually responsible for the recognition of the mRNPs as defective by the QC system [8-12].

In recent years, we implemented a new assay to study nuclear mRNP surveillance in *S. cerevisiae* which is based on perturbation of mRNP biogenesis by the RNA-dependent helicase/ translocase activity of the bacterial Rho factor. The heterologous expression of Rho in yeast induces a dose-dependent growth defect that stems from its interference in the normal co-transcriptional recruitment of mRNA processing and packaging factors yielding mRNPs that are recognized as aberrant and eliminated by the nuclear QC apparatus. In this experimental approach, sufficient amounts of defective mRNPs can be produced by Rho action within the cell nucleus to serve as substrates for the QC, which facilitates deep investigations of the surveillance process [13-15]. In this paper, we provide a full description of this new approach and outline its use to dissect the mechanism and reveal the intervening actors of nuclear mRNP QC from the co-transcriptional recognition of malformed mRNPs to their destruction by the RNA degradation machinery.

## MATERIALS AND METHODS

*S. cerevisiae* cells were grown according to standard procedures at 25 °C in synthetic complete medium with bases and amino acids omitted as necessary for selection and with glucose (2%) as carbon source. The cell growth was monitored by measuring the optical density (OD) at 600 nm. The yeast strains with gene deletions were generated either by one-step gene replacement technique [16] after PCR amplification on plasmid pFA6a-kanMX4 using appropriate primers designed for homologous recombination or by crossing the appropriate BY4741 *MATa* genomic deletion strain (Euroscarf collection) with BMA41 *MATα* and selection for $Kan^R$ progeny after sporulation. The strains expressing the various C-terminally tagged proteins were constructed by using the PCR-based one- step tagging technique with appropriate cassette plasmids and oligonucleotides [17].

The plasmids expressing Rho-NLS or Rho-ΔNLS under the control of the tetracycline promoter ($TetO_7$) were constructed by PCR amplification of the Rho open reading frame from *E. coli* genomic DNA with appropriate primers designed to generate Not I restriction sites at both ends. Following Not I hydrolysis, the PCR fragments were cloned into the pCM185 (TRP1 Marker) or pCM189 (URA3 Marker) vectors which harbor the constitutively expressed tTA transactivator [18]. The expression of Rho in the yeast was repressed by growing the cells in the presence of

the tetracycline analogue, Doxycycline (Doxy), at a final concentration of 1 μg/ml, whereas the omission of Doxy in the growth medium allowed maximum expression. To induce Rho for the ChIP and RNA analyses experiments, the cells bearing the expression plasmid were first pre-grown with 0.5 μg/ml of Doxy for 7 hours. The cells were then washed three to four times with a medium lacking Doxy, diluted, and allowed to grow overnight in the selective medium lacking Doxy to achieve maximum Rho expression. After the overnight growth (14-16 h), the Rho-induced cells were typically at an $OD_{600}$ of 0.25-0.3, whereas the non-induced samples were at an $OD_{600}$ of 0.8. To analyze the samples, the non-induced cultures were diluted with a fresh medium to adjust the $OD_{600}$ to the induced cultures before harvesting the cells (RNA extractions) or crosslinking (ChIP).

For RNA-FISH experiments, yeast cells harboring the Rho expression plasmid were grown under repressing (+ Doxy) or inducing (- Doxy) conditions in selective medium containing adenine (100 ng/ml) for 16 h as described above. Cells collected at low log-phase ($OD_{600}$ ~ 0.3) were prepared for analysis as described in [19] and hybridized with an oligo $d(T)_{40}$ labeled with Cy3 (Amersham) according to manufacturer's instructions. Fluorescent images acquired (as in [19] with a Leica DM6000 epifluorescence wide field microscope equipped with a Coolsnap HQ2 camera and a Leica 100X PL APO 1.4-0.7 oil NA objective) were analyzed with the MetaMorph software.

For the RNA analyses, yeast cells were grown with or without Rho induction and then the total RNAs were extracted by the hot phenol method [20] and quantified by nanodrop spectrophotometer. Northern blot analyses and hybridizations were conducted with the appropriate oligonucleotide probes as described in [14]. The RT-PCR analyses were also performed as described in [15]. For the ChIP experiments, the cells were fixed with 1% formaldehyde for 20 min at room temperature. After glycine addition, to stop the reaction, the cells were washed and lysed with glass beads to isolate chromatin. The isolated chromatin was then sheared by sonication and processed for immunoprecipitation and qPCR quantification using a Roche Light Cycler 480 with the Maxima SYBER Green qPCR Master mix detection kit from Fermentas as described in [15]. The qPCR and RT-qPCR data sets were analyzed using the ΔΔCt method. Amplifications were done in duplicate for each sample and at least three independent experiments were analyzed.

## RESULTS

### Expression of *E. coli* Rho factor in yeast induces a growth defect phenotype

Bacterial Rho factor, which has no functional homolog in eukaryotes, belongs to the family of ring-shaped homohexameric helicases and is involved in 50% of the transcription termination events in *E. coli*. Current models for Rho-dependent transcription termination in bacteria suggest that the termination process starts when Rho loads onto a naked and C-rich portion of the nascent transcript that becomes accessible following ribosome release. This binding activates the RNA-dependent ATPase activity of the protein leading to its translocation along the transcript in a 5' to 3' direction. When Rho encounters a transcription elongation complex delayed at a pause site, it brings about transcript release. Numerous studies in our laboratory have shown that Rho has a fairly loose sequence requirement for binding to a transcript. Any RNA sequence with relatively well spaced Cs can serve for Rho loading [21]. Also, certain RNA structures such as hairpins can serve as efficient loading sites [22]. Rho is able to compete with other proteins for binding, a process widely used in prokaryotic gene regulation [23]. Furthermore, Rho functions as a powerful helicase/translocase that can track on an RNA chain and melt nucleic acid base pairs or disrupt a protein-RNA complex present in its path [24-26].

