Pbp1 Is Involved in Ccr4- and Khd1-Mediated Regulation of Cell Growth through Association with Ribosomal Proteins Rpl12a and Rpl12b

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The Saccharomyces cerevisiae Pbp1 [poly(A)-binding protein (Pab1)-binding protein] is believed to be involved in RNA metabolism and regulation of translation, since Pbp1 regulates a length of poly(A) tail and is involved in stress granule (SG) formation. However, a physiological function of Pbp1 remains unclear, since the pbp1Δ mutation has no obvious effect on cell growth. In this study, we showed that PBP1 genetically interacts with CCR4 and KHD1, which encode a cytoplasmic deadenylase and an RNA-binding protein, respectively. Ccr4 and Khd1 modulate a signal from Rho1 in the cell wall integrity pathway by regulating the expression of RhoGEF and RhoGAP, and the double deletion of CCR4 and KHD1 confers a severe growth defect displaying cell lysis. We found that the pbp1Δ mutation suppressed the growth defect caused by the ccr4Δ khd1Δ mutation. The pbp1Δ mutation also suppressed the growth defect caused by double deletion of POPL2, encoding another cytoplasmic deadenylase, and KHD1. Deletion of the gene encoding previously known Pbp1-interacting factor Lsm12, Pbp4, or Mkt1 did not suppress the growth defect of the ccr4Δ khd1Δ mutant, suggesting that Pbp1 acts independently of these factors in this process. We then screened novel Pbp1-interacting factors and found that Pbp1 interacts with ribosomal proteins Rpl12a and Rpl12b. Similarly to the pbp1Δ mutation, the rpl12aΔ and rpl12bΔ mutations also suppressed the growth defect caused by the ccr4Δ khd1Δ mutation. Our results suggest that Pbp1 is involved in the Ccr4- and Khd1-mediated regulation of cell growth through the association with Rpl12a and Rpl12b.

Regulation of gene expression is achieved not only at the transcriptional level but also at the posttranscriptional level. Posttranscriptional regulation of gene expression includes the proper regulation of translation and mRNA degradation. The 5′ cap and 3′ poly(A) tail structures of mRNAs have important roles in both translation and mRNA degradation. Translation initiation is promoted by binding of the translation initiation complex eIF4F, which contains eIF4E, eIF4A, and eIF4G, to the 5′ cap structure. eIF4E directly binds to the 5′ cap structure, eIF4A is an RNA helicase, and eIF4G serves as a scaffold for the complex. Binding of the eIF4F complex to the 5′ cap structure recruits the 43S preinitiation complex, which includes the small ribosomal subunit, the initiator tRNA, and additional initiation factors (1). Translation initiation is also enhanced by the 3′ poly(A) tail and the poly(A)-binding protein that interacts with eIF4G.

Cytoplasmic degradation of mRNAs occurs by two general pathways, both of which are initiated by shortening of the 3′ poly(A) tail in a process referred to as deadenylation (2). Deadenylation is carried out by the Pan2/Pan3 complex as well as by the Ccr4/Pop2/Not complex in yeast. Following deadenylation, the mRNAs are decapped by the Dcp1/Dcp2 decapping enzyme and then subjected to 5′-to-3′ degradation by Xrn1 exonuclease. The deadenylated mRNAs are also subjected to 3′-to-5′ degradation by the exosome complex.

The yeast poly(A)-binding protein, Pab1, is relatively abundant and is present in both the nucleus and the cytoplasm of the cell (1, 3). The PAB1 gene is essential for cell growth on rich media, and deletion of PAB1 promotes misregulation of poly(A) addition, inhibits translation initiation and poly(A) shortening, and delays the onset of mRNA decay. A Pab1-binding protein, Pbp1, was identified as a protein that interacts with the C-terminal domain of Pab1 and was also shown to exist with both the translating and nontranslating pools of mRNAs (4, 5). The pbp1Δ mutation suppresses the lethality caused by the pab1Δ mutation (4). Pbp1 is yeast ortholog of human Ataxin 2, which is causal factor of spinal-cerebellar degeneration type 2. Both yeast Pbp1 and human Ataxin 2 localize to stress granules (SGs) and promote their formation (6, 7, 8). Pbp1 contains the Like-Sm domain (Lsm), Lsm-associated domain (LsmAD), and self-interacting region (4, 6, 8). Lsm of Pbp1 interacts with Lsm12 and Pbp4, relating to RNA processing (5). The self-interacting region of Pbp1 is needed for interaction with Pab1 and itself. Pab1 recruits Pan2 to the poly(A) tail, which results in poly(A) shortening. On the other hand, Pbp1 increases polyadenylation of the poly(A) tail by disturbing the Pab1-Pan2 interaction (5). Pbp1 was also reported to regulate expression of the HO gene, encoding an endonuclease involved in mating-type switching with Mkt1 (9). However, a physiological function of Pbp1 remains unclear, since the pbp1Δ mutation has no obvious effect on cell growth. Although Pbp1 was also shown to codistribute with polysomes and to exist with both the translating and nontranslating pools of mRNAs (5, 9), it also remains unclear how Pbp1 contributes to the translation.

Ccr4 is a cytoplasmic deadenylase and also a component of the Ccr4-Not complex (10). The ccr4Δ mutant shows pleiotropic phenotypes, including a weak cell lysis, a defect in checkpoint control, and a defect in cell cycle progression, and abnormal morphology...
pressed the growth defect of ccr4 mRNA, and deletion of CRT1 suppresses the checkpoint defect of the ccr4Δ mutant (14). For cell cycle progression, Ccr4 negatively regulates the WHI5 mRNA, and loss of WHI5 suppresses the cell cycle defect of the ccr4Δ mutant (12). We have found that Ccr4 negatively regulates the LRG1 mRNA encoding a GTPase-activating protein (GAP) for Rho1 in the cell wall integrity (CWI) pathway (15, 16). Loss of LRG1 suppressed the cell lysis of the ccr4Δ mutant. We also found that the ccr4Δ mutation causes a more severe cell lysis when combined with the deletion of the KHD1 gene, encoding an RNA-binding protein, and that Ccr4 together with Khd1 positively regulates expression of the ROM2 mRNA, encoding a guanine nucleotide exchange factor (GEF) for Rho1 (16). RNA-binding protein Khd1 associates with hundreds of mRNAs comprising almost 20% of the yeast's transcriptome, and a significant fraction of the potential Khd1 mRNA targets encode proteins localized to the cell periphery, such as the cell wall and plasma membrane, and also nuclear proteins involved in transcriptional regulation (17, 18).

