Rpm2p, a protein subunit of mitochondrial RNase P, physically and genetically interacts with cytoplasmic processing bodies

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ABSTRACT

The RPM2 gene of Saccharomyces cerevisiae codes for a protein subunit of mitochondrial RNase P and has another unknown essential function. We previously demonstrated that Rpm2p localizes to the nucleus and acts as a transcriptional activator. Rpm2p influences the level of mRNAs that encode components of the mitochondrial import apparatus and essential mitochondrial chaperones. Evidence is presented here that Rpm2p interacts with Dcp2p, a subunit of mRNA decapping enzyme in the two-hybrid assay, and is enriched in cytoplasmic P bodies, the sites of mRNA degradation and storage in yeast and mammalian cells. When overexpressed, GFP-Rpm2p does not impact the number and size of P bodies; however, it prevents their disappearance when translation elongation is inhibited by cycloheximide. Proteasome mutants, ump1-2 and pre4-2, that bypass essential Rpm2p function, also stabilize P bodies. The stabilization of P bodies by Rpm2p may occur through reduced protein degradation since GFP-Rpm2p expressing cells have lower levels of ubiquitin. Genetic analysis revealed that overexpression of Dhh1p (a DEAD box helicase localized to P bodies) suppresses temperature-sensitive growth of the rpm2-100 mutant. Overexpression of Pab1p (a poly (A)-binding protein) also suppresses rpm2-100, suggesting that Rpm2p functions in at least two aspects of mRNA metabolism. The results presented here, and the transcriptional activation function demonstrated earlier, implicate Rpm2p as a coordinator of transcription and mRNA storage/decay in P bodies.

INTRODUCTION

RPM2 is a nuclear gene encoding a multifunctional protein that can localize to both mitochondria and the nucleus. Together with the mitochondrially encoded RNA subunit, Rpm1r, Rpm2p functions as a protein subunit of mitochondrial RNAse P (1–4). Rpm2p is also required for the maturation of the RNAse P RNA subunit, Rpm1r (5), and separate domains of Rpm2p promote tRNA and Rpm1r maturation (6). Analysis of the rpm2-100 mutant revealed that Rpm2p has a role, independent of RNAse P activity, in translation of mitochondrially encoded cytochrome c oxidase subunits Cox1p, Cox2p and Cox3p (7). In addition, a synthetic lethal interaction has been found between the rpm2-100 mutant and the loss of wild-type mitochondrial DNA (mtDNA). Cells with either the rpm2-100 mutation or a deletion of mtDNA grow on glucose, but when both alterations occur in the same cell there is no growth on any carbon source (7).

Recently, we demonstrated that Rpm2p can localize to the nucleus, has a transcriptional activation domain, and plays a role in defining steady-state levels of mRNAs for some nuclear-encoded mitochondrial components, such as the TOM complex and the mitochondrial heat-shock proteins, such as Hsp60p and Hsp10p (8). It is likely that induction of the TOM components and the essential chaperones in cells lacking mtDNA is an adaptation to maintain efficient protein import upon reduction in membrane potential caused by the loss of mtDNA. Therefore, Rpm2p has emerged as a regulatory protein critical to maintaining viability in a retrograde fashion, when cells lose their mitochondrial genome. This observation may explain why a complete deletion of RPM2 is lethal in Saccharomyces cerevisiae (9).

Retrograde signaling is a pathway of communication from mitochondria to the nucleus (for review, see 10). Four percent of yeast genes reproducibly alter transcript levels in glucose grown yeast cells devoid of mtDNA (11).
Many genes with elevated expression in cells lacking mtDNA encode proteins involved in mitochondrial biogenesis and function (11), including those found to be dependent on Rpm2p (8).

The work presented here demonstrates that Rpm2p can localize to cytoplasmic processing bodies (P bodies) and genetically interacts with Dhh1p. In addition, we show a genetic interaction between Rpm2p and Pab1p. The presence of Rpm2p at sites of mRNA degradation and storage, as well as the relationship with Pab1p, suggest that changes in mRNA stability, in addition to changes in transcription may play a role in altering transcript levels in yeast cells devoid of mtDNA.

