Published online 31 January 2007 *Nucleic Acids Research, 2007, Vol. 35, No. 4* **1301–1311**
doi:10.1093/nar/gkm023

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

Vilius Stribinskis* and Kenneth S. Ramos

Department of Biochemistry and Molecular Biology and Center for Genetics and Molecular Medicine, School of Medicine, University of Louisville, Louisville, KY, USA

Received June 21, 2006; Revised December 21, 2006; Accepted January 3, 2007

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).

*To whom correspondence should be addressed. Tel: +1 502 852 7368; Fax: +1 502 852 3659; Email: v0stri01@louisville.edu

© 2007 The Author(s).
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/2.0/uk/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

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 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 *BamH*I 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 *Xho*I/*BamH*I 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 *BamH*I/*Sac*I 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 synthetic 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 [^{35}S-]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.

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 treated with anti-ubiquitin antibodies at 1 μg/ml (15). The affinity-purified rabbit polyclonal anti-ubiquitin antibody was a gift from Arthur Haas (Medical College of Wisconsin). The anti-GFP polyclonal were diluted 1:5000 (Molecular Probes); the anti-Rpm2p antibodies (7) were used at 1:2000 dilution.

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 Axioscope 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

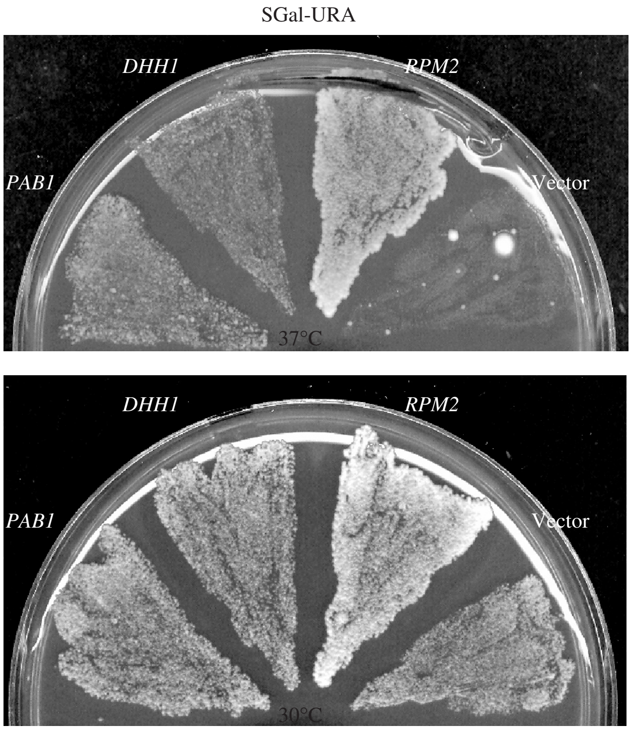

Figure 1. Growth of *rpm2-100* strains. The *rpm2-100* transformants carrying either *RPM2*, *DHH1* or *PAB1* genes under expression of *GAL1* promoter or vector alone were plated on synthetic plates containing galactose and scored for growth at 30 and 37°C.

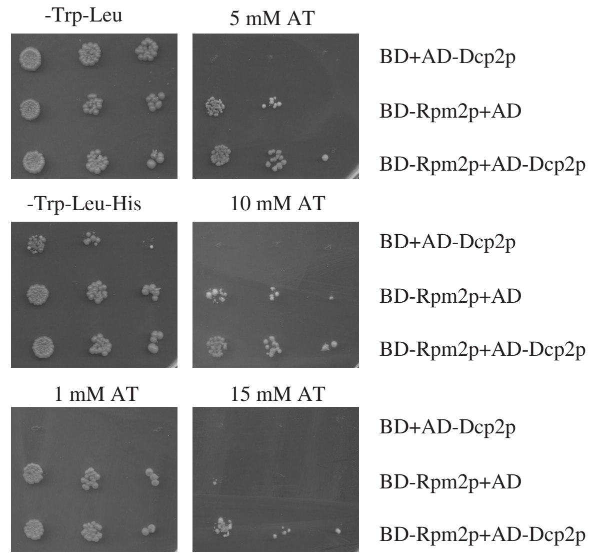

Figure 2. Rpm2p interacts with Dcp2p in the yeast two-hybrid assay. Yeast CG1945 strains expressing the combination Gal4p BD alone or fusion, and Gal4p AD alone or fusion were spotted on SD-Trp-Leu and SD-Trp-Leu-His (in the absence or presence of aminotriazole, AT) plates and incubated until visible colonies were formed.