In this study, we demonstrated that the pbp1Δ mutation suppressed the growth defect of ccr4Δ khd1Δ mutants. Deletion of the genes encoding previously known Pbp1-interacting factors Lsm12, Pbp4, and Mkt1 did not suppress the growth defect of the ccr4Δ khd1Δ mutant. We have found that Pbp1 interacts with the ribosomal large subunit, Rpl12a, and Rpl12b. Similarly to the pbp1Δ mutation, the rpl12aΔ and rpl12bΔ mutations also suppressed the growth defect caused by the ccr4Δ khd1Δ mutation. Our results suggest that Pbp1 is involved in the Ccr4- and Khd1-mediated regulation of cell growth through the association with Rpl12a and Rpl12b.

MATERIALS AND METHODS

Strains and general methods. Escherichia coli DH5α was used for DNA manipulations. The strains used in this study are described in Table 1. Standard procedures were followed for yeast manipulations (19). The media used in this study included rich medium (yeast extract-peptone-dextrose [YPD]), synthetic complete medium (SC), and synthetic minimal medium (SD) (19). SC media lacking amino acids or other nutrients (e.g., SC−Ura corresponds to SC lacking uracil) were used to select transformants. Recombinant DNA procedures were carried out as described previously (20).

Plasmids. The plasmids used in this study are described in Table 2. Plasmids pGBD-c1-PBP1 (amino acids [aa] 1 to 722), pGBD-c1-PBP1-n (aa 1 to 53), pGBD-c1-PBP1-Lsm (aa 54 to 130), pGBD-c1-PBP1-ad (aa 173 to 297), pGBD-PBP1-Lsm/ad (aa 298 to 722) were used for the yeast two-hybrid analysis. pGAD-c1-RPL12A, pGAD-c1-RPL12B, and pGAD-LSM12 were cloned from yeast two-hybrid libraries. Plasmid YCplac33-PBP1FLAG was used for the immunoprecipitation. Plasmids YCplac33-PBP1, YEpLac195-PBP1ΔLSM, YEpLac195-PBP1ΔAD, and YEpLac195-PBP1ΔLSMΔAD express the PBP1, PBP1ΔLSM, PBP1ΔLSMAD, and PBP1ΔLSMΔAD alleles, respectively. Plasmid YEp195-PAN2 express the PAN2 gene. Plasmids pCgLEU2, pCgHIS3, and pCgTRP1 are pUC19 carrying the Lsm12, LSM12myc, and LSM12myc untranslated region (UTR) and pFa6a-13myc-kanMX6.

Determination of cell lysis. Cell lysis was determined for aliquots of cell cultures as previously described (26) using propidium iodide staining. A minimum of 200 cells were counted for each sample.

Northern blot analysis. Total RNA was prepared from cells using Isogen reagent (Nippongene) and the RNasey minikit (Qiagen). RNA samples were separated by 1.5% denaturing agarose gel electrophoresis and transferred to nylon membrane. RNA was then hybridized using digoxigenin (DIG)-labeled antisense probe. The primer set j259 (ATGAT TCAAAATTCTCTGCTGTTA) and j260 (GCCAATATTTATGAAATTCCACAAT) was used to detect transcript containing LRG1. After washing and blocking, the membrane was incubated with alkaline phosphatase-conjugated anti-DIG antibody, and the signal was detected by enhanced chemiluminescence.

Western blot analysis. Extracts were prepared as described previously (17, 18). Extracts were subjected to SDS-PAGE on 8% acrylamide gels followed by electroblotting onto an Immobilon membrane (Millipore). To detect the tandem affinity purification-tagged proteins, the blots were blocked for 30 min at room temperature with TBS-M buffer (20 mM Tris-HCl [pH 7.5], 150 mM NaCl, 3% nonfat dry milk) and further incubated with 1:4,000-diluted peroxidase-antiperoxidase soluble complex (PAP) (Sigma) in TBS-M buffer overnight at 4°C. After three final washes with TBS buffer (20 mM Tris-HCl [pH 7.5], 150 mM NaCl), blots were developed with the enhanced chemiluminescence detection kit (Millipore). To detect hemagglutinin (HA)-tagged proteins, the membrane was incubated with anti-HA antibody (Santa Cruz Biotechnology Inc.; 1:2,000) and then with horseradish peroxidase (HRP)-labeled secondary antibody (Calbiochem; 1:4,000). To control for equal loading of the lanes, the blots were probed with anti-Mcm2 antibody (Santa Cruz Biotechnology Inc.; 1:1,000) and peroxidase-conjugated secondary antibody (Calbiochem; 1:3,000).

Yeast two-hybrid assays. Two-hybrid screening with pGBD-PBP1 and a yeast genomic two-hybrid library was done as previously described (9, 27). P69−4A harboring pGBD-PBP1 was transformed with the yeast two-hybrid library. Transformants were plated on SC−LeuTrpHis plates containing 1 mM 3-aminotriazole and incubated at 30°C for 4 days. The plates were replica plated to SC−LeuTrp Ade plates and continuously incubated at 30°C for 3 days. Twenty-three transformants showed a His+ Ade+ phenotype. The corresponding library plasmids were isolated from the transformants, and those that conferred the ability to interact with Pbp1 were confirmed by retransformation. Sequencing of the insert DNAs of the 23 recovered plasmids revealed that 18 contained the RPL12A gene, two contained the RPL12B gene, and three contained the LSM12 gene.

Immunoprecipitation of Pbp1-FLAG. Cells were grown in SC−Ura medium at 30°C to mid-log phase and harvested by centrifugation. The cells were washed twice in 400 mM potassium acetate, 2 mM EDTA, 0.1% Triton X-100, 5% glycerol) and re-suspended in XT buffer (20 mM Tris-HCl [pH 7.5], 20 mM potassium acetate, 2 mM EDTA, 0.1% Triton X-100, 5% glycerol) and resuspended in XT buffer containing protease inhibitors, phenylmethylsulfonyl fluoride (PMSF), aprotinin, and leupeptin. Glass beads were added, and the cells were broken by rigid vortexing at 4°C (4 times at 3,500 rpm for 30 s each). The supernatant was removed and centrifuged for 10 min at 5,000 × g. To immunoprecipitate Pbp1-FLAG, 200 μl of extract was incubated with anti-FLAG antibody (M2) coupled to protein G-Sepharose beads (20 μl; GE Healthcare) for 2 h at 4°C. Beads were washed three times with 400 μl XT buffer, and bound material was eluted with 50 μl elution buffer (0.1 μg/μl 3 × FLAG peptide in XT buffer) for 10 min at 4°C. Western blotting was performed using anti-FLAG antibody (M2).
TABLE 1 Strains used in this study