Given these functional features, we surmised that once in the nucleus of a eukaryotic cell, Rho could bind to nascent transcripts by competing with endogenous mRNA processing and packaging factors and even displace them during translocation along the RNA chain. Such action would potentially lead to the production of mRNPs, aberrantly processed or depleted of some crucial proteins. To this end, we expressed Rho in the yeast nucleus under the control of a Doxycyclin-regulated $TetO_7$ (Tet off) promoter within a

centromeric plasmid harboring the constitutively expressed transactivator (tTA). The gene encoding *E. coli* Rho was cloned by PCR amplification using primers designed to add a Nuclear Localization Signal (NLS) at the C-terminus of the protein (Figure 1A). The growth of *S. cerevisiae* BMA41 strain transformed with the empty plasmid vector or Rho expression constructs was monitored by serial dilutions on agar plates in the presence or absence of Doxycyclin (Figure 1B). The growth of yeast cells expressing Rho-NLS protein is severely reduced under inducing conditions (- Doxy). However, the growth defect is totally relieved when the expressed protein lacks the NLS or when the Rho-specific antibiotic (Bicyclomycin), which inhibits its ATPase activity and thus impedes its function as a helicase/translocase, is added in the medium. The tight regulation of the expression system can be seen by monitoring yeast growth in liquid medium containing different concentrations of the effector (Figure 1C). The gradual activation of the Tet promoter by decreasing the Doxy concentration in the medium progressively slowed the growth of the yeast cells harboring the Rho-NLS construct. This growth inhibition trend parallels the amount of Rho protein produced in the cells as revealed by Western blot (Figure 1D). Together, these results indicated that the Rho-induced growth defect in yeast relies on the nuclear localization of the protein and is linked to its functional activity as an RNA-dependent helicase/translocase.

**Rho expression in yeast affects steady-state mRNA levels by interfering with mRNP biogenesis**

The fact that the growth defect induced by Rho in yeast requires the RNA helicase/translocase activity of the protein as well as its nuclear localization strongly suggested that the growth inhibition effect is probably linked to Rho interference in RNA metabolism either directly at the transcription level or by affecting mRNP biogenesis. This hypothesis was evaluated first by measuring the steady-state levels of different RNAs produced in cells bearing the Rho expression plasmid and grown at different concentrations of Doxy. As shown in Figure 2A, the analyses by Northern blotting revealed that the levels of RNAP I-transcribed rRNAs (18S and 5.8S) as well as the RNAs synthesized by RNAP III (scR1, 5S and $tRNA^{met}$) were unchanged under Rho expression conditions. In contrast, the steady-state levels of a set of mRNA transcripts synthesized by RNAP II were clearly affected by increasing the extent of Rho expression. Among the mRNAs tested, the greatest effect of Rho expression was observed for PMA1 mRNA with a 60 to 70% reduction, which led us to use it as a model transcript for further investigations.

To find out if the reduction in mRNA level results from a direct action of Rho on the transcription elongation complex that would induce premature termination, we conducted Chromatin Immuno-Precipitation (ChIP) experiments. The results ruled out this hypothesis by showing no significant decrease of RNAP II occupancy along the *PMA1* gene upon Rho expression [15]. However, the ChIP analyses revealed that the recruitment to chromatin of some factors such as the THO subunit Mft1p was altered in the presence of Rho. This suggested that Rho action resides at the level of mRNP biogenesis by interfering with the binding of mRNA processing and packaging factors, yielding mRNPs which are recognized as defective and eliminated by the QC system. Since Rho has a loose sequence requirement for its loading, it is expected to impact the biogenesis of a large spectrum of mRNPs. In this process, each Rho-interference that produces a defective mRNP will hinder the corresponding metabolic pathway, adding extra impairment to the cell fitness. Thus, the cumulative effect of all Rho-induced interferences produces the growth defect phenotype. Additional support to our conclusion was provided by fluorescent *in situ* hybridization (FISH) experiments using a fluorescently labeled oligo dT probe. The results shown in Figure 2B clearly indicated that Rho action leads to the accumulation of polyA transcripts within the nucleus. Such nuclear retention of the polyA transcripts is a hallmark of QC-related phenotypes in yeast [8, 19].

**The targeting of aberrant mRNPs is mediated by Nrd1p co-transcriptional recruitment**

Nrd1p is involved in transcription termination and processing of short non-coding RNAs in yeast (snRNAs, snoRNAs and CUTs). It is known to interact at promoter proximal parts of genes with the CTD of RNAP II through its CTD Interacting Domain (CID) and with RNA by recognizing sequence elements within the nascent transcript