MATERIALS AND METHODS

Strains, media and reagents

Rich media included 1% Bacto-yeast extract, 2% Bacto-peptone and 2% glucose (YPD) or 3% glycerol and 2% ethanol (GE) instead of glucose. Synthetic complete (SC) media lacking appropriate amino acids for plasmid retention contained 0.67% Bacto-nitrogen base and either 2% glucose or 2% galactose. Solid media for plates included 2% Bacto-agar. Culture media reagents were Fisher Scientific or Difco. Yeast strains used in this study, yVS100 (MATa ade2-1 ade3 trp1-1 ura3-1 can1-100) (12) and isogenic mutants were Fisher Scientific or Difco. Yeast strains used in this study, yVS100 (MATa ade2-1 ade3Δ22 his3-11, 15 leu2-3,11 trp1-1 ura3-1 can1-100 Δrpm2::rpm2-100) (this study); YMW1 (MATa ade2-1 ade3Δ22 his3-11, 15 leu2-3,11 trp1-1 ura3-1 can1-100) (12) and isogenic mutants (MATa ade2-1 ade3Δ22 his3-11, 15 leu2-3,11 trp1-1 ura3-1 can1-100 ump1-2), (MATa ade2-1 ade3Δ22 his3-11, 15 leu2-3,11 trp1-1 ura3-1 can1-100 Δrpm2::kanMX) (13); BY4741 (MATa his3Δ leu2Δ lys2Δ met15Δ ura3Δ) and isogenic haploid strains containing LSM1, and XRN1 disruptions generated by the S. cerevisiae genome deletion project consortium were obtained from Research Genetics; yRP1358 (MATa his4-539 leu2-3112 lys2-201 trp1 ura3-52 dcp2::TRP1) strain containing DCP2 disruption was a gift from Roy Parker (University of Arizona). The GAL1-regulated yeast cDNA library in a centromeric shuttle vector was kindly provided by Anthony Bretscher, Cornell University (14); the GFP-Rpm2p expressing plasmid was described (8); the plasmids expressing the Dcp2p-RFP (pRP1155), the Lsm1p-RFP (pRP1185) and pAD-Dcp2p (pRP1359) were kindly provided by Roy Parker.

Plasmid construction

For the yeast two-hybrid analysis, a PCR product of the RPM2 coding region lacking the first 41 amino acids was cloned into the BamHI site of pGBT9. To construct plasmid expressing GFP-RPM2 under control of RPM2 promoter, RS315-EP-GFP-RPM2, first, a PCR product of the RPM2 promoter region (~560 + 1) was cloned into XhoI/BamHI sites of pRS316. Second, a PCR product encoding GFP-RPM2 was obtained from GFP-Rpm2p expressing plasmid (8) and cloned downstream of the promoter region into BamHI/SacI sites. The integrity of a new fusion gene was confirmed by sequencing. The oligonucleotide sequences are available upon request.

Library screening

To screen for proteins that suppress the rpm2-100 mutant, the strain yVS100 was transformed with the yeast S. cerevisiae cDNA library under control of the GAL1-inducible promoter on a centromeric shuttle vector. Transformants were plated on synthetic-complete medium lacking uracil and screened for growth at 37°C. Plasmids from growers at 37°C were isolated and tested again for suppression of the rpm2-100 mutant under the same conditions.

Protein synthesis assay

Yeast transformants carrying either the DCP2-RFP fusion gene, or both, DCP2-RFP and GFP-RPM2, on plasmids were grown in synthetic-complete selective medium containing glucose. Cultures were shifted to galactose selective medium for 6 h to induce GFP-Rpm2p. After two washes with sterile water, cultures were incubated in methionine-free medium for 30 min, treated with 100 μg of cycloheximide for 30 min, (control left untreated), and an equivalent number of cells were incubated in the presence of 50 μCi of [35S]-methionine for 30 min. Ten microliters of each sample was spotted on filters pretreated with 50% TCA. Dried filters were boiled for 5 min in 10% TCA, washed twice in 10% TCA, once with water and either spotted directly onto glass slides or fixed before spotting for imaging on a Zeiss Axioscope 200 microscope.