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 in an independent genetic screen (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 3-aminotriazole (AT), a competitive inhibitor of the *HIS3* gene product. Figure 2 shows that cells expressing both the AD-Dcp2p and BD can grow on medium 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 1mM 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 Δ*dcp2* mutant to grow, albeit poorly at all temperatures (32). To determine whether Rpm2p colocalizes with Dcp2p, the yeast strain yRP1358 was transformed with *GFP-RPM2* 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 cytoplasmic 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 transformants examined by

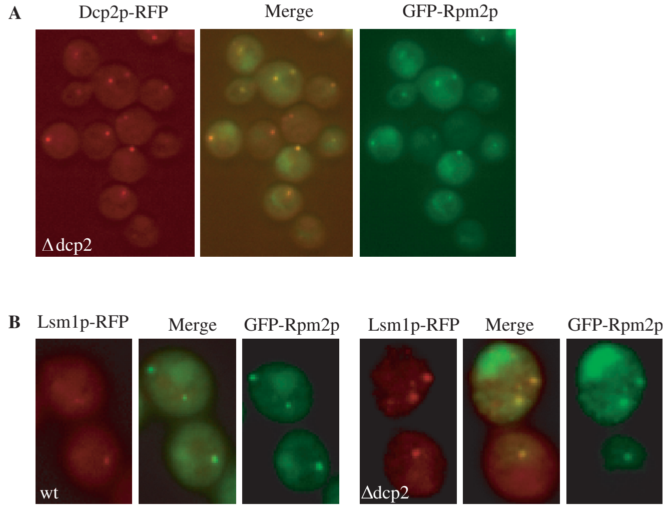

Figure 3. Rpm2p and Dcp2p colocalize to P bodies. Cells carrying both GFP-*RPM2* and *DCP2*-RFP in Δ*dcp2* strain (**A**); and GFP-*RPM2* and *LSM1*-RFP in either wt or Δ*dcp2* strain (**B**), were grown in selective medium in the presence of galactose for 6 h, fixed and fluorescence determined using microscope.

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 Dcp2p-RFP to P bodies is not observed after cycloheximide treatment. In contrast, Dcp2p-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 $OD_{600} = 0.5$ (early-log phase) to $OD_{600} = 4.0$ (end of log phase), both Dcp2p and Rpm2p fluorescent-tagged fusions were present in P bodies after cycloheximide

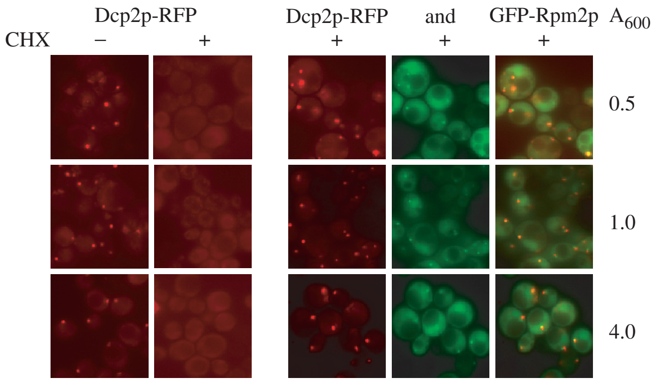

Figure 4. Disassembly of P bodies by translational inhibitor cycloheximide (CHX) does not occur in cells overexpressing GFP-Rpm2p. Cells expressing either Dcp2p-RFP or both, Dcp2p-RFP and GFP-Rpm2p were grown in galactose synthetic medium and P bodies visualized at different cell culture densities after incubation with or without CHX for 30 min. The different cell densities at A_{600} are indicated at the right side. For observation, cells were washed three times in water with or without cycloheximide without fixation.

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).