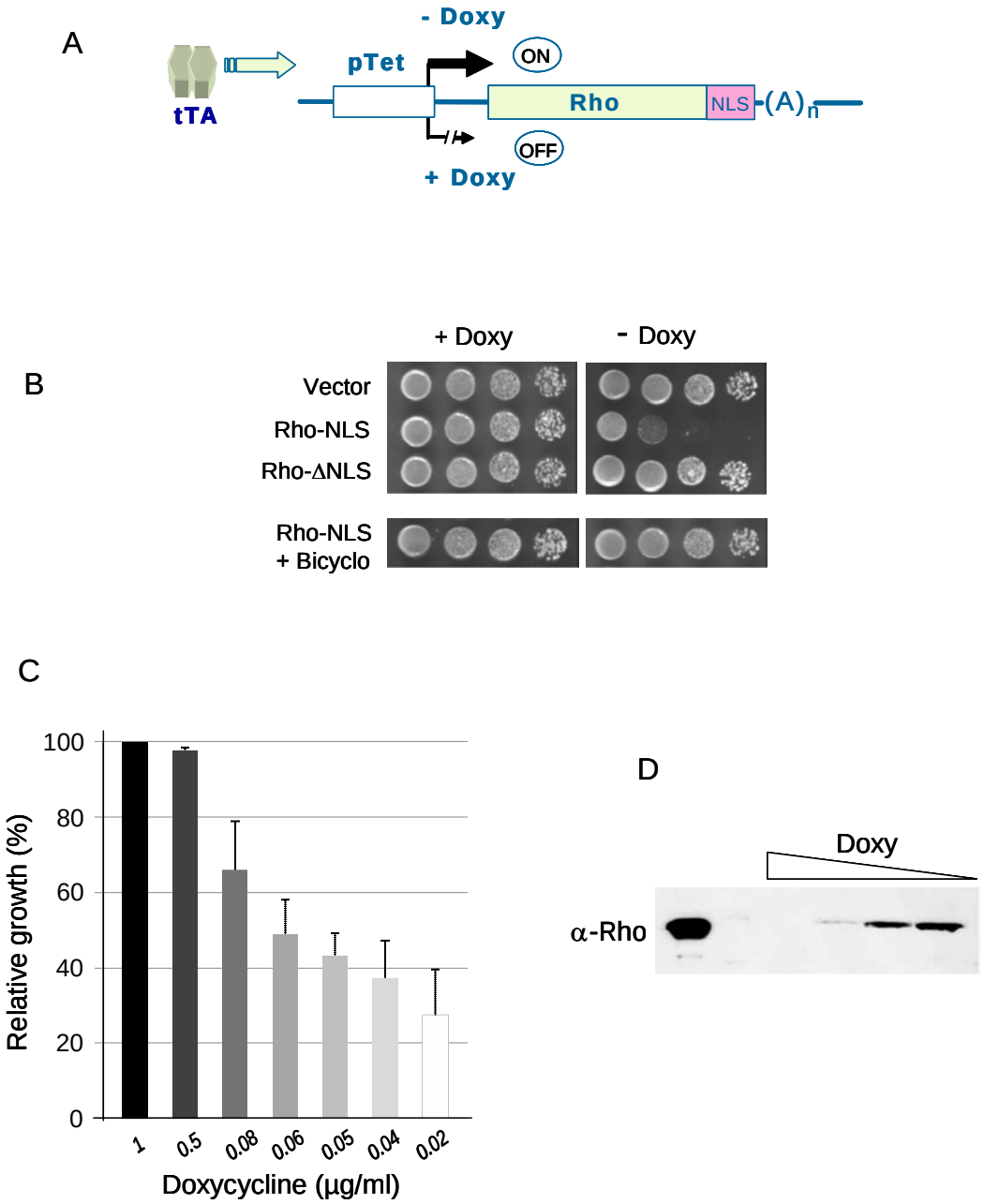


**Figure 1.** Rho expression in the nucleus of yeast cells induces a dose-dependent growth defect phenotype. **(A)** Schematic representation of the relevant region of the centromeric plasmid expressing Rho under the control of the $TetO_7$ promoter. The plasmid harbors the constitutively expressed tTA transactivator that mediates transcription activation of the $TetO_7$ promoter in the absence of Doxy. **(B)** Ten-fold serial dilutions of cells transformed with the empty vector or the indicated plasmids expressing Rho-NLS or Rho-ΔNLS were spotted on agarose plates in the presence or absence of 1 µg/ml Doxy and photographed after 3 days growth at 25 °C. For the appropriate experiment, Bicyclomycin was added in the plates at a final concentration of 60 µg/ml. **(C)** The Rho-induced growth defect is dose-dependent. Cells transformed with the Rho-NLS expression plasmid were grown in liquid medium with Doxy concentrations ranging from 1 to 0.02 µg/ml. Relative growth which is set arbitrarily to 100 for the sample under Rho-repressing conditions (1 µg/ml Doxy) corresponds to the $OD_{600}$ after 16 h growth at 25 °C. **(D)** Western blot using anti-Rho antibodies showing Rho production as a function of Doxy concentration. The brightest spot on the left lane detects purified recombinant Rho which was used as control.

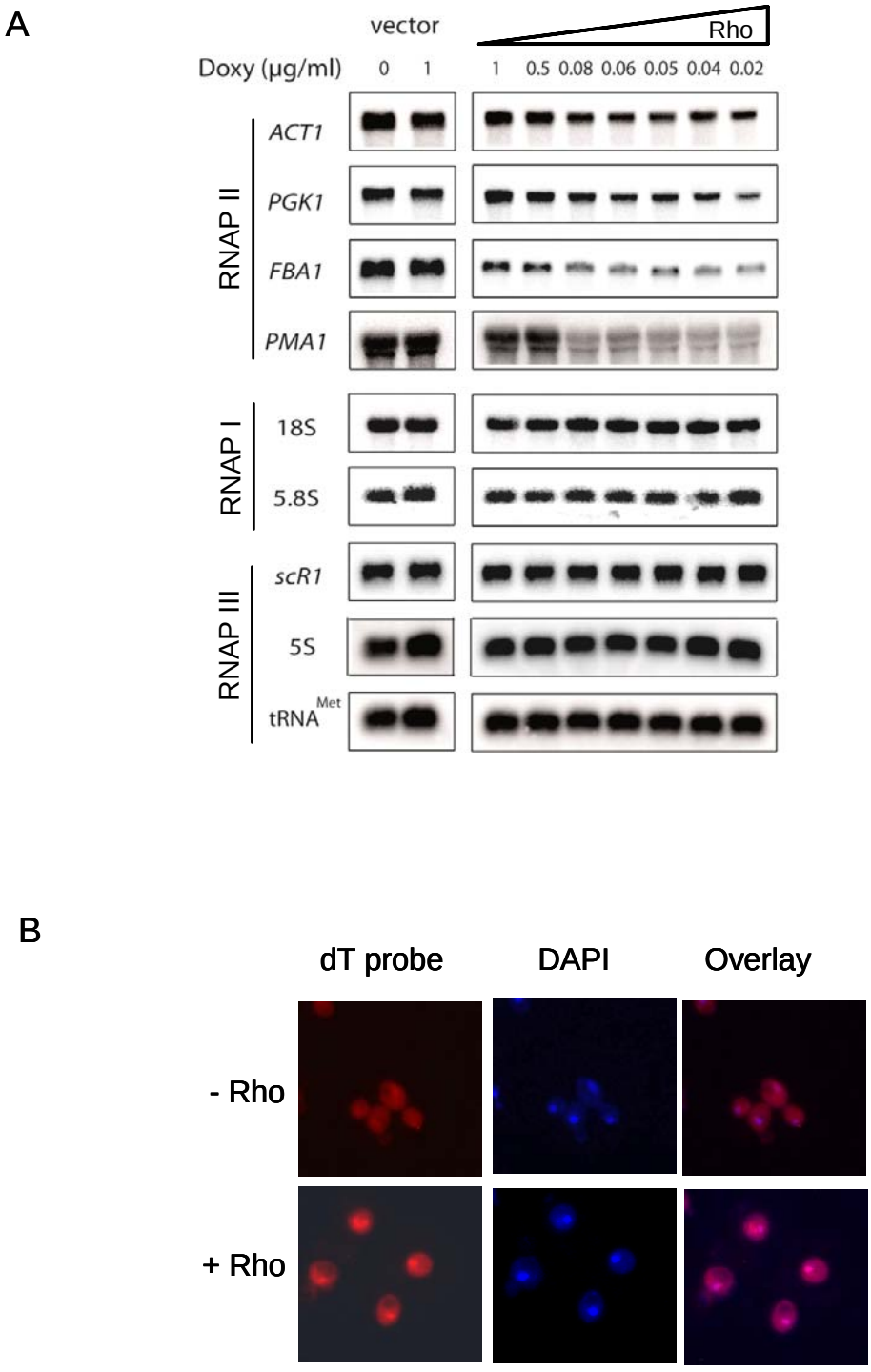


**Figure 2.** The Rho-induced growth defect is linked to its action on mRNA metabolism. **(A)** Northern blot analyses of total RNAs extracted from BMA41 cells transformed with the empty vector or the Rho-NLS expression plasmid and grown at different concentrations of Doxy. Samples of total RNAs (6 µg) were fractionated on an agarose gel, transferred onto a nylon membrane and then the steady-state level of each RNA species was detected sequentially by hybridization using specific 5'-end-labeled oligonucleotide probes. **(B)** Nuclear retention of mRNA in cells expressing Rho-NLS. The mRNA was detected using a Cy3-labeled 40-mer d(T) oligonucleotide as a probe directed against the poly(A) tails. DNA localizing the nucleus was stained with DAPI.