Western analysis

Total protein extracts were made using YBB buffer (Q-BIOgene) and glass beads in the presence of protease inhibitors (Boehringer). Proteins were separated on a 4–12% Bis-Tris gel (Invitrogen), transferred to an Immobilon-P membrane (Millipore, Bedford, MA) and protease inhibitors (Boehringer). Proteins were separated on a 4–12% Bis-Tris gel (Invitrogen), transferred to an Immobilon-P membrane (Millipore, Bedford, MA) and treated with anti-ubiquitin antibodies at 1 mCi of [35S]-methionine for 30 min. Ten microliters of each sample was spotted on filters pretreated with 50% TCA. Dried filters were boiled for 5 min in 10% TCA, washed twice in 10% TCA, once in ethanol, dried and subjected to scintillation counting.

Microscopy

Yeast transformants expressing fusion proteins were grown to mid-log phase in synthetic-complete medium lacking appropriate amino acids for plasmid retention. Cultures were then shifted to galactose-containing selective medium and grown for either 6 h to induce GFP-Rpm2p synthesis, or cultivated up to 24 h to obtain high-density cultures. Cells were harvested, washed three times with water and either spotted directly onto glass slides or fixed before spotting for imaging on a Zeiss Axioskope 200 microscope. In the cycloheximide treatments, washes also included 100 μg/ml of cycloheximide.

RESULTS

Dhh1p and Pab1p overexpression suppresses rpm2-100 temperature-sensitive growth

Previous studies have showed that the rpm2-100 mutation causes loss of cell osmotic integrity at the non-permissive
temperature, and this phenotype can be suppressed by increasing osmolarity of the growth medium (Stribinskis et al., manuscript in preparation). We have used this temperature-sensitive growth phenotype to isolate high-copy suppressors that allow rpm2-100 mutant cells to grow at the restrictive temperature. The yeast S. cerevisiae cDNA library under control of the GAL1-regulated promoter (14) was introduced into rpm2-100 cells; transformants were plated on synthetic plates containing galactose as the sole carbon source and incubated at 37°C. The screen revealed that in addition to Rpm2p, Dhh1p, an RNA helicase (16) and Pab1p, poly (A)-binding protein (17,18), are high-copy suppressors of rpm2-100 temperature-sensitive growth (Figure 1). Interestingly, PAB1 was found as a high-copy suppressor of an rpm2 deletion strain (Nancy C. Martin, personal communication). Pab1 is a multifunctional protein that plays a role in stabilization of mRNAs, brings the 5' and 3' ends of mRNAs into proximity by binding eIF4G, and stimulates translation (19–21). In addition, recent observations demonstrate a role for Pab1p in mRNA export from the nucleus (22,23).

Unlike PAB1 and SEF1 (24), overexpression of DHH1 does not compensate for the rpm2 deletion (data not shown). Dhh1p belongs to the family of DEAD-box proteins, which are ATP-dependent RNA helicases found in a variety of organisms (25). DHH1 was identified as a high-copy suppressor of the POP2 and CCR4 transcriptional complex and physically and functionally associates with Pop2p and Ccr4p (26). In addition, Dhh1p can stimulate decapping (27,28), and recent evidence indicates a role for Dhh1p in translational repression at an early step in mRNA decay (29). Overexpression of Dhh1p inhibits cell growth (29); however, as we show here, it has no appreciable inhibitory effect on cells of YMW (the derivative of W303) genetic background (Figure 1).

The finding that overexpression of DHH1 and PAB1 can suppress temperature-sensitive growth of the rpm2-100 mutant strongly suggests that Rpm2p functions in some aspects of RNA metabolism outside the mitochondria.