| Strain          | Genotype                                      | Source or reference |
|-----------------|-----------------------------------------------|---------------------|
| 10B             | MATa ade2 trp1 can1 leu2 his3 ura3 GAL psi¹   | 36                  |
| 10BD            | MATa/Mata ade2/ade2 trp1 can1 can1 leu2/leu2 his3/his3 ura3/ura3 | 36                  |
| 10BD-c163       | MATa/Mata ade2/ade2 trp1 can1 can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1Δ::CgTRP1 CCR4/cr4Δ::CgLEU2 | 16                  |
| 10BD-p163       | MATa/Mata ade2/ade2 trp1 can1 can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1Δ::CgTRP1 POP2/pop2Δ::CgLEU2 | This study          |
| 10BD-c163-p1    | MATa/Mata ade2/ade2 trp1 can1 can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1Δ::CgTRP1 CCR4/cr4Δ::CgLEU2 | This study          |
| 10BD-c163-11    | MATa/Mata ade2/ade2 trp1 can1 can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1Δ::CgTRP1 CCR4/cr4Δ::CgLEU2 | This study          |
| 10BD-p163-p1    | MATa/Mata ade2/ade2 trp1 can1 can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1Δ::CgTRP1 CCR4/cr4Δ::CgLEU2 | This study          |
| 10BD-d163-p1    | MATa/Mata ade2/ade2 trp1 can1 can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1Δ::CgTRP1 CCR4/cr4Δ::CgLEU2 | This study          |
| 10BD-c163-p1    | MATa/Mata ade2/ade2 trp1 can1 can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1Δ::CgTRP1 CCR4/cr4Δ::CgLEU2 | This study          |
| 10BD-c163-m1    | MATa/Mata ade2/ade2 trp1 can1 can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1Δ::CgTRP1 CCR4/cr4Δ::CgLEU2 | This study          |
| 10BD-c163-p4    | MATa/Mata ade2/ade2 trp1 can1 can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1Δ::CgTRP1 CCR4/cr4Δ::CgLEU2 | This study          |
| 10BD-c163-112   | MATa/Mata ade2/ade2 trp1 can1 can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1Δ::CgTRP1 CCR4/cr4Δ::CgLEU2 | This study          |
| 10BD-c163-12a   | MATa/Mata ade2/ade2 trp1 can1 can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1Δ::CgTRP1 CCR4/cr4Δ::CgLEU2 | This study          |
| 10BD-c163-12b   | MATa/Mata ade2/ade2 trp1 can1 can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1Δ::CgTRP1 CCR4/cr4Δ::CgLEU2 | This study          |
| 10BD-c163-pan2  | MATa/Mata ade2/ade2 trp1 can1 can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1Δ::CgTRP1 CCR4/cr4Δ::CgLEU2 | This study          |
| 10BD-c163-p1-pan2| MATa/Mata ade2/ade2 trp1 can1 can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1Δ::CgTRP1 CCR4/cr4Δ::CgLEU2 | This study          |
| c163-p1-1       | MATa ade2 trp1 can1 leu2 his3 ura3 | This study          |
| c163-p1-2       | Mata ade2 trp1 can1 leu2 his3 ura3 ccr4Δ::CgLEU2 | This study          |
| c163-p1-3       | Mata ade2 trp1 can1 leu2 his3 ura3 khd1Δ::CgTRP1 | This study          |
| c163-p1-4       | Mata ade2 trp1 can1 leu2 his3 ura3 pop2Δ::CgHIS3 | This study          |
| c163-p1-5       | Mata ade2 trp1 can1 leu2 his3 ura3 ccr4Δ::CgLEU2 pbp1Δ::CgHIS3 | This study          |
| c163-p1-6       | Mata ade2 trp1 can1 leu2 his3 ura3 khd1Δ::CgTRP1 pbp1Δ::CgHIS3 | This study          |
| c163-p1-7       | Mata ade2 trp1 can1 leu2 his3 ura3 khd1Δ::CgTRP1 ccr4Δ::CgLEU2 | This study          |
| c163-p1-8       | Mata ade2 trp1 can1 leu2 his3 ura3 khd1Δ::CgTRP1 ccr4Δ::CgLEU2 pbp1Δ::CgHIS3 | This study          |
| c163-p1-11      | Mata ade2 trp1 can1 leu2 his3 ura3 LRG1-3HA-LRG1 3’UTR::kanMX6 | This study          |
| c163-p1-12      | Mata ade2 trp1 can1 leu2 his3 ura3 LRG1-3HA-LRG1 3’UTR::kanMX6 | This study          |
| c163-p1-13      | Mata ade2 trp1 can1 leu2 his3 ura3 LRG1-3HA-LRG1 3’UTR::kanMX6 | This study          |
| c163-112-1      | Mata ade2 trp1 can1 leu2 his3 ura3 LSM2-12A::kanMX6 | This study          |
| c163-12a-1      | Mata ade2 trp1 can1 leu2 his3 ura3 RPL12A-1::kanMX6 | This study          |
| c163-12b-1      | Mata ade2 trp1 can1 leu2 his3 ura3 RPL12B-1::kanMX6 | This study          |
| P169-4A         | Mata ade2 trp1 can1 leu2 his3 ura3 PBP1::GAL::LEU2 | This study          |