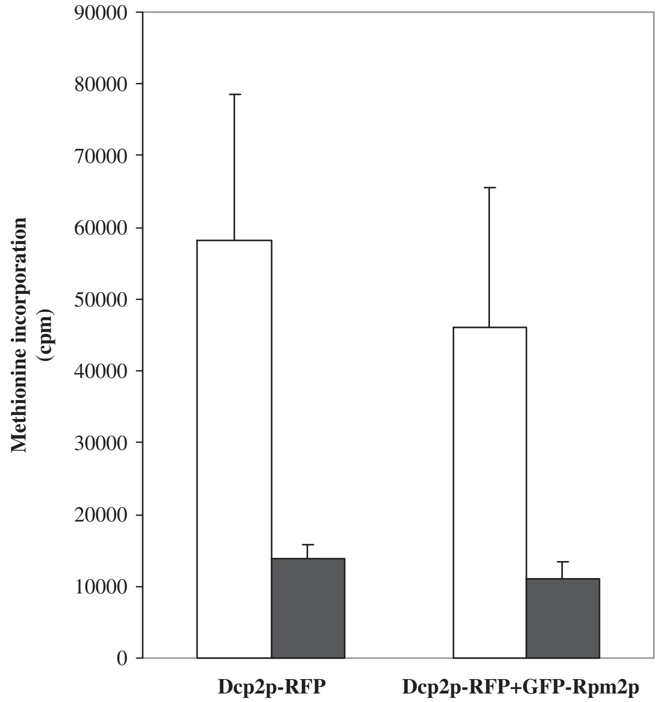

Figure 5. Inhibition of protein synthesis by cycloheximide. Equal numbers of mid-log phase cells, expressing either Dcp2p-RFP or both Dcp2p-RFP and GFP-Rpm2p, pretreated for 30 min with 100 µg/ml cycloheximide, were incubated with ^{35}S-labeled methionine for 30 min, without (open bars) or with (black bars) cycloheximide pretreatment for 30 min. TCA-insoluble material was measured by scintillation counting. Columns represent an average mean of the representative experiment performed in triplicate of three biological samples.

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). We made several observations (Figure 6). First, some P bodies are substantially larger in proteasome mutants compared to wild-type cells under normal growth conditions, indicating that the proteasome impacts the size of P body. Second, inhibition of translation elongation with cycloheximide does not eliminate P bodies in a majority of cells containing either proteasome mutant. Third, P bodies are present in the absence of Rpm2p, suggesting that Rpm2p itself is not required for the formation of P bodies, at least under conditions when proteasome activity is reduced. Together these results provide, for the first time, a link between proteasome function and P bodies.

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

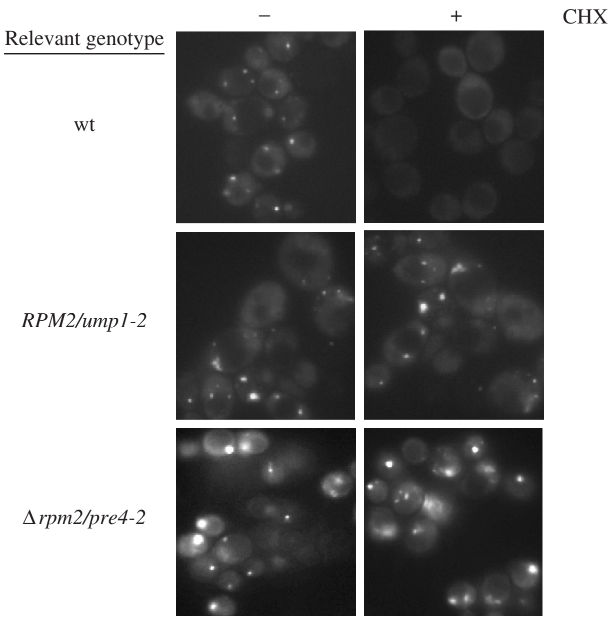

Figure 6. P bodies are affected by proteasome mutants. The wild-type strain, a strain carrying a mutation in *UMP1*, and a strain lacking *RPM2* but carrying a mutation in the *PRE4* all expressing Dcp2p-RFP were grown in glucose synthetic medium and P bodies visualized before and after incubation with cycloheximide for 30 min.

