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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 *pbp1*Δ 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 *pbp1*Δ mutation suppressed the growth defect caused by the *ccr4*Δ *khd1*Δ mutation. The *pbp1*Δ mutation also suppressed the growth defect caused by double deletion of *POP2*, 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 *ccr4*Δ *khd1*Δ 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 *pbp1*Δ mutation, the *rpl12a*Δ and *rpl12b*Δ mutations also suppressed the growth defect caused by the *ccr4*Δ *khd1*Δ 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 *pbp1*Δ mutation suppresses the lethality caused by the *pab1*Δ mutation (4). Pbp1 is yeast ortholog of human Ataxin 2, which is causal factor of spinocerebellar degeneration type2. 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 *pbp1*Δ mutation has no obvious effect on cell growth. Although Pbp1 was also shown to cosediment 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 *ccr4*Δ mutant shows pleiotropic phenotypes, including a weak cell lysis, a defect in checkpoint control, and a defect in cell cycle progression, and abnormal morphology

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(11, 12, 13, 14). Previous studies have revealed the target mRNAs for Ccr4 that account for these phenotypes of the *ccr4*Δ mutant. For checkpoint control, Ccr4 negatively regulates the *CRT1* mRNA, and deletion of *CRT1* suppresses the checkpoint defect of the *ccr4*Δ mutant (14). For cell cycle progression, Ccr4 negatively regulates the *WHI5* mRNA, and loss of *WHI5* suppresses the cell cycle defect of the *ccr4*Δ 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 *ccr4*Δ mutant. We also found that the *ccr4*Δ 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 *pbp1*Δ mutation suppressed the growth defect of *ccr4*Δ *khd1*Δ mutants. Deletion of the genes encoding previously known Pbp1-interacting factors Lsm12, Pbp4, and Mkt1 did not suppress the growth defect of the *ccr4*Δ *khd1*Δ mutant. We have found that Pbp1 interacts with the ribosomal large subunit, Rpl12a, and Rpl12b. Similarly to the *pbp1*Δ mutation, the *rpl12a*Δ and *rpl12b*Δ mutations also suppressed the growth defect caused by the *ccr4*Δ *khd1*Δ 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 54 to 297), and pGBD-PBP1-c (aa 298 to 722) were used for the yeast two-hybrid analysis. pGAD-c1-RPL12A, pGAD-c1-RPL12B, and pGAD-c1-LSM12 were cloned from yeast two-hybrid libraries. Plasmid YCplac33-PBP1FLAG was used for the immunoprecipitation. Plasmids YCplac33-PBP1, YEplac195-PBP1 ΔLSM, YEplac195-PBP1 ΔAD, and YEplac195-PBP1 ΔLSM ΔAD express the *PBP1*, *PBP1* Δ*LSM*, *PBP1* Δ*LSMAD*, and *PBP1* Δ*LSM* Δ*LSMAD* alleles, respectively. Plasmid YEp195-PAN2 express the *PAN2* gene. Plasmids pCgLEU2, pCgHIS3, and pCgTRP1 are pUC19 carrying the *Candida glabrata LEU2*, *HIS3*, and *TRP1* genes, respectively (21).