RESULTS

The pbp1Δ mutation suppressed the growth defect of the cr4Δ khd1Δ mutant. Woolstencroft et al. have shown that cr4Δ mutant cells are sensitive to hydroxyurea (HU) and that Ccr4 functions in tolerance of replication stress (14). In the same paper, they have also shown that the deletion of CRT1 and PBPI, encoding the transcriptional repressor of the DNA damage-induced gene regulator and Pab1-binding protein, respectively, suppress the HU sensitivity of the cr4Δ mutant (14). We have previously shown that cr4Δ mutant cells show slower growth than wild-type cells on rich medium and that cr4Δ khd1Δ double mutant cells show a more severe growth defect than cr4Δ single mutant cells (Fig. 1A) (16). We then examined whether the deletion of CRT1 and PBPI also suppresses the growth defect of the cr4Δ single or cr4Δ khd1Δ double mutant. We performed a standard genetic analysis of diploid strains that were heterozygous for the cr4Δ, khd1Δ, and cr1Δ or pbp1Δ alleles. Tetrad analysis revealed that the cr4Δ pbp1Δ double mutant cells grew better than the cr4Δ single mutant cells and that the cr4Δ khd1Δ pbp1Δ triple mutant cells also grew better than the cr4Δ khd1Δ double mutant cells (Fig. 1A). While the cr4Δ khd1Δ double mutant cells failed to grow at elevated temperature (37°C), the cr4Δ khd1Δ pbp1Δ triple mutant cells could grow at 37°C (Fig. 1B). Since the cr4Δ khd1Δ double mutant shows severe cell lysis (16), the cr4Δ khd1Δ double mutant cells grew a little better on YPD sorbitol plates containing 1 M sorbitol as an osmotic stabilizer than on YPD plates. It should be noted that the pbp1Δ mutation suppressed the growth defect of the cr4Δ khd1Δ mutant on YPD and YPD sorbitol plates (Fig. 1C). On the other hand, deletion of CRT1 did not affect the growth of the cr4Δ single or cr4Δ khd1Δ double mutant (data not shown). Thus, the pbp1Δ mutation, but not the cr1Δ mutation, suppressed the growth defect of the cr4Δ khd1Δ mutant. We then confirmed this suppression by the pbp1Δ mutation using the PBPI
gene expressed on the plasmid. The cer4Δ khd1Δ pbp1Δ triple mutant cells harboring the PBPl gene on the plasmid grew slower than the cer4Δ khd1Δ pbp1Δ triple mutant cells harboring the empty vector (see Fig. 10). These results indicated that Pbp1 has an inhibitory role in cell growth of the cer4Δ khd1Δ mutant.

The pbp1Δ mutation only partially suppresses the cell lysis of cer4Δ khd1Δ mutants. The cer4Δ mutant shows a temperature-sensitive cell lysis (11), and the cer4Δ khd1Δ double mutant shows more severe cell lysis than the cer4Δ single mutant (16). Cell lysis of the cer4Δ khd1Δ double mutant was caused by the decreased Rho1 activity that was a result of the decreased expression of RhoGGEF and Rom2 and the increased expression of RhoGAP and Lrg1 (16). Since deletion of PBPl suppressed the growth defect of the cer4Δ single and cer4Δ khd1Δ double mutants, we expected that deletion of PBPl would also suppress the cell lysis phenotype of these mutants. Unexpectedly, deletion of PBPl only partially suppressed the cell lysis of the cer4Δ and cer4Δ khd1Δ mutants (Fig. 2). The pbp1Δ mutant itself did not show cell lysis. These results suggest that the pbp1Δ mutation does not suppress the growth defect of the cer4Δ khd1Δ mutant simply by removing cell wall biogenesis. This result is consistent with the observation that the cer4Δ khd1Δ pbp1Δ triple mutant cells grew better than the cer4Δ khd1Δ double mutant cells on YPD sorbitol plates (Fig. 1C).

We have previously shown that a deletion of LRGl suppresses the growth defect of khd1Δ cer4Δ mutant at elevated temperatures (37°C) (16). While the cer4Δ khd1Δ double mutant cells failed to grow at 37°C, the cer4Δ khd1Δ lrg1Δ triple mutant cells grew at 37°C. To compare the effect of the lrg1Δ mutation with that of the pbp1Δ mutation, we reexamined the growth of cer4Δ khd1Δ lrg1Δ triple mutant cells by tetrad analysis. We found that the cer4Δ khd1Δ lrg1Δ triple mutant cells grew a little better than the cer4Δ khd1Δ double mutant cells on YPD plates (Fig. 1D). The cer4Δ khd1Δ lrg1Δ triple mutant cells grew similarly to the cer4Δ khd1Δ double mutant cells on YPD sorbitol plates (Fig. 1E). This is in contrast to the cer4Δ khd1Δ pbp1Δ triple mutant cells growing better than the cer4Δ khd1Δ double mutant cells on both YPD and YPD sorbitol plates (Fig. 1A and C). Therefore, while the pbp1Δ mutation suppressed both the slow-growth phenotype at room temperature and the growth defect at 37°C of the cer4Δ khd1Δ double mutant, the lrg1Δ mutation suppressed only the growth defect at 37°C. Thus, the suppression by the pbp1Δ mutation was not identical to that by the lrg1Δ mutation, suggesting that the suppression by the pbp1Δ mutation might not involve the LRG1 gene.

We further examined the LRGl mRNA and Lrg1 protein levels in wild-type, cer4Δ khd1Δ double mutant, and cer4Δ khd1Δ pbp1Δ triple mutant cells. While the LRGl mRNA level was increased in the cer4Δ khd1Δ double mutant cells compared to wild-type cells, the pbp1Δ mutation did not decrease the LRGl mRNA level (Fig. 3A). The Lrg1 protein level was also increased in the cer4Δ khd1Δ double mutant cells compared to wild-type cells (Fig. 3B). The pbp1Δ mutation did not decrease the Lrg1 protein level. Taking the results together, the suppression by the pbp1Δ mutation might not involve the LRGl gene.

The pbp1Δ mutation suppresses the growth defect of the pop2Δ mutant but not that of the dhh1Δ mutant. We have previously showed that the dhh1Δ mutation also showed a synthetic growth defect with a deletion mutation in POP2, which encodes another catalytic subunit of cytoplasmic deadenylase (10, 16). The pop2Δ mutant cells show slow growth on rich medium, and the pop2Δ khd1Δ double mutant cells show more severe slow growth with cell lysis (Fig. 4A) (16). We then examined whether the deletion of PBPl also suppresses the growth defect of the pop2Δ single or pop2Δ khd1Δ double mutant. Tetrad analysis revealed that the pop2Δ pbp1Δ double mutant cells grew better than the pop2Δ