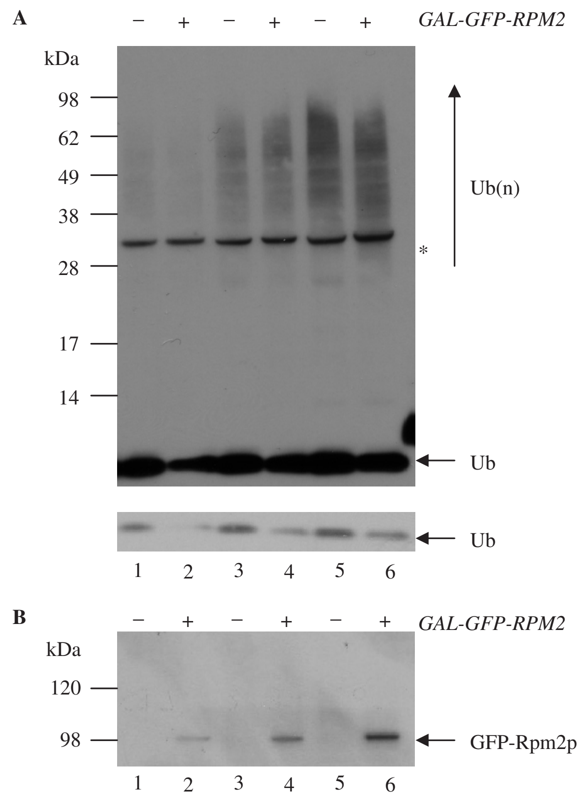

Figure 7. Depletion of ubiquitin in cells expressing GFP-Rpm2p. (**A**) Cells expressing either Dcp2p-RFP or Dcp2p-RFP and GFP-Rpm2p were grown in synthetic galactose medium in the exponential phase, and total protein extracts were analyzed by Western analysis with anti-ubiquitin antibodies after gel-separation and transfer to the membrane. Increasing amounts of protein extracts were used: 2 µg, lanes 1 and 2; 4 µg, lanes 3 and 4; 8 µg, lanes 5 and 6. ∗, unknown protein recognized by anti-ubiquitin antibodies serves as a loading control. A part of the membrane where free ubiquitin is detected was underexposed (bottom panel). (**B**) The same membrane was reprobed with anti-GFP antibodies.

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 *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.

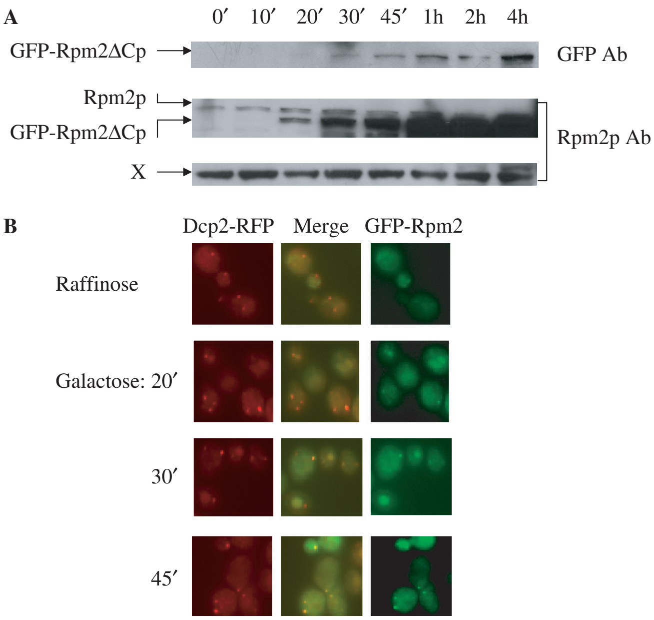

Figure 8. Kinetics of the induction and localization of GFP-Rpm2p fusion protein. (**A**) Western analysis of GFP-Rpm2p fusion protein before and after addition of galactose. The protein was detected using both anti-GFP and Rpm2p antibodies. Rpm2p shows the migration of endogenous Rpm2p. X, unknown protein recognized by anti-Rpm2p antibodies serving as a loading control. (**B**) Kinetics of GFP-Rpm2p colocalization with Dcp2p-RFP before and after addition of galactose.

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 Δ*lsm1* and Δ*xrn1* 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 (Δ*lsm1* cells) or the 5′ to 3′ mRNA degradation (Δ*xrn1* 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