Gene deletion and protein tagging. Deletions of *KHD1*, *CCR4*, *POP2*, *DHH1*, *PBP1*, *MKT1*, *PBP4*, *LSM12*, *RPL12A*, and *RPL12B* were constructed by the PCR-based gene deletion method (21–25). Primer sets were designed such that 46 bases at the 5′ end of the primers were complementary to those at the corresponding region of the target gene and 20 bases at their 3′ end were complementary to the pUC19 sequence outside the polylinker region in plasmid pCgLEU2, pCgHIS3, or pCgTRP1. Primer sets for PCR were designed to delete the open reading frame (ORF) completely. The PCR products were transformed into the wild-type strain and selected for Leu^+, His^+, or Trp^+. The *LRG1-HA*-harboring *LRG1 3′UTR*, *LSM12myc*, *RPL12Amyc*, and *RPL12Amyc* strains were prepared by the method of Longtine et al. (23) using pFA6a-3HA-kanMX6-LRG1-3 harboring the *LRG1* 3′ 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 RNeasy 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 TCAAAATTCTGCTGGTTA) and j260 (GCCAATATTTATGAATTCCA TAAC) 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, 5% 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). PJ69-4A 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−LeuTrpAde 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 XT buffer (50 mM HEPES-KOH [pH 7.3], 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	*MAT*α *ade2 trp1 can1 leu2 his3 ura3 GAL psi*$^{+}$ *HOp-ADE2-HO 3′UTR*	36
10BD	*MAT***a**/*MAT*α *ade2/ade2 trp1/trp1 can1/can1 leu2/leu2 his3/his3 ura3/ura3*	36
10BD-c163	*MAT***a**/*MAT*α *ade2/ade2 trp1/trp1 can1/can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1*Δ::*CgTRP1 CCR4/ccr4*Δ::*CgLEU2*	16
10BD-p163	*MAT***a**/*MAT*α *ade2/ade2 trp1/trp1 can1/can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1*Δ::*CgTRP1 POP2/pop2*Δ::*CgLEU2*	This study
10BD-d163	*MAT***a**/*MAT*α *ade2/ade2 trp1/trp1 can1/can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1*Δ::*CgTRP1 DHH1/dhh1*Δ::*CgLEU2*	This study
10BD-c163-p1	*MAT***a**/*MAT*α *ade2/ade2 trp1/trp1 can1/can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1*Δ::*CgTRP1 CCR4/ccr4*Δ::*CgLEU2 PBP1/pbp1*Δ::*CgHIS3*	This study
10BD-c163-11	*MAT***a**/*MAT*α *ade2/ade2 trp1/trp1 can1/can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1*Δ::*CgTRP1 CCR4/ccr4*Δ::*CgLEU2 LRG1/lrg1*Δ::*CgHIS3*	This study
10BD-p163-p1	*MAT***a**/*MAT*α *ade2/ade2 trp1/trp1 can1/can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1*Δ::*CgTRP1 POP2/pop2*Δ::*CgLEU2 PBP1/pbp1*Δ::*CgHIS3*	This study
10BD-d163-p1	*MAT***a**/*MAT*α *ade2/ade2 trp1/trp1 can1/can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1*Δ::*CgTRP1 DHH1/dhh1*Δ::*CgLEU2 PBP1/pbp1*Δ::*CgHIS3*	This study
10BD-c163-m1	*MAT***a**/*MAT*α *ade2/ade2 trp1/trp1 can1/can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1*Δ::*CgTRP1 CCR4/ccr4*Δ::*CgLEU2 MKT1/mkt1*Δ::*CgHIS3*	This study
10BD-c163-p4	*MAT***a**/*MAT*α *ade2/ade2 trp1/trp1 can1/can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1*Δ::*CgTRP1 CCR4/ccr4*Δ::*CgLEU2 PBP4/pbp4*Δ::*CgHIS3*	This study
10BD-c163-112	*MAT***a**/*MAT*α *ade2/ade2 trp1/trp1 can1/can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1*Δ::*CgTRP1 CCR4/ccr4*Δ::*CgLEU2 LSM12/lsm12*Δ::*CgHIS3*	This study
10BD-c163-12a	*MAT***a**/*MAT*α *ade2/ade2 trp1/trp1 can1/can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1*Δ::*CgTRP1 CCR4/ccr4*Δ::*CgLEU2 RPL12A/rpl12a*Δ::*CgHIS3*	This study
10BD-c163-12b	*MAT***a**/*MAT*α *ade2/ade2 trp1/trp1 can1/can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1*Δ::*CgTRP1 CCR4/ccr4*Δ::*CgLEU2 RPL12B/rpl12b*Δ::*CgHIS3*	This study
10BD-c163-pan2	*MAT***a**/*MAT*α *ade2/ade2 trp1/trp1 can1/can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1*Δ::*CgTRP1 CCR4/ccr4*Δ::*CgLEU2 PAN2/pan2*Δ::*CgHIS3*	This study
10BD-c163-p1-pan2	*MAT***a**/*MAT*α *ade2/ade2 trp1/trp1 can1/can1 leu2/leu2 his3/his3 ura3/ura3 KHD1/khd1*Δ::*CgTRP1 CCR4/ccr4*Δ::*CgLEU2 PAN2/pan2*Δ::*CgHIS3 PBP1/pbp1*Δ::*KlURA3*	This study
c163-p1-1	*MAT*α *ade2 trp1 can1 leu2 his3 ura3*	This study
c163-p1-2	*MAT***a** *ade2 trp1 can1 leu2 his3 ura3 ccr4*Δ::*CgLEU2*	This study
c163-p1-3	*MAT***a** *ade2 trp1 can1 leu2 his3 ura3 khd1*Δ::*CgTRP1*	This study
c163-p1-4	*MAT*α *ade2 trp1 can1 leu2 his3 ura3 pbp1*Δ::*CgHIS3*	This study
c163-p1-5	*MAT***a** *ade2 trp1 can1 leu2 his3 ura3 ccr4*Δ::*CgLEU2 pbp1*Δ::*CgHIS3*	This study
c163-p1-6	*MAT***a** *ade2 trp1 can1 leu2 his3 ura3 khd1*Δ::*CgTRP1 pbp1*Δ::*CgHIS3*	This study
c163-p1-7	*MAT*α *ade2 trp1 can1 leu2 his3 ura3 khd1*Δ::*CgTRP1 ccr4*Δ::*CgLEU2*	This study
c163-p1-8	*MAT*α *ade2 trp1 can1 leu2 his3 ura3 khd1*Δ::*CgTRP1 ccr4*Δ::*CgLEU2 pbp1*Δ::*CgHIS3*	This study
c163-p1-L1	*MAT*α *ade2 trp1 can1 leu2 his3 ura3 LRG1-3HA-LRG1 3′UTR::kanMX6*	This study
c163-p1-L2	*MAT*α *ade2 trp1 can1 leu2 his3 ura3 khd1*Δ::*CgTRP1 ccr4*Δ::*CgLEU2 LRG1-3HA-LRG1 3′UTR::kanMX6*	This study
c163-p1-L3	*MAT*α *ade2 trp1 can1 leu2 his3 ura3 khd1*Δ::*CgTRP1 ccr4*Δ::*CgLEU2 pbp1*Δ::*CgHIS3 LRG1-3HA-LRG1 3′UTR::kanMX6*	This study
c163-112-1	*MAT*α *ade2 trp1 can1 leu2 his3 ura3 LSM12-myc::kanMX6*	This study
c163-12a-1	*MAT*α *ade2 trp1 can1 leu2 his3 ura3 RPL12A-myc::kanMX6*	This study
c163-12b-1	*MAT*α *ade2 trp1 can1 leu2 his3 ura3 RPL12B-myc::kanMX6*	This study
PJ69-4A	*MAT***a** *trp1-901 leu2-3,112 ura3-52 his3-200 gal4*Δ *gal80*Δ *GAL2-ADE2 LYS2::GAL1-HIS3 met2::GAL7-lacZ*	27