TABLE 2 Plasmids used in this study

| Plasmid                  | Relevant markers | Source or reference |
|-------------------------|------------------|---------------------|
| YCplac3                 | URA3, CEN-ARS    | 37                  |
| YCplac33-PBP1FLAG       | URA3, CEN-ARS, PBP1FLAG | This study |
| YEpplac195              | URA3, 2µ         | 37                  |
| YEpplac195-PAN2         | URA3, 2µ, PAN2   | This study          |
| YEpplac195-PBP1         | URA3, 2µ, PBP1   | This study          |
| YEpplac195-PBP1 ΔLSM    | URA3, 2µ, PBP1 ΔLSM | This study |
| YEpplac195-PBP1 ΔAD     | URA3, 2µ, PBP1 ΔLSM | This study |
| YEpplac195-PBP1 ΔLSM ΔAD| URA3, 2µ, PBP1 ΔLSM ΔLSMAD | This study |
| pGBD-c1                 | TRP1, 2µ, GAL4-BD sequence behind ADH1 promoter | 27 |
| pGAD-c1-PBP1            | BD-PBP1 sequence in pGAD-c1 | This study |
| pGAD-c1-PBP1-n          | BD-PBP1 (amino acids 1–53) sequence in pGAD-c1 | This study |
| pGAD-c1-PBP1-lsm        | BD-PBP1 (amino acids 54–130) sequence in pGAD-c1 | This study |
| pGAD-c1-PBP1-ad         | BD-PBP1 (amino acids 173–297) sequence in pGAD-c1 | This study |
| pGAD-c1-PBP1-lsm/ad     | BD-PBP1 (amino acids 54–297) sequence in pGAD-c1 | This study |
| pGAD-c1-PBP1-c          | BD-PBP1 (amino acids 298–722) sequence in pGAD-c1 | This study |
| pGAD-c1                 | LEU2, 2µ, GAL4-AD sequence behind ADH1 promoter | 27 |
| pGAD-c1-RPL12A          | AD-RPL12A sequence in pGAD-c1 | This study |
| pGAD-c1-RPL12B          | AD-RPL12B sequence in pGAD-c1 | This study |
| pGAD-c1-LSM12           | AD-LSM12 sequence in pGAD-c1 | This study |
| pClglEU2                | C. glabrata LEU2 in pUC19 | 21 |
| pClgHis3                | C. glabrata HIS3 in pUC19 | 21 |
| pClgTRP1                | C. glabrata TRP1 in pUC19 | 21 |
| pFA6a-3HA-kanMX6-LRG1-3 | 3HA-LRG1 3’ UTR-kanMX6 | This study |
| pFA6a-13myc-kanMX6      | 13myc-ADH 3’ UTR-kanMX6 | 23 |
single mutant cells and that the pop2Δ khd1Δ pbp1Δ triple mutant cells also grew better than the pop2Δ khd1Δ double mutant cells (Fig. 4A). While the pop2Δ khd1Δ double mutant cells failed to grow at elevated temperature (37°C), the pop2Δ khd1Δ pbp1Δ triple mutant cells could grow at 37°C (data not shown).

RNA helicase Dhh1 is a stimulator for the decapping enzymes Dcp1 and Dcp2 (2), and previous studies indicate that Dhh1 functions downstream of Ccr4 and Pop2 (11, 28). Since dhh1Δ mutant cells show the slow growth on rich medium (Fig. 4B), we next examined whether the deletion of PBPI also suppresses the growth defect of the dhh1Δ mutant. Tetrad analysis revealed that the dhh1Δ pbp1Δ double mutant cells did not grow better but grew worse than the dhh1Δ single mutant cells (Fig. 4B). The dhh1Δ single and dhh1Δ pbp1Δ double mutant cells failed to grow at elevated temperature (37°C) (data not shown). Thus, the pbp1Δ mutation suppressed the growth defect of the ccr4Δ and pop2Δ mutants but not that of the dhh1Δ mutant. These results suggest that Pbp1 acts downstream of Ccr4 and Pop2 but upstream of Dhh1.

The pbp1Δ mutation suppresses the growth defect of the ccr4Δ mutant in a PAN2-dependent and -independent manner. It has been suggested that Pbp1 is a negative regulator of poly(A) nuclease Pan2, since the shorter poly(A) tails in the pbp1Δ extracts are not observed in the absence of Pan2 (5). Therefore, the suppression of the ccr4Δ and khd1Δ mutations by the pbp1Δ mutation might be due to the shorter poly(A) tails caused by the pbp1Δ mutation. To examine the involvement of Pan2 in the pbp1Δ mutation-mediated suppression, we first examined whether overexpression of PAN2 suppressed the growth defect of the ccr4Δ single or ccr4Δ khd1Δ double mutant. If the pbp1Δ mutation increased PAN2 activity, overexpression of PAN2 could suppress the growth defect of the ccr4Δ single or ccr4Δ khd1Δ double mutant. We found that the ccr4Δ khd1Δ double mutant harboring the YEppAN2 plasmid grew better than the ccr4Δ khd1Δ double mutant without plasmid (Fig. 5A).

We then examined whether the PAN2 gene is required for the suppression by the pbp1Δ mutation. If PBPI was required for the suppression, the suppression by the pbp1Δ mutation would not be observed in the pbp1Δ background. Tetrad analysis revealed that the ccr4Δ khd1Δ pbp1Δ pan2Δ quadruple mutant grew worse than the ccr4Δ khd1Δ pbp1Δ triple mutant (Fig. 5B). Thus, the pan2Δ mutation prevented the efficient suppression of cell growth by the pbp1Δ mutation. However, we also found in the same tetrad analysis that the ccr4Δ khd1Δ pbp1Δ pan2Δ quadruple mutant grew better than the ccr4Δ khd1Δ pan2Δ triple mutant (Fig. 5B). The ccr4Δ pbp1Δ pan2Δ triple mutant grew a little better than the ccr4Δ pan2Δ double mutant. These results suggest that the pbp1Δ mutation suppressed the growth defect of the ccr4Δ mutant in both a PAN2-dependent and -independent manner.

Pbp1 functions in the Ccr4- and Khd1-mediated pathway independently of its binding partners Lsm12, Pbp4, and Mkt1. Pbp1 is reported to interact with Lsm12 and Pbp4, which function in the control of mRNA metabolism (4, 5, 29, 30, 31). Pbp1 is also reported to interact with Mkt1 in the regulation of HO expression (9). We next examined whether the mkt1Δ, pbp4Δ, or lsm12Δ mutation also suppresses the growth defect of the ccr4Δ khd1Δ mutant similarly to the pbp1Δ mutation. Tetrad analysis revealed that the ccr4Δ khd1Δ mkt1Δ, ccr4Δ khd1Δ pbp4Δ, and ccr4Δ
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TABLE 3 Colony sizes of mutants

| Relevant genotype | Colony size $^a$ |
|-------------------|-----------------|
| Wild type         | ++ + + + + + +   |
| khd1Δ             | ++ + + + + + +   |
| khd1Δ pbp1Δ       | ++ + + + + + +   |
| khd1Δ pan2Δ       | ++ + + + + + +   |
| pbp1Δ pan2Δ       | ++ + + + + + +   |
| pbp1Δ             | ++ + + + + + +   |
| pan2Δ             | ++ + + + + + +   |
| pan1Δ             | ++ + + + + + +   |
| lrg1Δ             | ++ + + + + + +   |
| lsm12Δ            | ++ + + + + + +   |
| mkt1Δ             | ++ + + + + + +   |
| pbp4Δ             | ++ + + + + + +   |
| pop2Δ pbp1Δ       | ++ + + + + + +   |
| rpl12aΔ           | ++ + + + + + +   |
| cr4Δ khd1Δ pbp1Δ  | ++ + + + + + +   |
| cr4Δ pbp1Δ pan2Δ  | ++ + + + + + +   |
| cr4Δ khd1Δ rpl12aΔ| ++ + + + + + +   |
| pop2Δ khd1Δ pbp1Δ | ++ + + + + + +   |
| cr4Δ              | ++ + + + + + +   |
| cr4Δ pan2Δ        | ++ + + + + + +   |
| dbh1Δ             | ++ + + + + + +   |
| pop2Δ             | ++ + + + + + +   |