### Rpm2p interacts with Dcp2p in vivo

In a systematic analysis of protein complexes in yeast, Rpm2p was found in the same complex with Dcp2p by affinity capture using Dcp2p as bait in two independent attempts (30,31). Dcp2p is a subunit of the decapping enzyme and can be found in cytoplasmic processing bodies (32,31). To determine whether Rpm2p and Dcp2p interact in vivo, we performed the yeast two-hybrid analysis using Rpm2p as bait. We employed a reporter gene, HIS3, which is under control of the yeast GAL DNA-binding domain. To make the bait, we fused the RPM2 coding region lacking the first 41 amino acids downstream of the GAL DNA-binding domain (BD-Rpm2p). We introduced this plasmid into a reporter strain together with another plasmid expressing either Dcp2p fused to the GAL DNA-activation domain (AD-Dcp2p) or GAL DNA-activation domain alone (AD). Transformants were spotted on selective plates and scored for the ability to grow on medium lacking histidine and containing aminotriazole, AT, a competitive inhibitor of the HIS3 gene product.
lacking histidine, but growth ceases in the presence of 1 mM AT. This background growth is likely attributed to the leaky expression of the HIS3 gene. Cells expressing BD-Rpm2p and AD could grow in the presence of 1 mM AT, however their growth was diminished in the presence of 10 mM AT and completely abolished at 15 mM AT. This is because Rpm2p itself has a transactivation domain containing two putative leucine zippers, and shows robust reporter activity in the absence of the carboxy-terminal domain, which is not required for growth by fermentation (8). However, in the presence of an intact carboxy-terminus in the construct used here, Rpm2p transactivation is moderate and can be abolished at lower concentrations of AT than used in a previous study (8). In contrast, cells expressing both, BD-Rpm2p and AD-Dcp2p show increased fitness compared to cells expressing BD-Rpm2p and AD, at all concentrations of AT tested. This result indicates that Rpm2p and Dcp2p can interact in vivo and substantiates a model that assigned Rpm2p as the attachment protein together with Xrn1p (a 5’ to 3’ exonuclease) and a protein of unknown function, Ybr094p, to a module (Edc3p-Dcp1p-Dcp2p) known as the mRNA-decapping complex (Gavin et al. (36)).

Rpm2p colocalizes with Dcp2p in cytoplasmic processing bodies in vivo

We found that the essential portion of Rpm2p, which can also support mitochondrial RNase P activity under respiratory growth conditions (6), expressed as a fusion protein with GFP, concentrates in the nucleus and localizes to distinct foci in the cytoplasm that did not appear to colocalize with any organelle (8). The observation that Rpm2p interacts with Dcp2p, which resides in cytoplasmic mRNA processing bodies, P bodies (33), suggested that these foci might be P bodies. DCP2 is an essential gene, however, yeast strain yRP1358 has an unknown genetic variation that allows the Δdcp2 mutant to grow, albeit poorly at all temperatures (32). To determine whether Rpm2p colocalizes with Dcp2p, the yeast strain yRP1358 was transformed with GFP-Rpm2p and DCP2-RFP constructs and their localization after induced expression of GFP-Rpm2p with galactose was determined by fluorescence microscopy. Both proteins colocalize in discrete cytoplasmatic foci, and colocalization occurs in all cells that have both, green and red fluorescence (Figure 3A). In addition, overexpression of GFP-Rpm2p from a powerful, galactose-inducible promoter, neither leads to an increase in P body size nor number. The same result was also obtained in two other widely used laboratory yeast strains W303 and BY4741 (not shown). Therefore, Rpm2p, lacking a mitochondrial leader sequence, but able to support the essential function, localizes to P bodies.

Since Rpm2p interacts with Dcp2p, it was important to determine whether localization of Rpm2p depends on its interaction with Dcp2p. To address this question, yRP1358 cells lacking endogenous Dcp2p were cotransformed with plasmids expressing GFP-Rpm2p and Lsm1p-RFP, and the transforms were examined by fluorescence microscopy. Lsm1p is a component of P bodies and forms a heteroheptameric complex with Lsm2-Lsm7 proteins, which is involved in mRNA deadenylation-dependent decapping (34). Surprisingly, we could not observe P bodies in the vast majority of cells lacking Dcp2 protein, even after prolonged incubation in water, which is known to promote formation of P bodies (33). This result indicates that Dcp2 protein is necessary for P body formation in the majority of cells. However, in a small fraction of cells that contained P bodies, as visualized using Lsm1p-RFP, GFP-Rpm2p is present in a P body (Figure 3B). This result indicates that localization of Rpm2p to P bodies does not require Dcp2p, at least in a small fraction of cells that contain P bodies.