RESULTS

The *pbp1*Δ mutation suppressed the growth defect of the *ccr4*Δ *khd1*Δ mutant. Woolstencroft et al. have shown that *ccr4*Δ 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 *PBP1*, encoding the transcriptional repressor of the DNA damage-induced gene regulon and Pab1-binding protein, respectively, suppress the HU sensitivity of the *ccr4*Δ mutant (14). We have previously shown that *ccr4*Δ mutant cells show slower growth than wild-type cells on rich medium and that *ccr4*Δ *khd1*Δ double mutant cells show a more severe growth defect than *ccr4*Δ single mutant cells (Fig. 1A) (16). We then examined whether the deletion of *CRT1* and *PBP1* also suppresses the growth defect of the *ccr4*Δ single or *ccr4*Δ *khd1*Δ double mutant. We performed a standard genetic analysis of diploid strains that were heterozygous for the *ccr4*Δ, *khd1*Δ, and *crt1*Δ or *pbp1*Δ alleles. Tetrad analysis revealed that the *ccr4*Δ *pbp1*Δ double mutant cells grew better than the *ccr4*Δ single mutant cells and that the *ccr4*Δ *khd1*Δ *pbp1*Δ triple mutant cells also grew better than the *ccr4*Δ *khd1*Δ double mutant cells (Fig. 1A). While the *ccr4*Δ *khd1*Δ double mutant cells failed to grow at elevated temperature (37°C), the *ccr4*Δ *khd1*Δ *pbp1*Δ triple mutant cells could grow at 37°C (Fig. 1B). Since the *ccr4*Δ *khd1*Δ double mutant shows severe cell lysis (16), the *ccr4*Δ *khd1*Δ 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 *pbp1*Δ mutation suppressed the growth defect of the *ccr4*Δ *khd1*Δ mutant on YPD and YPD sorbitol plates (Fig. 1C). On the other hand, deletion of *CRT1* did not affect the growth of the *ccr4*Δ single or *ccr4*Δ *khd1*Δ double mutant (data not shown). Thus, the *pbp1*Δ mutation, but not the *crt1*Δ mutation, suppressed the growth defect of the *ccr4*Δ *khd1*Δ mutant. We then confirmed this suppression by the *pbp1*Δ mutation using the *PBP1*

TABLE 2 Plasmids used in this study

Plasmid	Relevant markers	Source or reference
YCplac33	*URA3*, *CEN-ARS*	37
YCplac33-PBP1FLAG	*URA3*, *CEN-ARS*, *PBP1FLAG*	This study
YEplac195	*URA3*, 2μ	37
YEplac195-PAN2	*URA3*, 2μ, *PAN2*	This study
YEplac195-PBP1	*URA3*, 2μ, *PBP1*	This study
YEplac195-PBP1 ΔLSM	*URA3*, 2μ, *PBP1* Δ*LSM*	This study
YEplac195-PBP1 ΔAD	*URA3*, 2μ, *PBP1* Δ*LSMAD*	This study
YEplac195-PBP1 ΔLSM ΔAD	*URA3*, 2μ, *PBP1* Δ*LSM* Δ*LSMAD*	This study
pGBD-c1	*TRP1*, 2μ, *GAL4-BD* sequence behind *ADH1* promoter	27
pGBD-c1-PBP1	*BD-PBP1* sequence in pGBD-c1	This study
pGBD-c1-PBP1-n	*BD-PBP1* (amino acids 1–53) sequence in pGBD-c1	This study
pGBD-c1-PBP1-lsm	*BD-PBP1* (amino acids 54–130) sequence in pGBD-c1	This study
pGBD-c1-PBP1-ad	*BD-PBP1* (amino acids 173–297) sequence in pGBD-c1	This study
pGBD-c1-PBP1-lsm/ad	*BD-PBP1* (amino acids 54–297) sequence in pGBD-c1	This study
pGBD-c1-PBP1-c	*BD-PBP1* (amino acids 298–722) sequence in pGBD-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
pCgLEU2	*C. glabrata LEU2* in pUC19	21
pCgHIS3	*C. glabrata HIS3* in pUC19	21
pCgTRP1	*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

gene expressed on the plasmid. The *ccr4*Δ *khd1*Δ *pbp1*Δ triple mutant cells harboring the *PBP1* gene on the plasmid grew slower than the *ccr4*Δ *khd1*Δ *pbp1*Δ triple mutant cells harboring the empty vector (see Fig. 10). These results indicated that Pbp1 has an inhibitory role in cell growth of the *ccr4*Δ *khd1*Δ mutant.

The *pbp1*Δ mutation only partially suppresses the cell lysis of *ccr4*Δ *khd1*Δ mutants. The *ccr4*Δ mutant shows a temperature-sensitive cell lysis (11), and the *ccr4*Δ *khd1*Δ double mutant shows more severe cell lysis than the *ccr4*Δ single mutant (16). Cell lysis of the *ccr4*Δ *khd1*Δ double mutant was caused by the decreased Rho1 activity that was a result of the decreased expression of RhoGEF and Rom2 and the increased expression of RhoGAP and Lrg1 (16). Since deletion of *PBP1* suppressed the growth defect of the *ccr4*Δ single and *ccr4*Δ *khd1*Δ double mutants, we expected that deletion of *PBP1* would also suppress the cell lysis phenotype of these mutants. Unexpectedly, deletion of *PBP1* only partially suppressed the cell lysis of the *ccr4*Δ and *ccr4*Δ *khd1*Δ mutants (Fig. 2). The *pbp1*Δ mutant itself did not show cell lysis. These results suggest that the *pbp1*Δ mutation does not suppress the growth defect of the *ccr4*Δ *khd1*Δ mutant simply by recovering cell wall biogenesis. This result is consistent with the observation that the *ccr4*Δ *khd1*Δ *pbp1*Δ triple mutant cells grew better than the *ccr4*Δ *khd1*Δ double mutant cells on YPD sorbitol plates (Fig. 1C).