$^a$To evaluate the cell growth of each strain, the size of each colony after 5 days of incubation at 25°C was measured. Colony sizes were classified from ++ + + + + + (for the wild type) to + (for the cr4Δ khd1Δ pan2Δ triple mutant, which had the smallest size in this study).

khd1Δ lsm12Δ triple mutant cells grew similarly to the cr4Δ khd1Δ double mutant cells (Fig. 6). Thus, unlike the pbp1Δ mutation, the mkt1Δ, pbp4Δ, or lsm12Δ mutation did not suppress the growth defect of the cr4Δ khd1Δ mutant. These results suggest that Pbp1 functions in the Ccr4- and Khd1-mediated pathway independently of Lsm12, Pbp4, and Mkt1.

Pbp1 interacts with components of ribosomal large subunit, Rpl12a and Rpl12b. Since the above data suggest that Pbp1 functions in the Ccr4- and Khd1-mediated pathway independently of Lsm12, Pbp4, and Mkt1, we speculated about the possibility that Pbp1 functions with a previously unknown binding partner(s). To find novel Pbp1-interacting factors, we performed yeast two-hybrid screening with Pbp1 as a bait. As a result, we identified two ribosomal proteins, Rpl12a and Rpl12b, as well as Lsm12 (Fig. 7A) (for details of the screening, see Materials and Methods). Rpl12a and Rpl12b are components of the ribosomal large subunit; Rpl12 is the yeast ortholog of human L11, and there are two types in budding yeast. Rpl12a and Rpl12b interact with the P0/P1/P2 complex, which is needed for ribosomal translocation, and regulate the stability of the P-protein stalk (32, 33).

To confirm that Pbp1 binds Rpl12a and Rpl12b in vivo, we performed immunoprecipitation analyses (Fig. 7B, C, and D). We constructed strains harboring a FLAG-tagged version of Pbp1, Pbp1-FLAG, and Myc-tagged versions of Rpl12a and Rpl12b, Rpl12amyD and Rpl12bmyD, as described in Materials and Methods, as well as Lsm12myc. When Pbp1-FLAG was immunoprecipitated by anti-FLAG antibody, we detected Rpl12a and Rpl12b proteins. We also detected Lsm12myc protein when Lsm12myc was immunoprecipitated by anti-Myc antibody. These results suggest that Pbp1 interacts with Rpl12a and Rpl12b and Lsm12.

FIG 2 cr4Δ khd1Δ double mutant cells show severe cell lysis. Results of a cell lysis assay in YPD medium are shown. Wild-type (c163-p1-1), cr4Δ (c163-p1-2), cr4Δ pbp1Δ (c163-p1-5), cr4Δ khd1Δ (c163-p1-7), khd1Δ cr4Δ pbp1Δ (c163-p1-8), and pbp1Δ (c163-p1-4) cells were grown in YPD medium to mid-log phase at 25°C and shifted to 37°C for 4 h. Cell lysis was monitored by propidium iodide staining. The graph represents averages from three independent cultures for each strain. Error bars depict the standard deviation. At least 200 cells were counted per strain.

FIG 3 LRG1 mRNA and Lrg1 protein levels are not affected by the pbp1Δ mutation. (A) LRG1 mRNA levels in wild-type, cr4Δ khd1Δ, and cr4Δ khd1Δ pbp1Δ cells. Wild-type (c163-p1-1), cr4Δ khd1Δ (c163-p1-7), and cr4Δ khd1Δ pbp1Δ (c163-p1-8) cells were cultured to mid-logarithmic phase in YPD medium at room temperature and collected, and total RNA was prepared. The LRG1 transcripts were quantified by Northern blotting as described in Materials and Methods. rRNA was included as a quantity control. (B) Lrg1-HA protein levels in wild-type, cr4Δ khd1Δ, and cr4Δ khd1Δ pbp1Δ cells. Wild-type LRG1-3HA-LRG1 3′ UTR (c163-p1-1), cr4Δ khd1Δ LRG1-3HA-LRG1 3′ UTR (c163-p1-7), and cr4Δ khd1Δ pbp1Δ LRG1-3HA-LRG1 3′ UTR (c163-p1-8) cells were cultured to mid-logarithmic phase in YPD medium at room temperature and collected, and total protein was prepared. Western blot analysis was performed to quantitate the levels of Lrg1-HA protein (upper panel) and Mcm2 protein (lower panel) as a quantity control.
Lsm12myc was coimmunoprecipitated, as detected by anti-myc antibody in Western blots (Fig. 7B). Similarly, Rpl12amyc and Rpl12bmyc were coimmunoprecipitated with Pbp1-FLAG (Fig. 7B). Similarly, Rpl12amyc and Rpl12bmyc were coimmunoprecipitated with Pbp1-FLAG (Fig. 7B).

Fig 4 Growth of the pop2Δ khd1Δ pbp1Δ and dhhlΔ khd1Δ pbp1Δ mutant strains. (A) Strain 10BD-p163-p1, which is heterozygous for the pop2Δ khd1Δ pbp1Δ alleles, was sporulated, and tetrads were dissected onto YPD plates containing 10% sorbitol. Growth after 5 days at 25°C is shown. Genotypes are indicated on the sides. More than 50 tetrads were dissected, and representative data are shown. (B) Strain 10BD-d163-p1, which is heterozygous for the dhhlΔ khd1Δ pbp1Δ alleles, was sporulated, and tetrads were dissected onto YPD plates containing 10% sorbitol. Growth after 5 days at 25°C is shown. Genotypes are indicated on the sides. More than 50 tetrads were dissected, and representative data are shown.