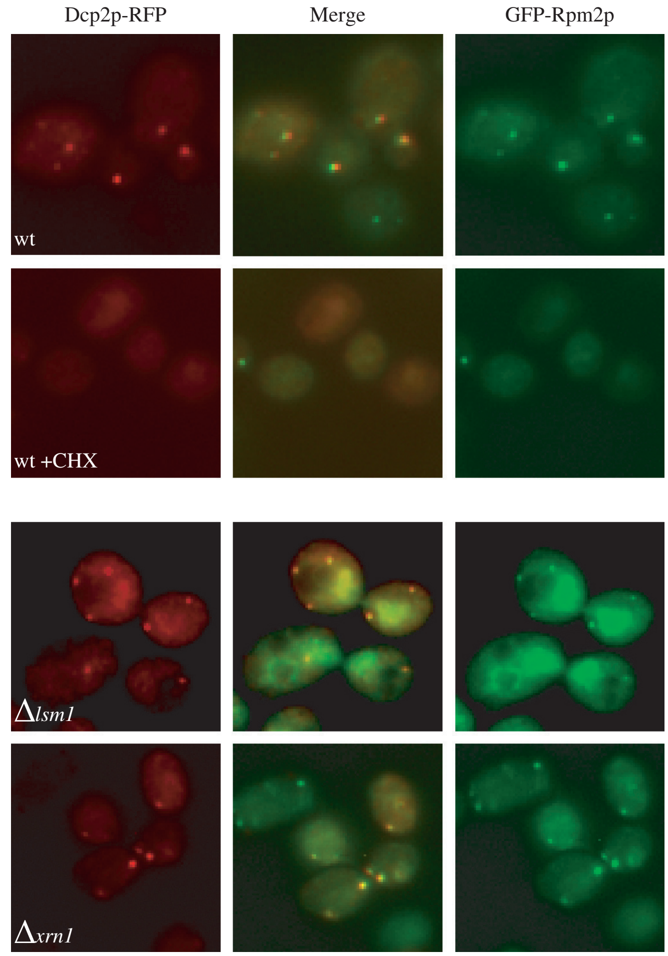

Figure 9. GFP-Rpm2p localization when expressed from its endogenous promoter. GFP-Rpm2p is observed in P bodies in the absence but not the presence of cycloheximide (CHX) (two upper panels). Localization of GFP-Rpm2p to P bodies does not require Lsm1p or Xrn1p, other components of P bodies (two bottom panels). The Dcp2p-RFP was used as a P body marker in colocalization studies.

that overexpression of Dhh1p, a known component of P bodies, in addition to Pab1p, 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 Sm-like proteins 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-1 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 of P bodies (45). The P bodies are the sites where mRNA decapping and degradation can occur, since mRNA decay intermediates, when trapped during degradation, are localized in P bodies (33). In addition, the formation of a P body is important for control of translational repression (29). The movement of mRNA from P bodies to the polysomes has been documented recently (46), indicating that P bodies are dynamic sites where mRNA can be either degraded or stored until appropriate conditions allow translation to resume.

Although we recently demonstrated that Rpm2p acts as transcriptional activator in the nucleus when not targeted to the mitochondria (9), the presence of Rpm2p in P bodies invokes a role in mRNA remodeling in the cytoplasm. Therefore, Rpm2p may function to coordinate transcription and mRNA decay. Several observations indicate that Rpm2p may directly interact with Dcp2p, a catalytic subunit of the decapping enzyme in P bodies. First, Rpm2p interacts with Dcp2p in affinity capture experiments (30,31). Second, as shown here, Rpm2p interacts with Dcp2p in the yeast two-hybrid system. Third, overexpression of Dhh1p, a component of P bodies that can regulate mRNA decapping (27,28), suppresses temperature-sensitive growth of the *rpm2-100* mutant.

DCP2 (formerly known as *PSU1*) 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, *ump1-2* 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-transcriptionally, 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).

ACKNOWLEDGEMENTS

We are thankful to Nancy Martin for her continuous interest and support of this work and for her advice; Anthony Bretscher for the yeast cDNA library; Roy Parker for strains and plasmids; Arthur Haas for affinity-purified polyclonal anti-ubiquitin antibody; Yong Li and Steven Ellis for helpful discussions; and Marlene Steffen for the help with Western analysis. Funding to pay the Open Access publication charge was provided by the Department of Biochemistry and Molecular Biology, School of Medicine, University of Louisville.

Conflict of interest statement. None declared.