We have previously shown that a deletion of *LRG1* suppresses the growth defect of *khd1*Δ *ccr4*Δ mutant at elevated temperatures (37°C) (16). While the *ccr4*Δ *khd1*Δ double mutant cells failed to grow at 37°C, the *ccr4*Δ *khd1*Δ *lrg1*Δ triple mutant cells grew at 37°C. To compare the effect of the *lrg1*Δ mutation with that of the *pbp1*Δ mutation, we reexamined the growth of *ccr4*Δ *khd1*Δ *lrg1*Δ triple mutant cells by tetrad analysis. We found that the *ccr4*Δ *khd1*Δ *lrg1*Δ triple mutant cells grew a little better than the *ccr4*Δ *khd1*Δ double mutant cells on YPD plates (Fig. 1D). The *ccr4*Δ *khd1*Δ *lrg1*Δ triple mutant cells grew similarly to the *ccr4*Δ *khd1*Δ double mutant cells on YPD sorbitol plates (Fig. 1E). This is in contrast to the *ccr4*Δ *khd1*Δ *pbp1*Δ triple mutant cells growing better than the *ccr4*Δ *khd1*Δ double mutant cells on both YPD and YPD sorbitol plates (Fig. 1A and C). Therefore, while the *pbp1*Δ mutation suppressed both the slow-growth phenotype at room temperature and the growth defect at 37°C of the *ccr4*Δ *khd1*Δ double mutant, the *lrg1*Δ mutation suppressed only the growth defect at 37°C. Thus, the suppression by the *pbp1*Δ mutation was not identical to that by the *lrg1*Δ mutation, suggesting that the suppression by the *pbp1*Δ mutation might not involve the *LRG1* gene.

We further examined the *LRG1* mRNA and Lrg1 protein levels in wild-type, *ccr4*Δ *khd1*Δ double mutant, and *ccr4*Δ *khd1*Δ *pbp1*Δ triple mutant cells. While the *LRG1* mRNA level was increased in the *ccr4*Δ *khd1*Δ double mutant cells compared to wild-type cells, the *pbp1*Δ mutation did not decrease the *LRG1* mRNA level (Fig. 3A). The Lrg1 protein level was also increased in the *ccr4*Δ *khd1*Δ double mutant cells compared to wild-type cells (Fig. 3B). The *pbp1*Δ mutation did not decrease the Lrg1 protein level. Taking the results together, the suppression by the *pbp1*Δ mutation might not involve the *LRG1* gene.

The *pbp1*Δ mutation suppresses the growth defect of the *pop2*Δ mutant but not that of the *dhh1*Δ mutant. We have previously showed that the *khd1*Δ mutation also showed a synthetic growth defect with a deletion mutation in *POP2*, which encodes another catalytic subunit of cytoplasmic deadenylase (10, 16). The *pop2*Δ mutant cells show slow growth on rich medium, and the *pop2*Δ *khd1*Δ double mutant cells show more severe slow growth with cell lysis (Fig. 4A) (16). We then examined whether the deletion of *PBP1* also suppresses the growth defect of the *pop2*Δ single or *pop2*Δ *khd1*Δ double mutant. Tetrad analysis revealed that the *pop2*Δ *pbp1*Δ double mutant cells grew better than the *pop2*Δ

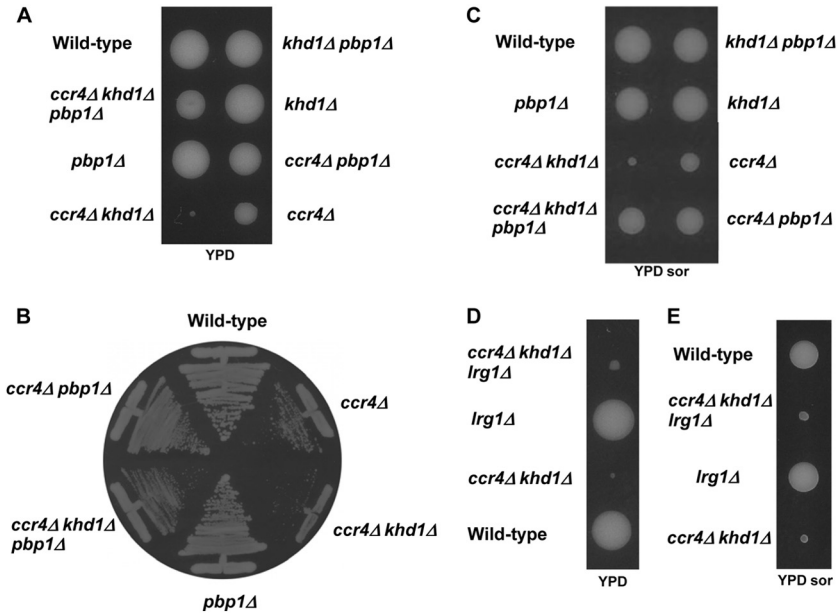

FIG 1 Growth of the *ccr4*Δ *khd1*Δ *pbp1*Δ mutant strain. (A) Strain 10BD-c163-p1, which is heterozygous for the *ccr4*Δ *khd1*Δ and *pbp1*Δ alleles, was sporulated, and tetrads were dissected onto YPD plates. Growth after 5 days at 25°C is shown. Genotypes are indicated on the sides. More than 50 tetrads were dissected, and representative data are shown. Relative colony sizes are listed in Table 3. (B) Each strain was streaked onto a YPD plate and grown for 2 days at 37°C. Yeast strains: wild type, c163-p1-1; *ccr4*Δ, c163-p1-2; *ccr4*Δ *khd1*Δ, c163-p1-7; *pbp1*Δ, c163-p1-4; *ccr4*Δ *pbp1*Δ, c163-p1-5; and *khd1*Δ *ccr4*Δ *pbp1*Δ, c163-p1-8. (C) Strain 10BD-c163-p1, which is heterozygous for the *ccr4*Δ *khd1*Δ and *pbp1*Δ 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. (D and E) Strain 10BD-c163-l1, which is heterozygous for the *ccr4*Δ *khd1*Δ and *lrg1*Δ alleles, was sporulated, and tetrads were dissected onto YPD plates (D) and YPD plates containing 10% sorbitol (E). 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.