Overexpression of GFP-Rpm2p prevents dissociation of P bodies upon inhibition of translation elongation

To determine whether association of Rpm2p with P bodies remains under conditions that promote dissociation of other known P body components, cells were exposed for 30 min to cycloheximide (100 μg/ml), an inhibitor of translation elongation. Cycloheximide induces dissociation of P bodies in both yeast and mammalian cells (33,35). Figure 4 shows that the typical localization of Dcp2-RFP to P bodies is not observed after cycloheximide treatment. In contrast, Dcp2-RFP remains in P bodies upon expression of GFP-Rpm2 protein in the presence of cycloheximide. Moreover, both proteins remain in P bodies under these conditions. We also examined whether the observed effect of Rpm2p on P body stability depends on the stage of growth of a yeast culture. We found that at each stage of growth, from OD₆₀₀ = 0.5 (early-log phase) to OD₆₀₀ = 4.0 (end of log phase), both Dcp2p and Rpm2p fluorescent-tagged fusions were present in P bodies after cycloheximide
treatment (Figure 4). Therefore, overexpression of GFP-Rpm2p from an inducible GAL promoter stabilizes P bodies against dissociation by cycloheximide.

One explanation for the Rpm2p effects on P bodies is that cells become resistant to cycloheximide. To test this prediction, protein synthesis levels before and after cycloheximide exposure in cells expressing either Dcp2p-RFP or both, Dcp2p-RFP and GFP-Rpm2p were measured. Both strains displayed comparable growth rates in synthetic galactose medium (310 min and 330 min division rates for Dcp2p-RFP and Dcp2-RFP/GFP-Rpm2p expressing strains, respectively). Although, the protein synthesis rate in the Dcp2p-RFP/GFP-Rpm2p strain is reduced by 20% compared to Dcp2p-RFP strain, the remaining protein synthesis in the presence of cycloheximide is reduced to the same extent in both strains indicating that overexpression of Rpm2p does not desensitize cells to cycloheximide (Figure 5).

Proteasome mutants affect P bodies
In addition to its role in the mitochondrial RNase P, Rpm2p is an essential protein under all growth conditions. It has previously been found that reduced proteasome activity in pre4-2 or ump1-2 mutants allows growth in the absence of Rpm2p, although the mechanism is unknown (13). Therefore, Rpm2p may have a role in controlling activity of the ubiquitin-proteasome pathway or the stability of a critical component of the P body that is rapidly degraded upon inhibition of translation elongation. If this were true, inhibition of proteasome activity should stabilize P bodies. To test this hypothesis, P body formation was determined in a wild-type strain expressing Dcp2p-RFP or in a strain carrying a mutation in the proteasome chaperone Ump1p, or in a strain lacking Rpm2p, but carrying a mutation in the proteasome catalytic subunit Pre4p, and determined P body formation. Note that these strains are in YMW genetic background, in which the essential function of Rpm2p can be suppressed by proteasome mutants (13).

Lower ubiquitin levels in cells overexpressing GFP-Rpm2p
Protein degradation by the ubiquitin-proteasome pathway is required for altering the levels of key regulators, as well as the degradation of misfolded and mutant proteins. Attachment of ubiquitin to proteins targets them to the 26S proteasome for degradation. The accumulation of high-molecular mass ubiquitinated proteins is a hallmark of reduced proteasome function. To establish a relationship between Rpm2p and the ubiquitin-proteasome pathway we examined the levels of ubiquitinated proteins by comparing cells expressing GFP-Rpm2p to those that do not. Total protein was isolated from mid-log phase grown cultures and Western
analysis performed with anti-ubiquitin antibodies. The results show no differences in the intensity of a diffused signal in the molecular mass range of 30–100 kDa, which reflects steady-state levels of ubiquitinated proteins, in either strain (Figure 7A). However, levels of free ubiquitin were reduced in cells expressing GFP-Rpm2p. To determine if overexpressed Rpm2p is using up the free ubiquitin for its own turnover, we reprobed the same membrane with anti-GFP antibodies and found no evidence for the accumulation of higher molecular mass GFP-Rpm2p species. Although the mechanism of the ubiquitin depletion in GFP-Rpm2p expressing cells is unclear, it is possible that depletion in free ubiquitin levels may reduce the availability of ubiquitin for conjugation and this reduction contributes to increased stability of some proteins involved in P body formation and/or maintenance.