REFERENCES

1. Morales,M.J., Dang,Y.L., Lou,Y.C., Sulo,P. and Martin,N.C. (1992) A 105-kDa protein is required for yeast mitochondrial RNase P activity. *Proc. Natl. Acad. Sci.U.S.A*, **89**, 9875–9879.
2. Dang,Y.L. and Martin,N.C. (1993) Yeast mitochondrial RNase P. Sequence of *RPM2* gene and demonstration that its product is a protein subunit of the enzyme. *J. Biol. Chem*., **268**, 19791–19796.
3. Underbrink-Lyon,K., Miller,D.L., Ross,N.A., Fukuhara,H. and Martin,N.C. (1983) Characterization of a yeast mitochondrial locus necessary for tRNA biogenesis. *Mol. Gen. Genet*., **191**, 512–518.
4. Hollingsworth,M.J. and Martin,N.C. (1986) RNase P activity in the mitochondria of *Saccharomyces cerevisiae* depends on both mitochondrial and nucleus-encoded components. *Mol. Cell. Biol*., **6**, 1058–1064.
5. Stribinskis,V., Gao,G-J., Sulo,P., Dang,Y.L. and Martin,N.C. (1996) Yeast mitochondrial RNase P RNA synthesis is altered in an RNase P protein subunit mutant: insights into the biogenesis of mitochondrial RNA-processing enzyme. *Mol. Cell. Biol*., **16**, 3429–3436.
6. Stribinskis,V., Gao,G-J., Sulo,P., Ellis,S.R. and Martin,N.C. (2001) Rpm2p: separate domains promote tRNA and Rpm1r maturation in *Saccharomyces cerevisiae* mitochondria. *Nucleic Acids Res*., **29**, 3631–3637.
7. Stribinskis,V., Gao,G-J., Ellis,S.R. and Martin,N.C. (2001) Rpm2, the protein subunit of mitochondrial RNase P in *Saccharomyces cerevisiae*, also has a role in the translation of mitochondrially encoded subunits of cytochrome c oxidase. *Genetics*, **158**, 573–585.
8. Stribinskis,V., Heyman,H-C., Ellis,S.R., Steffen,M.C. and Martin,N.C. (2005) Rpm2p, a component of yeast mitochondrial RNase P, acts as a transcriptional activator in the nucleus. *Mol. Cell. Biol*., **25**, 6546–6558.
9. Kassenbrock,C.K., Gao,G-J., Groom,K.R., Sulo,P., Douglas,M.G. and Martin,N.C. (1995) *RPM2*, independently of its mitochondrial RNase P function, suppresses *ISP42* mutant defective in mitochondrial import and essential for normal growth. *Mol. Cell. Biol*., **15**, 4763–4770.
10. Butow,R.A. and Avadhani,N.G. (2004) Mitochondrial signaling: the retrograde response. *Mol. Cell*, **14**, 1–15.
11. Traven,A., Wong,J.M., Xu,D., Sopta,M. and Ingles,C.J. (2001) Interorganelle communication. Altered nuclear gene expression profiles in a yeast mitochondrial DNA mutant. *J. Biol. Chem*., **276**, 4020–4027.
12. Zieler,H.A., Walberg,M. and Berg,P. (1995) Suppression of mutations in two *Saccharomyces cerevisiae* genes by the adenovirus E1A protein. *Mol. Cell. Biol*., **15**, 3227–3237.