single mutant cells and that the *pop2*Δ *khd1*Δ *pbp1*Δ triple mutant cells also grew better than the *pop2*Δ *khd1*Δ double mutant cells (Fig. 4A). While the *pop2*Δ *khd1*Δ double mutant cells failed to grow at elevated temperature (37°C), the *pop2*Δ *khd1*Δ *pbp1*Δ 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 *dhh1*Δ mutant cells show the slow growth on rich medium (Fig. 4B), we next examined whether the deletion of *PBP1* also suppresses the growth defect of the *dhh1*Δ mutant. Tetrad analysis revealed that the *dhh1*Δ *pbp1*Δ double mutant cells did not grew better but grew worse than the *dhh1*Δ single mutant cells (Fig. 4B). The *dhh1*Δ single and *dhh1*Δ *pbp1*Δ double mutant cells failed to grow at elevated temperature (37°C) (data not shown). Thus, the *pbp1*Δ mutation suppressed the growth defect of the *ccr4*Δ and *pop2*Δ mutants but not that of the *dhh1*Δ mutant. These results suggest that Pbp1 acts downstream of Ccr4 and Pop2 but upstream of Dhh1.

The *pbp1*Δ mutation suppresses the growth defect of the *ccr4*Δ 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 *pbp1*Δ extracts are not observed in the absence of Pan2 (5). Therefore, the suppression of the *ccr4*Δ and *khd1*Δ mutations by the *pbp1*Δ mutation might be due to the shorter poly(A) tails caused by the *pbp1*Δ mutation. To examine the involvement of Pan2 in the *pbp1*Δ mutation-mediated suppression, we first examined whether overexpression of *PAN2* suppressed the growth defect of the *ccr4*Δ single or *ccr4*Δ *khd1*Δ double mutant. If the *pbp1*Δ mutation increased *PAN2* activity, overexpression of *PAN2* could suppress the growth defect of the *ccr4*Δ single or *ccr4*Δ *khd1*Δ double mutant. We found that the *ccr4*Δ *khd1*Δ double mutant harboring the YEp-PAN2 plasmid grew better than the *ccr4*Δ *khd1*Δ double mutant without plasmid (Fig. 5A).

We then examined whether the *PAN2* gene is required for the suppression by the *pbp1*Δ mutation. If *PAN2* was required for the suppression, the suppression by the *pbp1*Δ mutation would not be observed in the *pan2*Δ background. Tetrad analysis revealed that the *ccr4*Δ *khd1*Δ *pbp1*Δ *pan2*Δ quadruple mutant grew worse than the *ccr4*Δ *khd1*Δ *pbp1*Δ triple mutant (Fig. 5B). Thus, the *pan2*Δ mutation prevented the efficient suppression of cell growth by the *pbp1*Δ mutation. However, we also found in the same tetrad analysis that the *ccr4*Δ *khd1*Δ *pbp1*Δ *pan2*Δ quadruple mutant grew better than the *ccr4*Δ *khd1*Δ *pan2*Δ triple mutant (Fig. 5B). The *ccr4*Δ *pbp1*Δ *pan2*Δ triple mutant grew a little better than the *ccr4*Δ *pan2*Δ double mutant. These results suggest that the *pbp1*Δ mutation suppressed the growth defect of the *ccr4*Δ 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 *mkt1*Δ, *pbp4*Δ, or *lsm12*Δ mutation also suppresses the growth defect of the *ccr4*Δ *khd1*Δ mutant similarly to the *pbp1*Δ mutation. Tetrad analysis revealed that the *ccr4*Δ *khd1*Δ *mkt1*Δ, *ccr4*Δ *khd1*Δ *pbp4*Δ, and *ccr4*Δ

TABLE 3 Colony sizes of mutants

Relevant genotype	Colony size[a]
Wild type	+++++++
*khd1*Δ	+++++++
*khd1*Δ *pbp1*Δ	+++++++
*khd1*Δ *pbp1*Δ *pan2*Δ	+++++++
*pbp1*Δ *pan2*Δ	+++++++
*pbp1*Δ	+++++++
*pan2*Δ	+++++++
*lrg1*Δ	+++++++
*lsm12*Δ	+++++++
*mkt1*Δ	+++++++
*pbp4*Δ	+++++++
*pop2*Δ *pbp1*Δ	++++++
*rpl12a*Δ	++++++
*ccr4*Δ *khd1*Δ *pbp1*Δ	+++++
*ccr4*Δ *pbp1*Δ *pan2*Δ	+++++
*ccr4*Δ *khd1*Δ *rpl12a*Δ	+++++
*pop2*Δ *khd1*Δ *pbp1*Δ	+++++
*ccr4*Δ	++++
*ccr4*Δ *pan2*Δ	++++
*dhh1*Δ	++++
*pop2*Δ	++++
*ccr4*Δ *khd1*Δ *pbp1*Δ *pan2*Δ	+++
*ccr4*Δ *khd1*Δ *lrg1*Δ	+++
*ccr4*Δ *khd1*Δ *rpl12b*Δ	+++
*dhh1*Δ *khd1*Δ *pbp1*Δ	+++
*dhh1*Δ *pbp1*Δ	+++
*rpl12b*Δ	+++
*ccr4*Δ *khd1*Δ	++
*ccr4*Δ *khd1*Δ *lsm12*Δ	++
*ccr4*Δ *khd1*Δ *mkt1*Δ	++
*ccr4*Δ *khd1*Δ *pbp4*Δ	++
*pop2*Δ *khd1*Δ	+
*ccr4*Δ *khd1*Δ *pan2*Δ	+

[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 *ccr4*Δ *khd1*Δ *pan2*Δ triple mutant, which had the smallest size in this study).