in association with Nab3p. The NRD1 (Nrd1p-Nab3p) complex was also shown to interact with the RNA exosome [27]. These properties led us to suspect Nrd1p as the possible factor that mediates the targeting of Rho-induced aberrant mRNPs during co-transcriptional biogenesis. As a first step to examine our prediction, we tested the sensitivity to Rho expression of a yeast strain harboring a hypomorphic allele of NRD1 lacking the CID (*nrd1Δ1-151*) which decreases the affinity of the protein for binding to RNAP II. The *nrd1Δ1-151* strain was transformed with Rho expression plasmid and the growth was monitored in parallel with an isogenic wild-type strain as control. The transformant spotted on agar plates recovered a full capacity to grow under Rho-inducing conditions (Figure 3A). In agreement with the growth results, the steady-state level of the model transcript (PMA1) in the presence of Rho was rescued significantly in the *nrd1Δ1-151* mutant strain (Figure 3B). Next, we analyzed the distribution of TAP-tagged Nrd1p along the *PMA1* gene by ChIP. As shown in Figure 3C, the ChIP signals in the absence of Rho are consistent with previous findings with a low occupancy of Nrd1p over the *PMA1* chromatin. However, the expression of Rho leads to a large increase (4-fold) of Nrd1p recruitment across the entire gene. The over-expression of isolated CID domains of Nrd1p or Pcf11p (a prominent component of the cleavage and polyadenylation complex) alleviated the high enrichment of Nrd1p along the *PMA1* gene through a competition for recruitment by the transcription complex [13]. Remarkably, the Rho-induced growth defect and the disappearance of PMA1 mRNA are also readily suppressed by the over-expression of the isolated CID domains [13]. Together, these results indicated that the stimulation of Nrd1p co-transcriptional recruitment coordinates the recognition of aberrant mRNPs and probably their subsequent elimination by promoting the attachment of the nuclear mRNA degradation machinery.

**The removal of Rho-induced aberrant mRNPs requires the co-transcriptional recruitment of catalytically active Rrp6p along with the cofactors Rrp47p and Mpp6p**

The nuclear-specific exosome component Rrp6p possesses a 3'-to-5' exonuclease activity and is involved in processing, maturation and surveillance of a large number of different RNAs. To find out if Rrp6p plays a role in the elimination of Rho-induced aberrant mRNPs, we expressed Rho in a strain in which the gene encoding the protein was deleted (*rrp6Δ*). Indeed, the absence of Rrp6p relieved the Rho-induced growth defect and restored the PMA1 mRNA to a nearly normal level (Figure 4). A similar relief of growth defect and rescue of PMA1 mRNA was obtained in the strain *rrp6-Y361A*, which harbors a catalytically inactive version of Rrp6p [8, 28] as the sole source of the protein expressed from its chromosomal locus. This indicated that the exonuclease function of Rrp6p is essential for the degradation of Rho-induced aberrant mRNPs. The involvement of Rrp6p was also revealed by ChIP experiments showing that Rho action triggers co-transcriptional recruitment of both the active and inactive forms of the protein (Figure 4C). Since the Rho-dependent disappearance of PMA1 mRNA is fully alleviated in the mutant strain, this indicated that the elimination of defective mRNPs cannot proceed if the recruited Rrp6p is catalytically inactive.

We also tested the contribution in the elimination process of the cofactors Rrp47p and Mpp6p which have been shown to promote the catalytic activity of Rrp6p-containing exosome complexes [29-31]. Using deletion strains depleted of Rrp47p or Mpp6p as well as strains harboring tagged versions of the proteins, we have shown that the participation of the two cofactors is essential for the targeting and elimination of Rho-induced aberrant mRNPs (Figure 5). The prominent suppression of Rho-induced defects conferred by the removal of either cofactor strongly suggested that they are actively involved in a common functional pathway with Rrp6p. Also, the two cofactors are co-transcriptionally recruited to *PMA1* chromatin as for Rrp6p with a clear physical association of Rrp47p with the exonuclease [15].

Similar experiments were carried out to test the possible involvement of the core exosome as well as the other catalytic subunit of the exosome complex, Dis3p [32, 33]. The results indicated that Rrp6p is the main hydrolytic activity responsible for the degradation of Rho-induced malformed mRNPs. Dis3p plays a rather minor role in the elimination process and ChIP