13. Lutz,M.S., Ellis,S.R. and Martin,N.C. (2000) Proteasome mutants, *pre-4-2* and *ump1-2*, suppress the essential function but not the mitochondrial RNase P function of the *Saccharomyces cereviaise* gene *RPM2*. *Genetics*, **154**, 1013–1023.
14. Liu,H., Krizek,J. and Bretcher,A. (1992) Construction of a *GAL1*-regulated yeast cDNA expression library and its application to the identification of genes whose overexpression causes lethality in yeast. *Genetics*, **132**, 665–673.
15. Haas,A.L. and Bright,P.M. (1985) The immunochemical detection and quantification of intracellular ubiquitin-protein conjugates. *J. Biol. Chem.*, **260**, 12464–12473.
16. Strahl-Bolsinger,S. and Tanner,W. (1993) A yeast gene encoding a putative RNA helicase of the 'DEAD'-box family. *Yeast*, **9**, 429–432.
17. Adam,S.A., Nakagawa,T., Swanson,M.S., Woodruff,T.K. and Dreyfuss,G. (1986) mRNA polyadenylate-binding protein: gene isolation and sequencing and identification of a ribonucleoprotein consensus sequence. *Mol. Cell. Biol.*, **6**, 2932–2943.
18. Sachs,A.B., Bond,M.W. and Kornbery,R.D. (1986) A single gene from yeast for both nuclear and cytoplasmic polyadenylate-binding proteins: domain structure and expression. *Cell*, **45**, 827–835.
19. Tarun,S.Z., Jr, Wells,S.E., Deardorff,J.A. and Sachs,A.B. (1997) Translation initiation factor eIF4G mediated *in vitro* poly (A) tail dependent translation. *Proc. Natl. Acad. Sci. U.S.A.*, **94**, 9046–9051.
20. Wells,S.E., Hillner,P.E., Vale,R.D. and Sachs,A.B. (1998) Circularization of mRNA by eukaryotic translation initiation factors. *Mol. Cell*, **2**, 135–140.
21. Gray,N.K., Coller,J.M., Dickson,K.S. and Wickens,M. (2000) Multiple portions of poly(A)-binding protein stimulate translation *in vivo*. *EMBO J.*, **19**, 4723–4733.
22. Brune,C., Munchel,S.E., Fisher,N., Podtelejnikov,A.V. and Weis,K. (2005) Yeast poly (A)-binding protein Pab1 shuttles between the nucleus and the cytoplasm and functions in mRNA export. *RNA*, **11**, 517–531.
23. Dunn,E.F., Hammell,C.M., Hodge,C.A. and Cole,C.N. (2005) Yeast poly (A)-binding protein, Pab1, and PAN, a poly (A) nuclease complex recruited by Pab1, connect mRNA biogenesis to export. *Genes Dev.*, **19**, 90–103.
24. Groom,K.R., Heyman,H.C., Steffen,M.C., Hawkins,L. and Martin,N.C. (1998) *Kluyveromyces lactis SEF1* and its *Saccharomyces cerevisae* homologue bypass the unknown essential function, but not the mitochondrial RNase P function, of the *S. cerevisiae RPM2* gene. *Yeast*, **14**, 77–87.
25. Tanner,N.K. and Linder,P. (2001) DEAD/H box RNA helicases: from generic motors to specific dissociation functions. *Mol. Cell*, **8**, 251–262.
26. Hata,H., Mitsui,H., Liu,H., Bai,Y., Dennis,C.L., Shimizu,Y. and Sakai,A. (1998) Dhh1p, a putative RNA helicase, associates with the general transcription factors Pop2 and Ccr4p from *Saccharomyces cerevisiae*. *Genetics*, **148**, 571–579.
27. Coller,J.M., Tucker,M., Sheth,U., Valencia-Sanchez,M.A. and Parker,R. (2001) The DEAD box helicase, Dhh1p, functions in mRNA decapping and deadenylase complexes. *RNA*, **7**, 7117–1727.
28. Fisher,N. and Weis,K. (2002) The DEAD box protein Dhh1 stimulates the decapping enzyme Dcp1. *EMBO J.*, **21**, 2788–2797.
29. Coller,J. and Parker,R. (2005) General translational repression by activators of mRNA decapping. *Cell*, **122**, 875–886.
30. Gavin,A.C., Bosche,M., Krause,R., Grandi,P., Marzioch,M., Bauer,A., Schultz,J., Rick,J.M., Michon,A.M. *et al.* (2002) Functional organization of yeast proteome by systematic analysis of protein complexes. *Nature*, **415**, 141–147.
31. Gavin,A.C., Aloy,P., Grandi,P., Krause,R., Boesche,M., Marzioch,M., Rau,C., Jensen,L.J., Bastuck,S. *et al.* (2006) Proteome survey reveals modulatority of the yeast cell machinery. *Nature*, **440**, 631–636.
32. Dunckley,T. and Parker,R. (1999) The DCP2 protein is required for mRNA decapping in *Saccharomyces cerevisiae* and contains a functional MutT motif. *EMBO J.*, **18**, 5411–5422.
33. Sheth,U. and Parker,R. (2003) Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. *Science*, **300**, 806–808.
34. Tharun,S., Muhlrad,D., Showdhury,A. and Parker,R. (2005) Mutations in the *Saccharomyces cerevisiae LSM1* gene that affect mRNA decapping and 3′ end protection. *Genetics*, **170**, 33–46.
35. Wilczynska,A., Aigueperse,C., Kress,M., Dautry,F. and Weil,D. (2005) The translational regulator SPEB1 provides a link between dcp1 bodies and stress granules. *J. Cell Sci.*, **118**, 981–992.
36. Cougot,N., Babajko,S. and Serapin,B. (2004) Cytoplasmic foci are sites of mRNA decay in human cells. *J. Cell Biol.*, **165**, 31–40.
37. Ingelfinger,D., Arndt-Jovin,D.J., Luhrmann,R. and Achsel,T. (2002) The human Lam1-7 proteins colocalize with mRNA-degrading enzymes Dcp1/2 and Xrn1 in distinct cytoplasmic foci. *RNA*, **8**, 1489–1501.
38. Kshirsagar,M. and Parker,R. (2004) Identification of Edc3p as an enhancer of mRNA decapping in *Saccharomyces cerevisiae*. *Genetics*, **166**, 729–739.
39. Liu,J., Valenzia-Sanchez,M.A., Hannon,G.J. and Parker,R. (2005) Micro-RNA dependent localization of targeted mRNAs to mammalian P-bodies. *Nature Cell Biol.*, **7**, 719–723.
40. Sen,G.L. and Blau,H.M. (2005) Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies. *Nature Cell Biol.*, **7**, 633–636.
41. Eystathioy,T., Jakymiw,A., Chan,E.K., Serapin,B., Cougot,N. and Fritzler,M.J. (2003) The G182 protein colocalizes with mRNA degradation associated proteins hDcp1 and hLsm4 in cytoplasmic GW bodies. *RNA*, **9**, 1171–1173.
42. Yu,J.H., Yang,W.H., Gulick,T., Bloch,K.D. and Block,D.B. (2005) Ge-1 is a central component of the mammalian mRNA processing body. *RNA*, **11**, 1795–1802.
43. Yang,W.H., Yu,J.H., Gulick,T., Bloch,K.D. and Block,D.B. (2006) RNA-associated protein 55 (RAP55) localizes to mRNA processing bodies and stress granules. *RNA*, **12**, 547–554.
44. Kedersha,N., Stoecklin,G., Ayodele,M., Yacono,P., Lykke-Andersen,J., Fritzler,M.J., Scheuner,D., Kaufman,R.J., Golan,D.E. *et al.* (2005) Stress granules and processing bodies are dynamically linked sites of mRNA remodeling. *J. Cell Biol.*, **169**, 871–884.
45. Texeira,D., Sheth,U., Valenzia-Sanchez,M.A., Brengues,M. and Parker,R. (2005) Processing bodies require RNA for assembly and contain nontranslating mRNAs. *RNA*, **11**, 371–382.
46. Brengues,M., Texeira,D. and Parker,R. (2005) Movement of eukaryotic mRNAs between polysomes and cytoplasmic processing bodies. *Science*, **310**, 486–489.
47. Schwartz,D., Decker,C.J. and Parker,R. (2003) The enhancer of decapping proteins, Edc1p and Edc2p, bind RNA and stimulate the activity of the decapping enzyme. *RNA*, **9**, 239–251.
48. Coller,J. and Parker,R. (2004) Eukaryotic mRNA degradation. *Annu. Rev. Biochem.*, **73**, 861–890.
49. Tijsterman,M., Ketting,R.F. and Plasterk,R.H. (2002) The genetics of RNA silencing. *Annu. Rev. Genet.*, **36**, 489–519.
50. Anderson,P. and Kedersha,N. (2002) Stressful initiations. *J. Cell Sci.*, **115**, 3227–3234.
51. Dunand-Sauthire,I., Walker,C., Wilkinson,C., Gordon,C., Crane,R., Norbury,C. and Humphrey,T. (2002) Sum1, a component of the fission yeast eIF3 translation initiation complex, is rapidly relocalized during environmental stress and interacts with components of the 26S proteasome. *Mol. Biol. Cell*, **13**, 1626–1640.
52. Minvielle-Sebastia,L.P., Preker,P.J., Wiederkher,T., Strahm,Y. and Keller,W. (1997) The major yeast poly (A)-binding protein is associated with cleavage factor IA and functions in premessenger RNA 3′-end formation. *Proc. Natl. Acad. Sci. U.S.A.*, **94**, 7897–7902.
53. Morrissey,J.P., Deardorff,J.A., Hebron,C. and Sachs,A.B. (1999) Decapping of stabilized, polyadenylated mRNA in yeast pab1 mutants. *Yeast*, **15**, 687–702.
54. Otero,L.J., Ashe,M.P. and Sachs,A.B. (1999) The yeast poly (A)-binding protein Pab1p stimulates *in vitro* poly (A)-dependent and cap-dependent translation by distinct mechanisms. *EMBO J.*, **18**, 3153–3163.
55. Sachs,A.B., Sarnow,P. and Hentze,M.W. (1997) Starting at the beginning, middle, and end: translation initiation in eukaryotes. *Cell*, **89**, 831–838.