*khd1*Δ *lsm12*Δ triple mutant cells grew similarly to the *ccr4*Δ *khd1*Δ double mutant cells (Fig. 6). Thus, unlike the *pbp1*Δ mutation, the *mkt1*Δ, *pbp4*Δ, or *lsm12*Δ mutation did not suppress the growth defect of the *ccr4*Δ *khd1*Δ 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

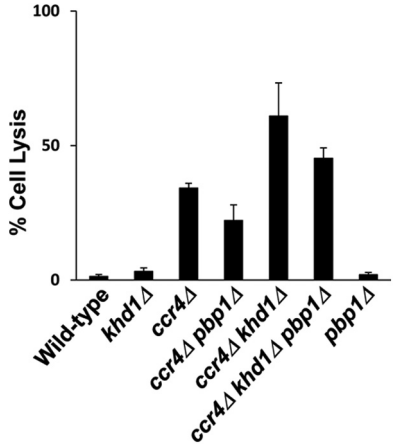

FIG 2 *ccr4*Δ *khd1*Δ double mutant cells show severe cell lysis. Results of a cell lysis assay in YPD medium are shown. Wild-type (c163-p1-1), *ccr4*Δ (c163-p1-2), *ccr4*Δ *pbp1*Δ (c163-p1-5), *ccr4*Δ *khd1*Δ (c163-p1-7), *khd1*Δ *ccr4*Δ *pbp1*Δ (c163-p1-8), and *pbp1*Δ (c163-p1-4) cells were grown 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.

constructed strains harboring a FLAG-tagged version of Pbp1, Pbp1-FLAG, and Myc-tagged versions of Rpl12a and Rpl12b, Rpl12amyc and Rpl12bmyc, as described in Materials and Methods, as well as Lsm12myc. When Pbp1-FLAG was immunopre-

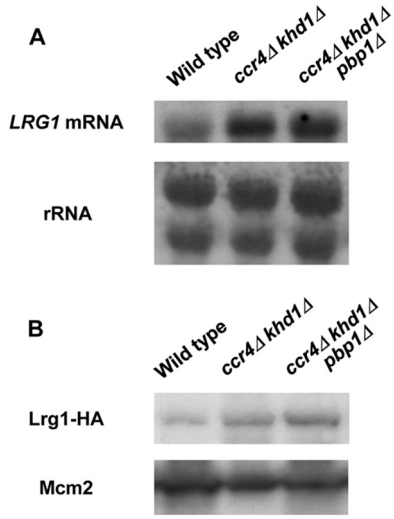

FIG 3 *LRG1* mRNA and Lrg1 protein levels are not affected by the *pbp1*Δ mutation. (A) *LRG1* mRNA levels in wild-type, *ccr4*Δ *khd1*Δ, and *ccr4*Δ *khd1*Δ *pbp1*Δ cells. Wild-type (c163-p1-1), *ccr4*Δ *khd1*Δ (c163-p1-7), and *ccr4*Δ *khd1*Δ *pbp1*Δ (c163-p1-8) cells were cultured to mid-logarithmic 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, *ccr4*Δ *khd1*Δ, and *ccr4*Δ *khd1*Δ *pbp1*Δ cells. Wild-type *LRG1-3HA-LRG1 3′UTR* (c163-p1-L1), *ccr4*Δ *khd1*Δ *LRG1-3HA-LRG1 3′UTR* (c163-p1-L2), and *ccr4*Δ *khd1*Δ *pbp1*Δ *LRG1-3HA-LRG1 3′UTR* (c163-p1-L3) 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.

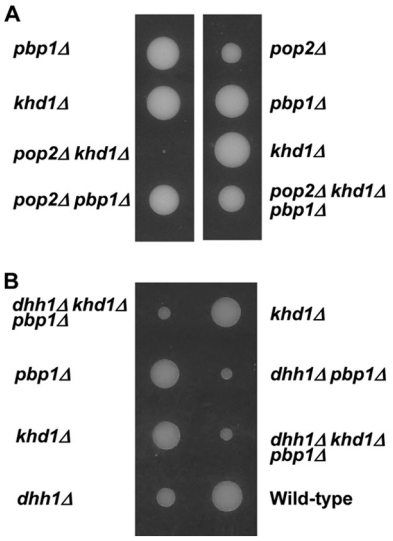

FIG 4 Growth of the *pop2*Δ *khd1*Δ *pbp1*Δ and *dhh1*Δ *khd1*Δ *pbp1*Δ mutant strains. (A) Strain 10BD-p163-p1, which is heterozygous for the *pop2*Δ *khd1*Δ *pbp1*Δ alleles, was sporulated, and tetrads is dissected onto YPD plates containing 10% sorbitol. Growth after 5 days at 25°C is shown. Genotypes 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 *dhh1*Δ *khd1*Δ *pbp1*Δ alleles, was sporulated, and tetrads is 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.