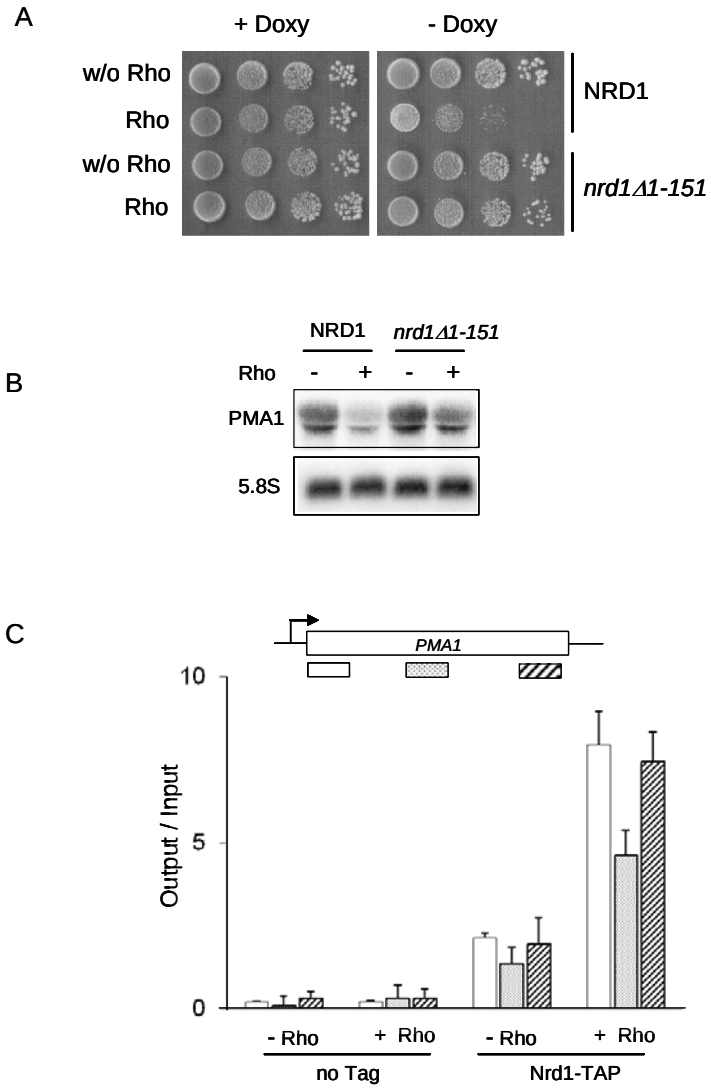


**Figure 3.** The targeting of Rho-induced aberrant mRNPs is mediated by Nrd1p. **(A)** Growth test showing the relief of Rho-induced growth defect in the strain having a deletion of the CID domain of Nrd1p (*nrd1Δ1-151*) as compared to the wild-type (w/o denotes without). **(B)** Northern blot showing the rescue of the model transcript PMA1 in the strain *nrd1Δ1-151* under Rho-inducing conditions. Specific hybridization to the 5.8S rRNA is shown as loading control. **(C)** Quantifications of the fold enrichment of *PMA1* DNA in Nrd1p-TAP immunoprecipitates (ChIP) from cells expressing Rho (+Rho) or not (-Rho). The no-Tag samples are from wild-type cells in which Nrd1p is not tagged. The *PMA1* gene is schematically represented on the top with the horizontal boxes denoting the positions of the PCR products used in the ChIP analyses. The average of three independent ChIP experiments is shown with error bars representing standard deviations.

experiments did not show any stimulation of its co-transcriptional recruitment to *PMA1* chromatin under Rho-inducing conditions. A similar conclusion was reached with the core exosomal subunits Rrp4p and Rrp41p which did not show any Rho-induced stimulation of recruitment to the *PMA1* gene [15].

### The TRAMP components Trf4p and Trf5p are both involved in the targeting of Rho-induced defective mRNPs

The TRAMP complex was shown to mediate the targeting and degradation of a large variety of RNA substrates by the nuclear exosome and it can also stimulate the exonuclease activity of Rrp6p

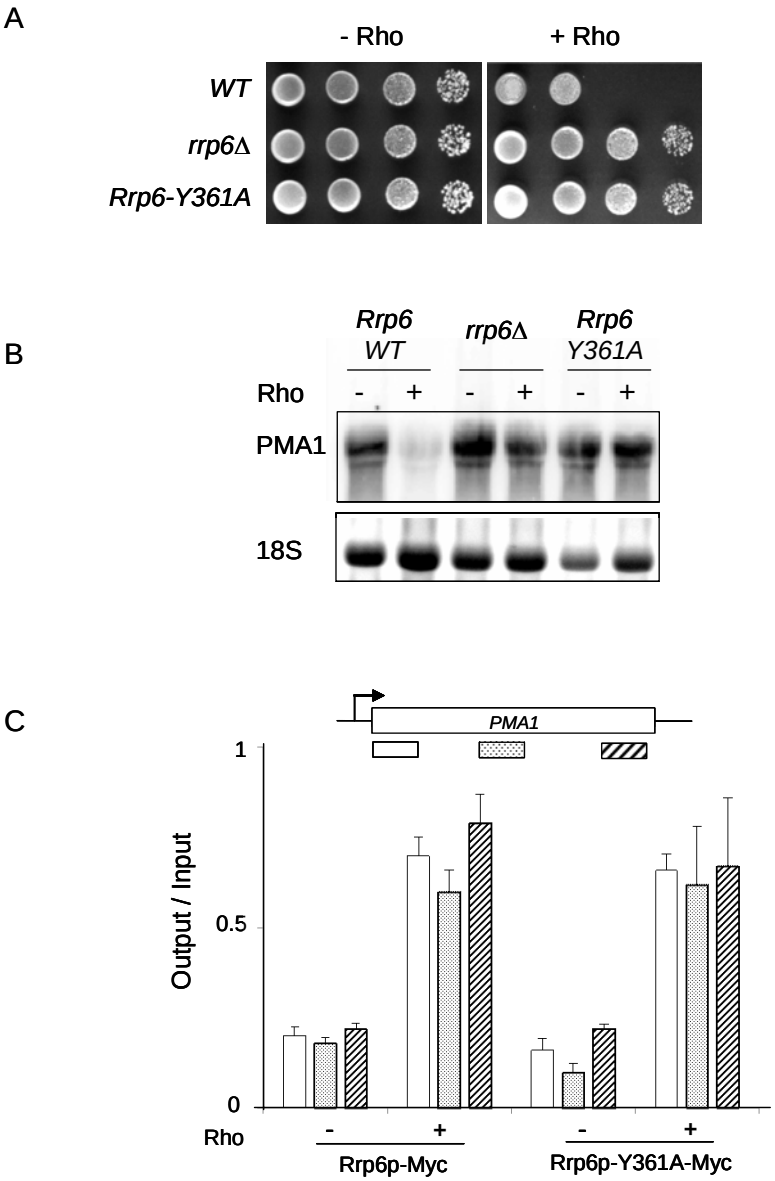


**Figure 4.** Depletion of Rrp6p or inactivation of its catalytic activity alleviates the Rho-induced defects. **(A)** Ten-fold serial dilutions of wild-type, *rrp6Δ* and *Rrp6-Y361A* strains transformed with the Rho expression plasmid and grown in the presence or absence of Doxy. **(B)** Northern blot analysis showing the restoration of PMA1 mRNA under Rho-inducing conditions in *rrp6Δ* and *Rrp6-Y361A* strains. Ethidium bromide staining of the gel before membrane transfer was performed to visualize the 18S rRNAs as a loading control. **(C)** ChIP analyses of Myc-tagged Rrp6p and Rrp6p-Y361A proteins in the presence or the absence of Rho. The analyses were conducted as in Figure 3C.

independently from the exosome *in vitro* [34-37]. The TRAMP complex is formed by one of two non-canonical poly(A) polymerases Trf4p or Trf5p, one of two homologous RNA binding proteins Air1p or Air2p and the RNA helicase Mtr4p. To investigate the possible role of the TRAMP complex and especially the contribution of each subunit in the targeting of Rho-induced aberrant mRNPs, we constructed single deletion strains of the *TRF4* and *TRF5* genes and tested their sensitivity to Rho action. The deletion of the *TRF4* gene mitigated the growth inhibition induced by Rho, whereas the deletion of *TRF5* did not show any relief of the growth defect (Figure 6A).