Fig 5 Growth of the pop2Δ khd1Δ pbp1Δ mutant strain. (A) Strain 10BD-c163-pan2, which is heterozygous for the pop2Δ khd1Δ pbp1Δ and pan2Δ alleles, containing YEpl195-PAN2 plasmid (YEPlPAN2) was sporulated, and tetrads were dissected onto YPD plates containing 10% sorbitol. Growth after 5 days at 25°C is shown. Genotypes are indicated on the sides. More than 50 tetrads were dissected, and representative data are shown. (B) Strain 10BD-c163-pan2, which is heterozygous for the pop2Δ khd1Δ pbp1Δ and pan2Δ alleles, containing YEpl195-PAN2 plasmid (YEPlPAN2) was sporulated, and tetrads were dissected onto YPD plates containing 10% sorbitol. Growth after 5 days at 25°C is shown. Genotypes are indicated on the sides. More than 50 tetrads were dissected, and representative data are shown. Relative colony sizes are listed in Table 3.

Fig 6 Growth of the ccr4Δ khd1Δ mkt1Δ, ccr4Δ khd1Δ pbp4Δ, and ccr4Δ khd1Δ lsm12Δ mutant strains. (A) Strain 10BD-c163-m1, which is heterozygous for the ccr4Δ khd1Δ and mkt1Δ alleles, was sporulated, and tetrads were dissected onto YPD plates containing 10% sorbitol. Growth after 5 days at 25°C is shown. Genotypes are indicated on the left. More than 50 tetrads were dissected, and representative data are shown. (B) Strain 10BD-c163-p4, which is heterozygous for the ccr4Δ khd1Δ and pbp4Δ alleles, was sporulated, and tetrads were dissected onto YPD plates containing 10% sorbitol. Growth after 5 days at 25°C is shown. Genotypes are indicated on the left. More than 50 tetrads were dissected, and representative data are shown. (C) Strain 10BD-c163-l12, which is heterozygous for the ccr4Δ khd1Δ and lsm12Δ alleles was sporulated, and tetrads were dissected onto YPD plates containing 10% sorbitol. Growth after 5 days at 25°C is shown. Genotypes are indicated on the left. More than 50 tetrads were dissected, and representative data are shown.
FIG 7 Interaction of Pbp1 with Rpl12a and Rpl12b. (A) Two-hybrid screening. Yeast strain PJ69-4A harboring GAL2p-ADE2 and GAL1p-HIS3 reporters was transformed with the indicated plasmids, and the transformants were streaked onto SC-Leu-Trp-His plates containing 1 mM 3-aminotriazole and incubated for 3 days at 30°C. The same results were obtained on SC-Leu-Trp-ade plates (data not shown). (B, C, and D) Coimmunoprecipitation analysis. The Lsm12myc, Rpl12amyc, and Rpl12bmyc strains were transformed with YCplac33 vector (untag) and YCplac33-PBP1FLAG (Pbp1FLAG). Pbp1FLAG protein was immunoprecipitated with anti-FLAG antibody coupled to protein G-Sepharose and eluted with FLAG peptides. The eluates (IP: anti-FLAG) were separated by SDS-10% PAGE, blotted, and probed with anti-FLAG or anti-myc antibody. Pbp1-Lsm12 interaction was used as a positive control. Yeast strains: LSM12-myc, c163-l12-1; RPL12A-myc, c163-12a-1; and RPL12B-myc, c163-12b-1.

7C and D). These results indicate that Pbp1 interacts with Rpl12a and Rpl12b in vivo.

The rpl12aΔ and rpl12bΔ mutations suppress the growth defect caused by the ccr4Δ khd1Δ mutation. We next examined whether the rpl12aΔ or rpl12bΔ mutation suppressed the growth defect of the ccr4Δ khd1Δ mutant. Tetrad analyses revealed that the ccr4Δ khd1Δ rpl12aΔ triple mutant cells grew better than the ccr4Δ khd1Δ double mutant cells (Fig. 8A). The ccr4Δ khd1Δ rpl12bΔ triple mutant cells also grew better than the ccr4Δ khd1Δ double mutant cells, although the rpl12bΔ mutant cells grew worse than the rpl12aΔ mutant cells (Fig. 8B). Thus, both the rpl12aΔ and rpl12bΔ mutations suppressed the growth defect of the ccr4Δ khd1Δ mutant. We could not evaluate whether the rpl12aΔ and rpl12bΔ mutations suppress the growth defect of the ccr4Δ single mutant, because the rpl12aΔ and rpl12bΔ single mutants showed slow growth (Fig. 8). These results suggest that Rpl12a and Rpl12b together with Pbp1 have a negative role in the cell growth of the ccr4Δ khd1Δ double mutant.

Both Rpl12a and Rpl12b bind to the Lsm domain and Lsm-associated domain of Pbp1. To reveal how Pbp1 regulates the cell growth through Rpl12a and Rpl12b, we examined the Rpl12 interaction region within Pbp1. Pbp1 contains Lsm, LsmAD, and the self-interacting region of the C terminus, and both Rpl12a and Rpl12b contain an HMM domain. We constructed various fragments of Pbp1 and then identified the interaction region of Rpl12a and Rpl12b by two-hybrid assay (Fig. 9). We found that the Lsm of Pbp1, but not the LsmAD, interacts with Rpl12a and Rpl12b. The region containing both the Lsm and the LsmAD of Pbp1 showed stronger interaction with Rpl12a and Rpl12b than the Lsm only (Fig. 9). These results suggest that Lsm is required for the binding of Pbp1 to Rpl12a and Rpl12b and that LsmAD enhances the binding of Lsm.