GFP-Rpm2p localizes to P bodies when expressed at normal levels

To determine whether localization of Rpm2p to P bodies is due to overexpression of the fusion protein, or is an intrinsic property of Rpm2p, we performed localization studies under conditions where a fusion protein is expressed at levels comparable to the endogenous levels of Rpm2p. In the first approach, cells expressing both the Dcp2p-RFP and GAL promoter-driven GFP-Rpm2p were grown on a fermentable, but not repressible, carbon source raffinose in the BY4741 yeast. We switched to the BY4741 strain because the yRP1358 strain, used in previous experiments, has leaky expression from the GAL promoter in the absence of galactose (not shown). We monitored the kinetics of GFP-Rpm2p localization after addition of galactose. Figure 8 demonstrates that GFP-Rpm2p is undetectable in extracts prepared from BY4741 cells grown in raffinose medium. However, its expression is induced after 20 min upon galactose addition, and the level of expression continues to rise up to 4 h. We found that when expression of GFP-Rpm2p reaches comparable levels to that of endogenous Rpm2p (20 min after galactose addition) (panel A), the fusion protein localizes together with Dcp2-RFP in cytoplasmic foci (panel B), indicating the rapid kinetics of Rpm2p localization to P bodies.

In the second approach, we constructed a plasmid where the expression of GFP-Rpm2p is under control of the RPM2 promoter. The transformants in BY4741 genetic background, harboring both GFP-RPM2 and DCP2-RFP fusion genes on centromeric plasmids, were grown in selective raffinose medium, fixed and subjected to fluorescent microscopy. Figure 9 shows that GFP-Rpm2p expressed from a centromeric plasmid under control of the RPM2 promoter, colocalizes with Dcp2p-RFP. Together, these results indicate that localization of Rpm2p to P bodies is not a consequence of protein overproduction, and occurs when Rpm2p is not targeted to the mitochondria.
We demonstrated that expression of GFP-Rpm2p from an inducible GAL promoter stabilizes P bodies against dissociation by cycloheximide (Figure 4). Although under conditions where Rpm2p protein levels are very high it was unclear whether the presence of Rpm2p in P bodies or the high levels of expression stabilize P bodies. To determine whether stabilization of P bodies against cycloheximide can occur when fusion protein is expressed under an endogenous RPM2 promoter, we exposed cells for 30 min to cycloheximide (100 μg/ml) in growth medium. Figure 9 shows that the typical localization of both Dcp2p-RFP and GFP-Rpm2p to discrete cytoplasmic foci is not observed after cycloheximide treatment, indicating that the presence of GFP-Rpm2p in P bodies is not sufficient to prevent dissociation of P bodies by cycloheximide.

To determine other requirements for Rpm2p localization to P bodies, we transformed GFP-Rpm2p expressing plasmid under control of the RPM2 promoter into Δlsml and Δxrn1 deletion strains (BY4741 background), lacking individual components of P bodies. We found that GFP-Rpm2p colocalizes with Dcp2p-RFP in P bodies in both mutants. This result indicates that defects in either decapping (Δlsml cells) or the 5’ to 3’ mRNA degradation (Δxrn1 cells) do not affect the association of Rpm2p with P bodies.

**DISCUSSION**

This work presents evidence that Rpm2p localizes to cytoplasmic processing bodies. Moreover, we found that overexpression of Dhh1p, a known component of P bodies, in addition to Pablp1, a poly (A)-binding protein, suppresses temperature-sensitive growth of the rpm2-100 mutant. These data indicate that Rpm2p has a role in cytoplasmic mRNA metabolism, in addition to its role in tRNA processing inside the mitochondria.