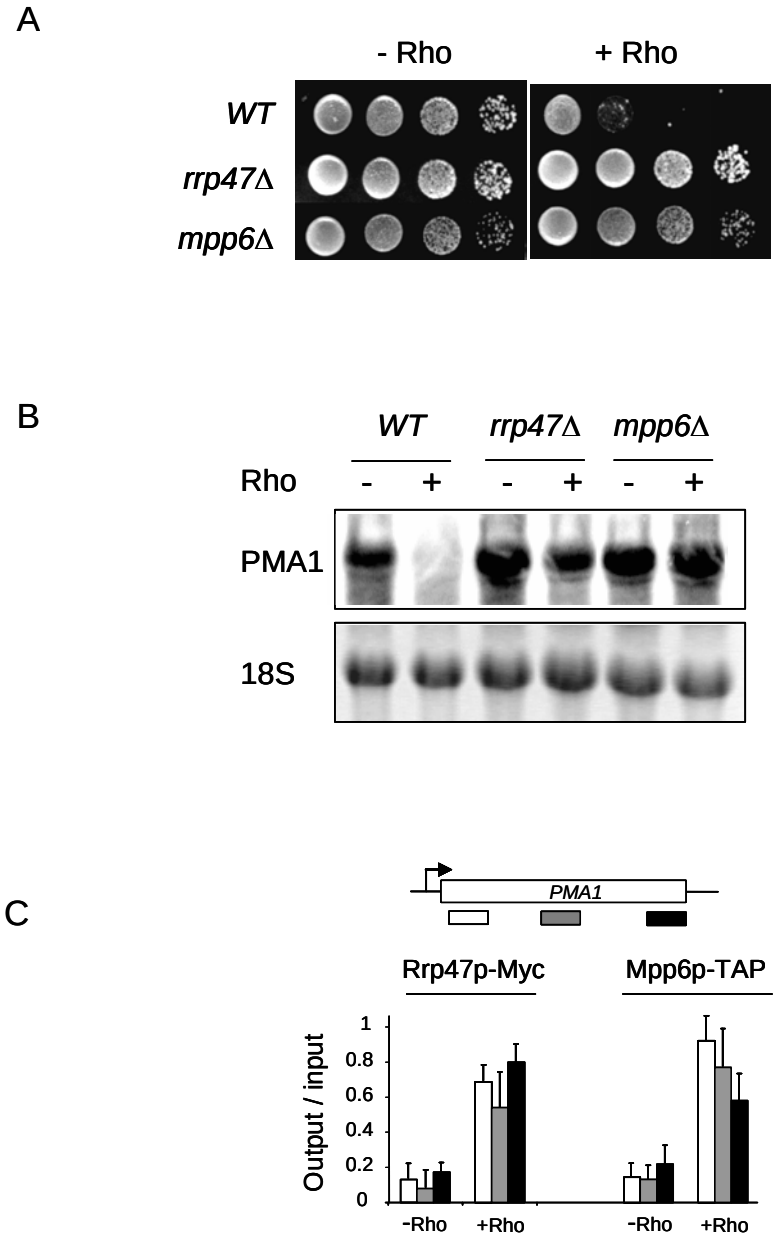


**Figure 5.** The exosome cofactors Rrp47p and Mpp6p contribute to the elimination of Rho-induced aberrant mRNPs. **(A)** Growth test of wild-type, *rrp47Δ* and *mpp6Δ* cells under Rho-repressing or inducing conditions as in Figure 1B. **(B)** Northern blot analysis showing the restoration of PMA1 mRNA under Rho inducing conditions in *rrp47Δ* and *mpp6Δ* strains. **(C)** ChIP analyses of Myc-tagged Rrp47p and TAP-tagged Mpp6p proteins in the presence or the absence of Rho. The analyses were conducted as in Figure 3C.

RT-PCR quantifications of the model transcript PMA1 (Figure 6B) revealed that a large part of the mRNA is recovered in the *trf4Δ* mutant under Rho expression conditions (a ratio of 0.9 as compared to 0.3 in the wild-type strain). In contrast, only a moderate fraction is recovered in the *trf5Δ* strain (a ratio of 0.5 as compared to 0.3 in the wild-type strain), which is in agreement with the growth tests because the minor transcript recovery is not sufficient to improve the relative growth, whereas the substantial rescue in the *trf4Δ* mutant is sufficient to mitigate the growth defect.