Lsm and LsmAD of Pbp1 are required for the Pbp1 function that inhibits growth of the ccr4Δ khd1Δ mutant. We then examined the necessity of the interaction of Pbp1 with Rpl12a and Rpl12b in the suppression of the ccr4Δ khd1Δ mutation. To address this question, we evaluated the effect of the Rpl12-interacting domains using PBP1 plasmids which lacked Lsm, LsmAD, and both domains. When the wild-type PBP1 gene was introduced into the ccr4Δ khd1Δ pBP1Δ mutant, the ccr4Δ khd1Δ pBP1Δ mu-
is known to confer a weak cell lysis that is caused by the increased expression of RhoGAP and Lrg1 (15, 16). The weak cell lysis phenotype is enhanced by the khd1Δ mutation, which confers the decreased expression of RhoGEF and Rom2, together with the ccr4Δ mutation (16). The ccr4Δ khd1Δ double mutant cells show a severe slow growth at room temperature and no growth with cell lysis at elevated temperature. While the lrg1Δ mutation suppressed the growth defect at 37°C, the lrg1Δ mutation only weakly suppressed the slow-growth phenotype of the ccr4Δ khd1Δ mutant on YPD plates. The lrg1Δ mutation did not suppress the slow-growth phenotype of the ccr4Δ khd1Δ mutant on YPD sorbitol plates. In contrast, the pbp1Δ mutation suppressed both the slow-growth phenotype on both YPD and YPD sorbitol plates at room temperature and the growth defect at 37°C of the ccr4Δ khd1Δ double mutant. Thus, the pbp1Δ mutation is unlikely to act to decrease the Lrg1 level or its activity to suppress the ccr4Δ khd1Δ double mutation. Consistently, the pbp1Δ mutation did not decrease the LRG1 mRNA and protein levels. Furthermore, while a deletion of LRG1 suppresses the growth defect of a dhh1Δ mutant at 37°C (our unpublished data), the pbp1Δ mutation did not suppress the growth defect of a dhh1Δ mutant at 37°C. Thus, the pbp1Δ-mediated suppression seems to involve genes other than LRG1. It has been reported that the deletion of CRT1 and PBPI suppresses the HU sensitivity of the ccr4Δ mutant (14). Our analysis has revealed that the pbp1Δ mutation, but not the crt1Δ mutation, suppressed the growth defect caused by the ccr4Δ khd1Δ double mutation. Furthermore, we found that unlike the case for LRG1 mRNA, the CRT1 mRNA level was not increased in the ccr4Δ mutants. In addition to the CRT1 mRNA, it has been reported that the WHI5 mRNA was negatively regulated by Ccr4. We also found that the WHI5 mRNA level was not increased in the ccr4Δ mutant. Thus, the pbp1Δ-mediated suppression seems to involve genes other than CRT1 and WHI5.

The rpl12aΔ and rpl12bΔ mutations as well as the pbp1Δ mutation suppressed the growth defect caused by the ccr4Δ khd1Δ double mutation, indicating that the Pbp1 and Rpl12 proteins have a negative effect on the cell growth in the ccr4Δ khd1Δ double mutant cells. Since LRG1 overexpression is toxic in the ccr4Δ single and the ccr4Δ khd1Δ double mutant cells, one could imagine that Lrg1 protein would be improperly upregulated by Pbp1 and Rpl12 proteins in these mutants. However, LRG1 overexpression was still toxic in the ccr4Δ pbp1Δ double mutant cells, and the pbp1Δ mutation did not decrease the LRG1 mRNA and protein levels. Again, Pbp1 and Rpl12 proteins regulate the expression of genes other than LRG1. We have previously found that a multi-copy SSD1 encoding an RNA-binding protein suppressed the growth defect caused by the ccr4Δ khd1Δ double mutation (16). Since the RNA-binding protein Ssd1 is involved in the regulation of many genes as well as Khd1 (17, 34, 35), the Pbp1 and Rpl12 proteins may also involve the expression of multiple genes in a negative effect on cell growth in the ccr4Δ khd1Δ double mutant cells. Since Rpl12a and Rpl12b stabilize the structure of the P-protein stalk by interacting with the P0/P1/P2 complex (32, 33), the rpl12aΔ and rpl12bΔ mutations as well as the pbp1Δ mutation decrease the translation of multiple target mRNAs that influence cell growth.

Previous reports demonstrated that Pbp1, as well as a subunit of cleavage and polyadenylation factor (CFI), Ref2, and a factor interacting with Ref2, Fih1, is required for the formation of a normal-length poly(A) tail on precleaved CYC1 pre-mRNA (5).
While the yeast extracts from ref2Δ mutant cells synthesize longer poly(A) tails than those synthesized by wild-type extracts, pbp1Δ and fir1Δ extracts synthesize shorter poly(A) tails (5). Since the shorter poly(A) tails in the pbp1Δ extracts are not observed in the absence of poly(A) nuclease Pan2, it has been suggested that Pbp1 is a negative regulator of Pan2 (5). Thus, the suppression of the ccr4Δ and khd1Δ mutations by the pbp1Δ mutation might be due to the shorter poly(A) tails caused by the pbp1Δ mutation. Indeed, we have found that overexpression of PAN2 suppressed the growth defect of the ccr4Δ khd1ΔΔ double mutants and that the pan2ΔΔ mutation prevented the efficient suppression of cell growth by the pbp1Δ mutation. On the other hand, we also found that the ccr4Δ khd1Δ pbp1Δ pan2Δ quadruple mutants grew better than the ccr4Δ khd1ΔΔ pan2ΔΔ triple mutants. Thus, the pbp1Δ mutation suppressed the growth defect of the ccr4Δ mutant in both a PAN2-dependent and -independent manner.

Since the rpl12aΔ and rpl12bΔ mutations as well as the pbp1Δ mutation suppressed the growth defect caused by the ccr4Δ khd1ΔΔ double mutation, Pbp1-1 mediated translation with Rpl12a and Rpl12b seems to involve the PAN2-independent suppression of the ccr4Δ khd1ΔΔ double mutation. The rpl12aΔ and rpl12bΔ mutant cells grow slower than wild-type cells, and the rpl12aΔ rpl12bΔ mutant cells show a more severe growth defect. However, the pbp1Δ mutant cells grow normally, and the pbp1Δ mutation does not affect the cell growth of the rpl12aΔ and rpl12bΔ mutant cells (data not shown). Thus, Pbp1 does not seem to be involved in normal ribosomal function of Rpl12 proteins. While we have genetic evidence that the rpl12aΔ and rpl12bΔ mutations as well as the pbp1Δ mutation suppressed the growth defect caused by the ccr4Δ khd1ΔΔ mutation, we have to further analyze the significance of the physical interaction between Pbp1 and Rpl12. On the other hand, the pbp1Δ dhh1ΔΔ double mutant cells grow worse than the dhh1ΔΔ single mutant cells. It has been reported that Pbp1 and Dhh1 overexpression inhibits cell growth and that both are involved in SG formation (7). Thus, Pbp1 might have an overlapping function with Dhh1 in translational control.

In this study, we found that Pbp1 interacts with Rpl12a and Rpl12b and that Pbp1 and Rpl12 proteins are involved in the Ccr4- and Khd1-mediated regulation of cell growth. What is the relevance of the regulation? We have previously shown that Pbp1 together with Mkt1 regulates HO expression (9). HO expression is also regulated posttranscriptionally by Mpt5/Puf5, Ccr4, Pop2, and Dhh1 (9, 25, 28). Although Mkt1 is not involved in the Ccr4- and Khd1-mediated regulation of cell growth, Pbp1 seems to have a positive role in the translational control of target mRNAs for Ccr4, such as HO mRNA. Thus, the Pbp1-mediated regulation of translation together with Rpl12a and Rpl12b may have an important role for target mRNAs for Ccr4.

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