P bodies have been described both in yeast and mammalian cells. They contain the decapping enzyme composed of Dcp1p and Dcp2p, the conserved 5’-3’ exonuclease Xrn1p, the 5m7G cap-binding protein Lsm1-Lsm7, the deadenylase hCcr4, the helicase Dhh1p, the enhancer of decapping Edc3p, Pat1p, the translational repressor, in addition to mRNAs associated with P bodies (33,36–38). In addition, in mammalian cells, P bodies contain components of the silencing complex, such as microRNAs and argonaute protein (39,40), the eIF4E-binding protein 4-ET, three autoantigens, GW182, Ge-l and RAP55 (41–43), and several RNA-binding proteins including TTP, BRF1, and CPEB (44). The mRNAs associated with P bodies are not actively involved in translation; however, they are required for the assembly...
mRNA remodeling through decapping is necessary for cell growth (47, 26). These observations suggest that stimulation of mRNA decapping, also have defects in respiration (47). The mutants lacking either DHH1 component of decapping enzyme, do not grow by respiratory growth defects. Cells that lack Dcp1p, a major protein of mRNA that can regulate mRNA decapping (27, 28), suppresses temperature-sensitive growth of the rpm2-100 mutant.

DCP2 (formerly known as PAB1) was originally identified as a suppressor of a nuclear petite mutant (petite mutants do not grow by respiration) (Tzagoloff, GenBank, accession #L43065). Later reports revealed that a variety of mutants defective in decapping also show respiratory growth defects. Cells that lack Dcp1p, a major component of decapping enzyme, do not grow by respiration at any temperature, but do grow by fermentation (47). The mutants lacking either DHH1 or EDC1 that stimulate mRNA decapping, also have defects in respiratory growth (47, 26). These observations suggest that mRNA remodeling through decapping is necessary for growth on respiratory carbon sources. Two different RPM2 mutants, rpm2-ΔC and rpm2-100 described previously (6, 7) have defects in the utilization of non-fermentable carbon sources. The rpm2-100 cells maintain normal mitochondrial RNAse P activity but grow extremely slowly on respiratory medium, due to a defect in the translation of all three mitochondrially encoded subunits of cytochrome c oxidase (7). The rpm2-ΔC mutant has a very peculiar property. If the ΔC mutation is introduced into cells that are grown on glucose, the cells lose mitochondrial DNA at a very high frequency and subsequently cannot grow by respiration. However, if the mutation is introduced into cells during respiratory growth, the cells continue to respire, albeit slowly due to defects in the processing of RNA subunit of mitochondrial RNAse P (6), indicating that the loss of mtDNA is conditional and depends on the carbon source. Therefore, if Rpm2p has a role in mRNA decapping, it might affect decapping under different growth conditions. Alternatively, Rpm2p may control decapping of specific mRNAs that are essential during a switch from a fermentable to non-fermentable carbon source. In addition, the same activity of Rpm2p may play a role in adaptation to high temperature and other stresses. For instance, the rpm2-100 mutant cells cannot withstand high temperatures (Figure 1), and cannot tolerate loss of mtDNA (7) but wild-type cells can lose mtDNA and are more resistant to different stresses, including high temperature (11).

We cannot exclude the possibility that Rpm2p possesses some nuclease activity in P bodies by itself or in combination with either protein or RNA, since in the mitochondria it functions as an endonuclease together with Rpm1r, the RNA subunit (2). Many eukaryotic mRNAs can be degraded by endonucleolytic cleavage (for references, see review in 48). In addition, RNA-mediated gene silencing involves endonucleolytic cleavage (for review, see 49), and can occur in P bodies in mammalian cells (39, 40).

The findings that Rpm2p can localize to P bodies and the isolation of PAB1 and DHH1 genes as high-copy suppressors of rpm2-100 suggest different roles for Rpm2p in RNA metabolism. It is surprising that two proteins apparently performing opposite functions in the cell can suppress rpm2-100 when overexpressed. In contrast to Pab1p, which stimulates translation complex assembly, Dhh1p promotes recruitment of mRNA to a repressed state (29). However, under certain stresses the human homolog of Dhh1p, rck/p54, localizes to stress granules, the cytoplasmic foci where translation preinitiation complexes assemble on mRNA and accumulate with various mRNA-binding proteins, including Pab1p (50, 35). These observations suggest that under some conditions both Dhh1p and Pab1p participate in translational silencing and mRNA storage. Although stress granule formation was not reported in the yeast S. cerevisiae, similar structures containing translation initiation complex eIF3 were found in the fission yeast Schizosaccharomyces pombe (51).