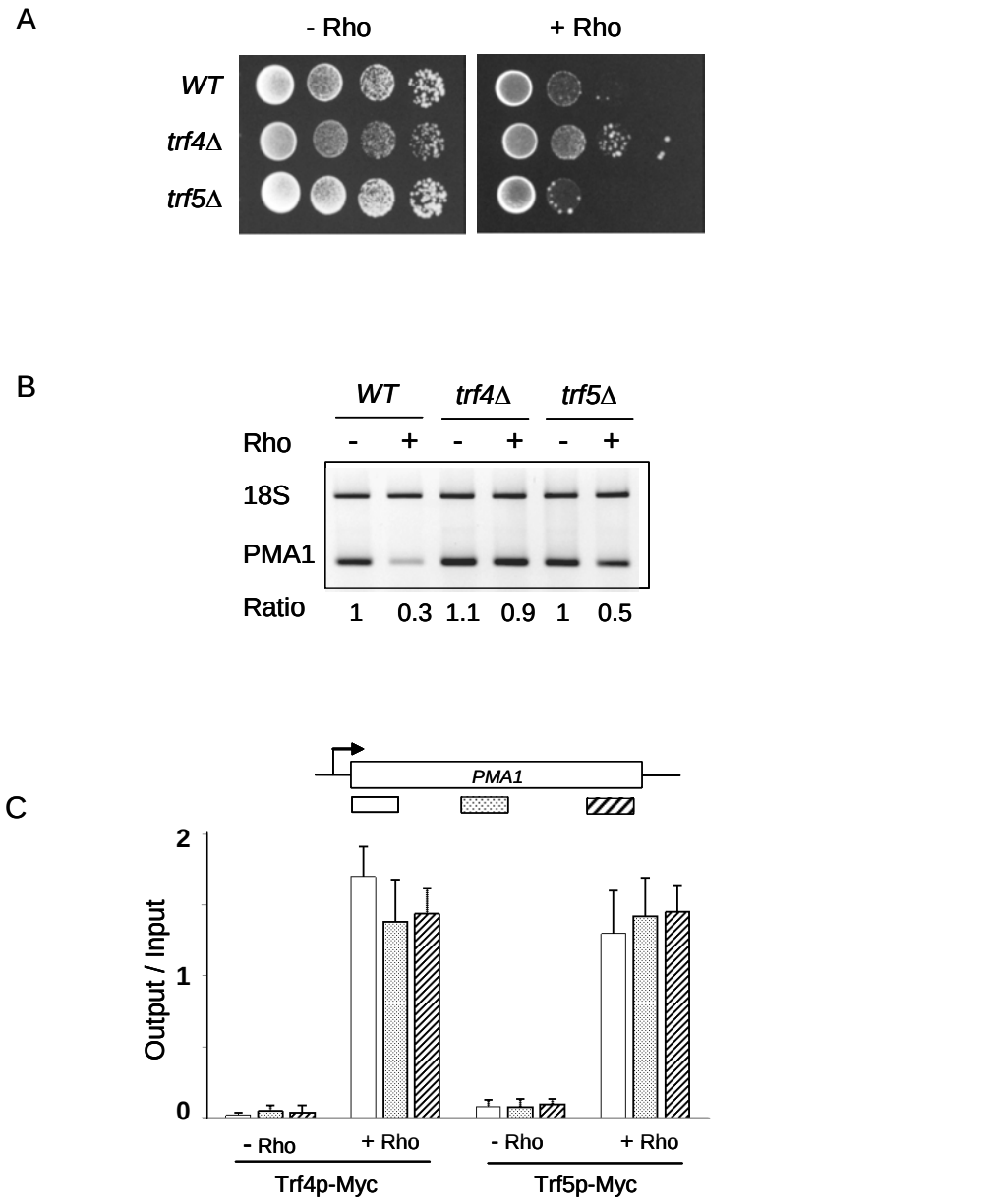


**Figure 6.** The removal of Rho-induced aberrant mRNPs requires the TRAMP components Trf4p and Trf5p. **(A)** Growth tests comparing the wild-type with *trf4Δ* and *trf5Δ* strains under Rho-repressing or inducing conditions as in Figure 1B. **(B)** Agarose gel showing semi-quantitative RT-PCR analyses to evaluate the level of mRNA under Rho-repressing or inducing conditions. Ratio denotes the level of PMA1 mRNA relative to 18S rRNA as determined by quantitative RT-PCR and by setting the value of PMA1 signal to 1 for the wild-type BMA41 strain in the absence of Rho expression. **(C)** ChIP analyses of Myc-tagged Trf4p and Trf5p proteins in the presence or the absence of Rho. The analyses were conducted as in Figure 3C.

These results suggested that the two TRAMP components Trf4p and Trf5p are involved in the surveillance process that confers the Rho-mediated phenotype. However, the fact that the absence of either component allows the restoration of only a fraction of the model transcript PMA1 suggested that they may intervene within distinct TRAMP complexes. Strains carrying Myc-tagged versions of Trf4p or Trf5p were constructed to assess the potential co-transcriptional recruitment of the

two components following Rho action. The ChIP analyses presented in Figure 6C show that Rho action triggers a large enrichment of both proteins across the whole *PMA1* gene. This led us to conclude that Trf4p and Trf5p are both involved in the targeting and elimination of Rho-induced aberrant mRNPs. They presumably act within two distinct forms of the TRAMP complex that are co-transcriptionally recruited.

**The removal of Rho-induced aberrant mRNPs requires Air2p but not Air1p**

Having found that Trf4p and Trf5p act within two distinct TRAMP complexes for the removal of Rho-induced defective mRNPs, we sought to evaluate the contributions of the two other homologous components of TRAMP, Air1p and Air2p. Single deletion mutant strains as well as the double mutant were constructed and their sensitivity to Rho action was tested by growth on agar plates. The growth results in Figure 7A show that the deletion of *AIR1* (*air1Δ*) alone did not relieve the sensitivity of yeast cells to Rho action, whereas the depletion of Air2p (*air2Δ*) induced a strong suppressing phenotype similar to the one observed with the double-mutant strain (*air1Δ air2Δ* in [15]). The relief of Rho-induced growth defect in the absence of Air2p was substantiated by the restoration of the model transcript PMA1 to a nearly wild-type level, as shown by Northern blotting in Figure 7B. These results indicated that, for the removal of Rho-induced aberrant mRNPs, there is a functional distinction between Air1p and Air2p since Air1p is not participating in the QC process. Thus, the two TRAMP complexes formed by Trf4p and Trf5p have both Air2p as component. ChIP analyses with Myc-tagged versions of the proteins supported this conclusion by showing a Rho-dependent co-transcriptional recruitment to the *PMA1* gene of Air2p but not Air1p [15].

## DISCUSSION

Eukaryotic cells have evolved an mRNP quality control (QC) system by which aberrant transcripts having processing and assembly defects are recognized as export-incompetent leading to their retention in the nucleus with ensuing degradation of their mRNA component by the RNA decay machinery. Numerous studies have suggested the interconnection of this surveillance system with transcription elongation and mRNP biogenesis. However, the process by which an integrated system recognizes aberrancies at each step of mRNP biogenesis and targets the defective molecules for destruction remained unclear. Interestingly, several lines of evidence suggested that the QC apparatus could be recruited directly to the transcription complex, a position from which it can scrutinize all mRNP processing and packaging reactions looking for faulty events.