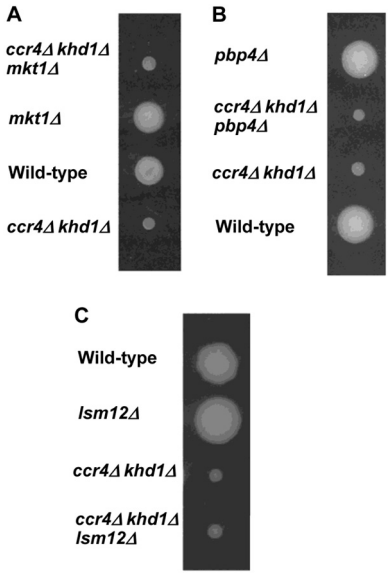

FIG 6 Growth of the *ccr4*Δ *khd1*Δ *mkt1*Δ, *ccr4*Δ *khd1*Δ *pbp4*Δ, and *ccr4*Δ *khd1*Δ *lsm12*Δ mutant strains. (A) Strain 10BD-c163-m1, which is heterozygous for the *ccr4*Δ *khd1*Δ and *mkt1*Δ alleles, was sporulated, and tetrads were dissected onto YPD plates containing 10% sorbitol. Growth after 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 *ccr4*Δ *khd1*Δ and *pbp4*Δ 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 *ccr4*Δ *khd1*Δ and *lsm12*Δ 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.

cipitated from cell extracts with an anti-FLAG antibody, Lsm12myc was coimmunoprecipitated, as detected by anti-myc antibody in Western blots (Fig. 7B). Similarly, Rpl12amyc and Rpl12bmyc were coimmunoprecipitated with Pbp1-FLAG (Fig.

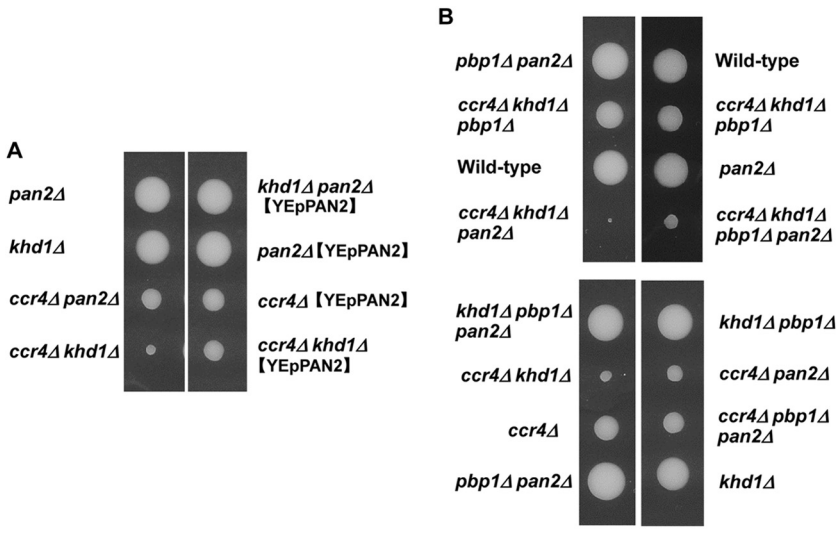

FIG 5 Growth of the *pop2*Δ *khd1*Δ *pbp1*Δ pan2Δ mutant strain. (A) Strain 10BD-c163-pan2, which is heterozygous for the *ccr4*Δ *khd1*Δ *pan2*Δ alleles, containing YEp195-PAN2 plasmid (YEpPAN2) was sporulated, and tetrads were dissected onto YPD plates containing 10% sorbitol. Growth after 5 days at 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-p1-pan2, which is heterozygous for the *ccr4*Δ *khd1*Δ *pbp1*Δ *pan2*Δ 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. Relative colony sizes are listed in Table 3.

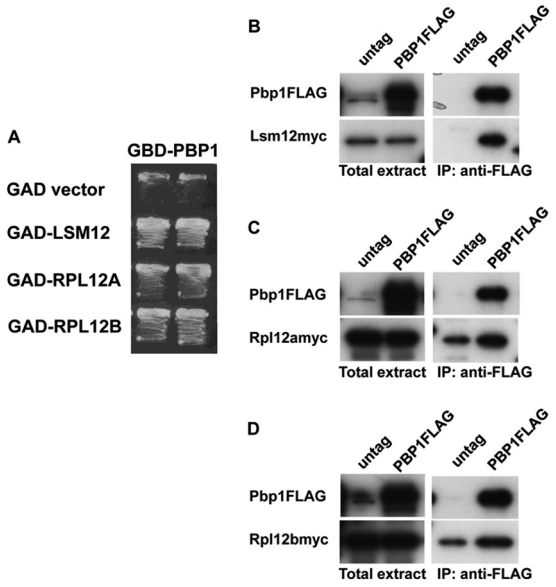

FIG 7 Interaction of Pbp1 with Rpl12a and Rpl12b. (A) Two-hybrid screening. Yeast strain PJ69-4A harboring *GAL2p-ADE2* and *GAL1p-HIS3* reporters was transformed with the indicated plasmids, and the transformants were streaked onto SC−LeuTrpHis plates containing 1 mM 3-aminotriazole, and incubated for 3 days at 30°C. The same results were obtained on SC−LeuTrpAde 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 for the presence of Pbp1FLAG, Lsm12myc, Rpl12amyc, and Rpl12bmyc proteins. Total extracts (Total extract) were also immunoblotted 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 *rpl12a*Δ and *rpl12b*Δ mutations suppress the growth defect caused by the *ccr4*Δ *khd1*Δ mutation. We next examined whether the *rpl12a*Δ or *rpl12b*Δ mutation suppressed the growth

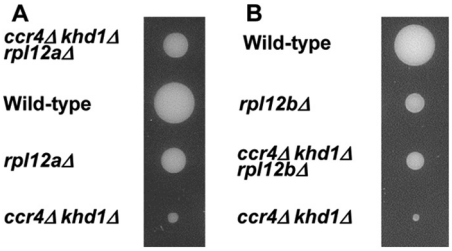

FIG 8 Growth of the *ccr4*Δ *khd1*Δ *rpl12a*Δ and *ccr4*Δ *khd1*Δ *rpl12b*Δ mutant strains. (A) Strain 10BD-c163-12a, which is heterozygous for the *ccr4*Δ *khd1*Δ and *rpl12a*Δ alleles, was sporulated, and tetrads were dissected onto YPD plates containing 10% sorbitol. Growth after 5 days at 25°C is shown. Genotypes are indicated on the left. More than 50 tetrads were dissected, and representative data are shown. (B) Strain 10BD-c163-12b, which is heterozygous for the *ccr4*Δ *khd1*Δ and *rpl12b*Δ 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.