56. Coller,J.M., Gray,N.K. and Wickens,M.P. (1998) mRNA stabilization by poly (A) binding protein is independent of poly (A) and requires translation. *Genes Dev.*, **12**, 3226–3235.
57. Schwartz,D.C. and Parker,R. (1999) Mutations in translation initiation factors lead to increased rates of deadenylation and decapping of mRNAs in *Sacharomyces cerevisiae*. *Mol. Cell. Biol.*, **19**, 5247–5256.
58. Brown,J.T., Yang,X. and Johnson,A.W. (2000) Inhibition of mRNA turnover in yeast by xrn1 mutation enhances the requirement for eIF4E binding to the eIF4G and for proper capping of transcripts by Ceg1p. *Genetics*, **155**, 31–42.
59. Swaminathan,S., Amerik,A.Y. and Hochstrasser,M. (1999) The Doa4 deubiquitinating enzyme is required for ubiquitin homeostasis in yeast. *Mol. Biol. Cell*, **10**, 2583–2594.
60. Krsmanović,T. and Kölling,R. (2004) The HECT E3 ubiquitin ligase Rsp5 is important for ubiquitin homeostasis in yeast. *FEBS Lett.*, **577**, 215–219.
61. Richardson,P.G. and Mitsiades,C. (2005) Bortezomib: proteasome inhibition as an effective anticancer therapy. *Future Oncol.*, **1**, 161–171.