While both Dhh1p and Pab1p are involved in translational control, only overexpression of Pab1p suppresses the rpm2 deletion (personal communication). Pab1p participates in many aspects of mRNA metabolism such as 3’ end processing, translation and decay (52–56). Translational enhancement by Pab1p is mediated by the binding of Pab1p to initiation factor eIF4G, which is a part of the mRNA cap-binding complex eIF4F (19). Binding of Pab1p stabilizes mRNA in a translation-dependent manner (56), and mutations in either the PAB1 or IF4F complex lead to destabilization of mRNA (57, 58). Recent observations show that Pab1p rapidly shuttles between the nucleus and the cytoplasm and is required for the efficient export of mRNA out of the nucleus (22, 23). Since Rpm2p has RNA-binding activity and can localize to the nucleus and the cytoplasm, it may also have a role in mRNA export and assembly of mRNPs into P bodies. The most interesting finding was that overexpression of GFP-Rpm2p prevents the disappearance of P bodies that occurs when translation elongation is inhibited by cycloheximide. This effect is independent of the growth stage because it was observed in cultures at both low and high cell densities. Usually all known components of P bodies dissociate upon cycloheximide treatment in both yeast and mammalian cells (33, 35, 45). Yeast P bodies contain mRNAs in equilibrium with polysomes and cycloheximide treatment, which prevents mRNAs
from exiting translation and entering P bodies, leads to the disappearance of P bodies (46). In contrast, inhibition of translation initiation by genetic means in yeast or by silencing in mammalian cells, results in increase in P bodies (33,45,29,35,36). For instance, overexpression of translational repressors, such as Dhh1p and Pat1p from an inducible galactose promoter represses translation, promotes dissociation of mRNA from polysomes and facilitates assembly of mRNAs into P bodies (29). This leads to a dramatic increase in P body abundance. However, overexpression of GFP-Rpm2p under the same conditions (expression from a galactose-inducible promoter in the same yeast genetic background used in the study by Coller and Parker (29)) does not impact the number and size of P bodies (Figure 2), arguing against a role for Rpm2p in translational repression, and suggesting that stabilization of P bodies against cycloheximide treatment involves some other mechanism. Since Rpm2p is also nuclear, it is plausible that Rpm2p may have a role in the increased flow of mRNAs directly to P bodies before mRNAs enter the translation apparatus. If this were true, stabilization of P bodies could occur, independent of those mRNAs that are trapped on polysomes upon cycloheximide treatment.

Evidence is also presented here that cells expressing GFP-Rpm2p have lower ubiquitin levels. Ubiquitin levels are important for a proper balance between all ubiquitin-dependent processes, and analysis of ubiquitin profiles in a variety of yeast proteasome mutants suggests the existence of a homeostatic mechanism that maintains free ubiquitin levels within a certain range (59). A decrease in ubiquitin levels to about half of the wild-type level, compromises degradation of all substrates tested (59,60). Although the mechanism of the ubiquitin depletion in GFP-Rpm2p-expressing cells is unclear, it is possible that depletion in free ubiquitin levels may reduce the availability of ubiquitin for conjugation and this reduction contributes to increased stability of some proteins involved in P body formation and/or maintenance. Consistent with this, we found that proteasome mutants, umpl-2 and pre4-2, which are defective either in proteasome maturation or function, respectively, stabilize P bodies under conditions where all known components dissociate from P bodies. Moreover, P bodies appear larger in proteasome mutants compared to wild-type under normal growth conditions. These observations, combined with previous work which demonstrated that the essential role of Rpm2p can be bypassed by inhibiting proteasome function (13), strongly argues for a genetic interaction amongst Rpm2p, the ubiquitin-proteasome pathway and P bodies.

This study identified Rpm2p as a new component of P bodies. It is clear that Rpm2p has RNA-binding activity and therefore such binding activity may be important for its P body function. The observation that Rpm2p localizes to the nucleus and activates gene expression (9) coupled with its presence in P bodies, suggests that Rpm2p may provide a link between mRNA transcription and turnover/storage. The connection reported between proteasome function and P bodies implies that inhibition of proteasome activity may change the abundance of some mRNAs post-translationally, due to changes in the stability of P bodies. If the same holds for mammalian cells, this may be of medical relevance since proteasome inhibitors are being tested and used in anticancer therapy (61).

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REFERENCES