Here, we describe the implementation of an experimental approach in which the activity of bacterial Rho helicase in the yeast nucleus impedes the mRNA processing and packaging reactions producing sufficient amounts of defective mRNPs that serve as substrates to study the surveillance mechanism. Using this approach, we have shown that the recognition and targeting for degradation of defective mRNPs is mediated by Nrd1p which is recruited co-transcriptionally to the malformed mRNP in association with the RNA decay machinery. The results obtained with this artificially designed system led us to propose a model that mimics the detection by the QC of aberrancies that may arise naturally during the coupled transcription and mRNP biogenesis (Figure 8). Upon its recruitment at the promoter-proximal region of a transcribed gene, Nrd1p is presumably weakly anchored to the transcription complex through interactions of its CID with the CTD of RNAP II. The loosely bound Nrd1p is usually outcompeted by newly recruited factors while RNAP II is transcribing further downstream within the body of the gene. However, the interaction of Nrd1p could be further stabilized by its binding in association with Nab3p to RNA segments uncovered by Rho helicase/translocase activity, which mimics a faulty event such as the misloading of an mRNP processing or assembly factor. The co-transcriptional recruitment and RNA binding of Nrd1p is presumably followed by the attachment of Rrp6p in association with the cofactors/activators Rrp47p, Mpp6p and the TRAMP complex as revealed by our ChIP experiments and supported by other studies showing physical interactions between Nrd1p and Rrp6p [27] as well as between Nrd1p and Trf4p [38]. This may represent the step at which a defect is detected and

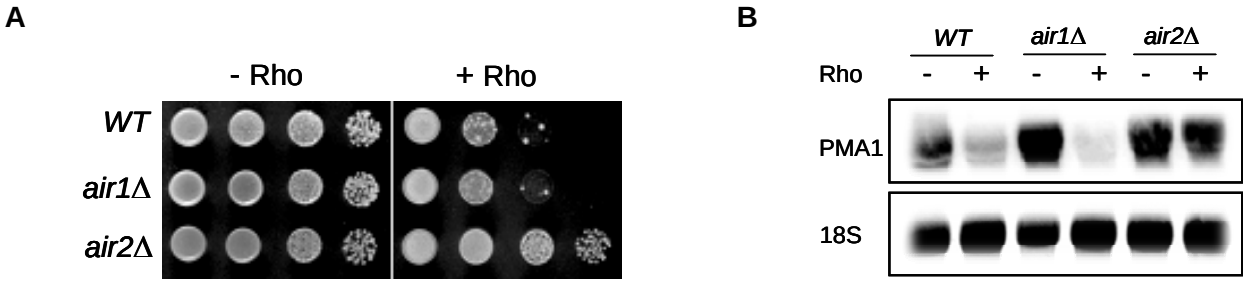


**Figure 7.** The removal of Rho-induced aberrant mRNPs requires the TRAMP component Air2p but not Air1p. **(A)** Growth tests comparing the wild-type with *air1Δ* and *air2Δ* strains under Rho-repressing or inducing conditions as in Figure 1B. **(B)** Northern blot analysis showing the restoration of PMA1 mRNA under Rho-inducing conditions in *air2Δ* but not in *air1Δ*strains.

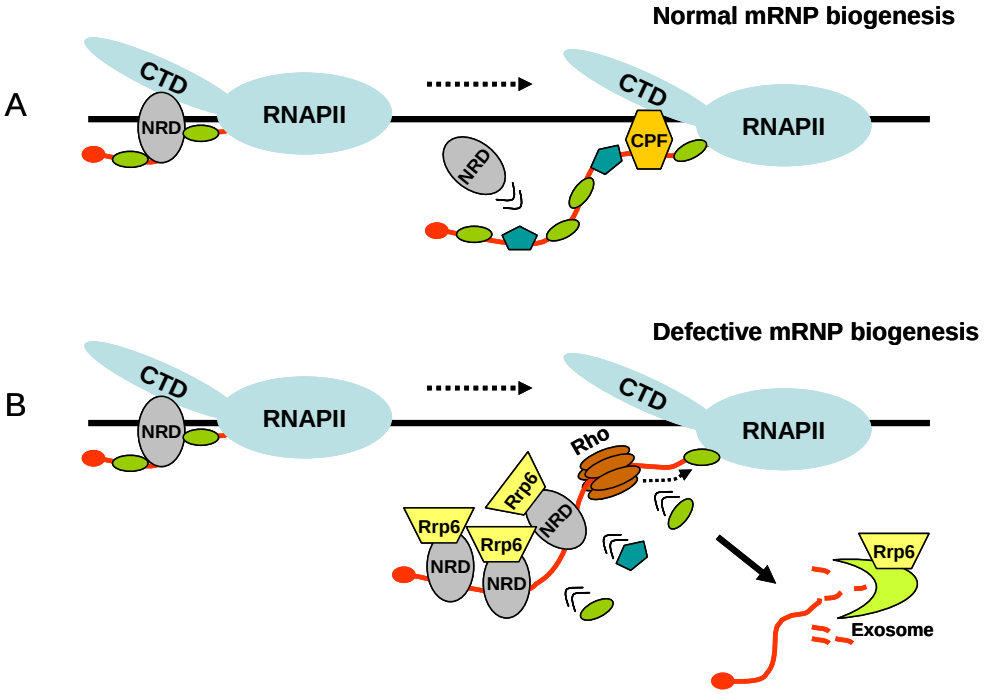


**Figure 8.** Model illustrating the Nrd1p-Rrp6p mediated recognition and targeting for degradation of Rho-induced aberrant mRNPs. **(A)** Normal conditions of mRNP biogenesis in which Nrd1p is recruited to the transcription complex at early elongation then is outcompeted by the recruitment of mRNA processing and binding proteins as RNAP II travels further downstream. **(B)** Rho loading and tracking along nascent transcript displaces processing and binding proteins uncovering specific RNA-binding sites that promote preferential recruitment of the NRD1 complex (Nrd1p-Nab3p), and association of nuclear RNA degradation machinery (Rrp6p and the cofactors/activators Rrp47p, Mpp6p and TRAMP) represented by Rrp6. Upon transcription completion, the aberrant mRNP would be retained in the nucleus and degraded by the 3'-to-5' exonuclease activity of Rrp6p either alone or in association with the core exosome scaffold.

the transcript is labeled as aberrant. Upon transcription completion, the labeled mRNP would be prevented from export and degraded by the 3'-to-5' exonuclease activity of Rrp6p either independently or in association with the core exosomal scaffold. This Nrd1p-Rrp6p tagging model could explain how a general QC system recognizes various aberrancies arising at different steps of mRNP biogenesis

such as, splicing termination and 3'end formation or assembly and export, then directs the defective molecules to the same outcome, destruction.

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**CONFLICT OF INTEREST STATEMENT**

The authors declare that they have no conflicts of interest.

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