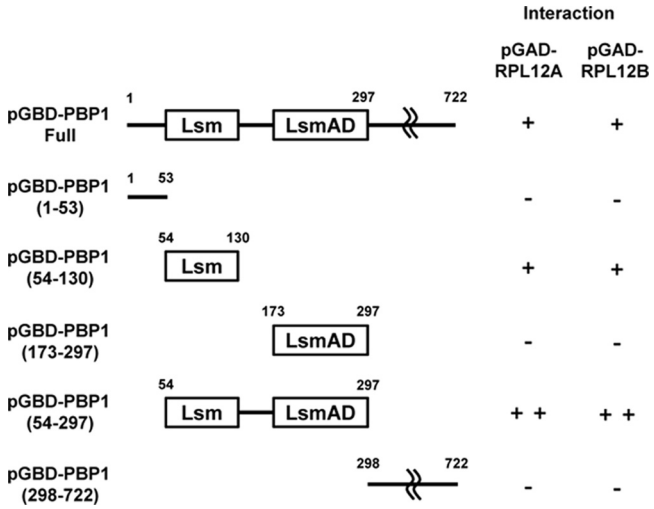

FIG 9 Interaction of Pbp1 with Rpl12a, and Rpl12b. Two-hybrid analysis is shown. Yeast strain PJ69-4A harboring *GAL2p-ADE2* and *GAL1p-HIS3* reporters was transformed with the indicated plasmids, and the transformants were streaked onto SC−LeuTrpHis plates containing 1 mM 3-aminotriazole and onto SC−LeuTrpAde plates and incubated for 3 days at 30°C. ++, stronger interaction; +, interaction; −, no interaction.

defect of the *ccr4*Δ *khd1*Δ mutant. Tetrad analyses revealed that the *ccr4*Δ *khd1*Δ *rpl12a*Δ triple mutant cells grew better than the *ccr4*Δ *khd1*Δ double mutant cells (Fig. 8A). The *ccr4*Δ *khd1*Δ *rpl12b*Δ triple mutant cells also grew better than the *ccr4*Δ *khd1*Δ double mutant cells, although the *rpl12b*Δ mutant cells grew worse than the *rpl12a*Δ mutant cells (Fig. 8B). Thus, both the *rpl12a*Δ and *rpl12b*Δ mutations suppressed the growth defect of the *ccr4*Δ *khd1*Δ mutant. We could not evaluate whether the *rpl12a*Δ and *rpl12b*Δ mutations suppress the growth defect of the *ccr4*Δ single mutant, because the *rpl12a*Δ and *rpl12b*Δ 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 *ccr4*Δ *khd1*Δ 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 *ccr4*Δ *khd1*Δ mutant. We then examined the necessity of the interaction of Pbp1 with Rpl12a and Rpl12b in the suppression of the *ccr4*Δ *khd1*Δ 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 *ccr4*Δ *khd1*Δ *pbp1*Δ mutant, the *ccr4*Δ *khd1*Δ *pbp1*Δ mu-

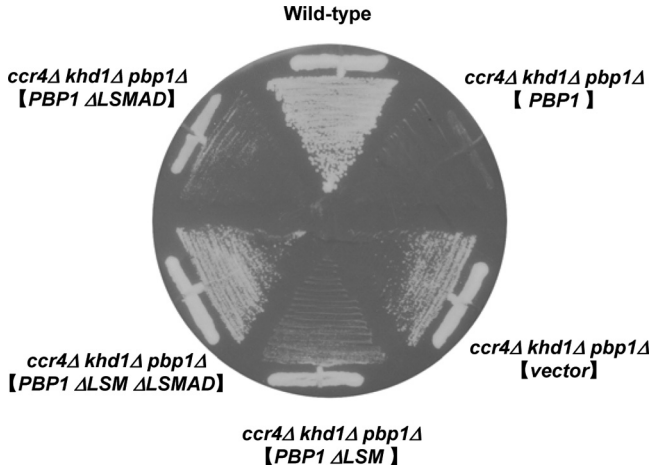

FIG 10 Growth of the *ccr4*Δ *khd1*Δ *pbp1*Δ mutant strain harboring *PBP1* plasmids. The yeast strain was transformed with the indicated plasmids. Transformants were streaked onto SD−Ura plates and grown at 37°C for 3 days. Yeast strains: wild type (vector), *ccr4*Δ *khd1*Δ *pbp1*Δ (YCplac33-PBP1), *ccr4*Δ *khd1*Δ *pbp1*Δ (vector), *ccr4*Δ *khd1*Δ *pbp1*Δ (YEplac195-PBP1 ΔLSM), *ccr4*Δ *khd1*Δ *pbp1*Δ (YEplac195-PBP1 ΔLSMAD), and *ccr4*Δ *khd1*Δ *pbp1*Δ (YEplac195-PBP1 ΔLSM ΔLSMAD).

tant harboring the wild-type *PBP1* gene on the plasmid did not grow at 37°C (Fig. 10). The *ccr4*Δ *khd1*Δ *pbp1*Δ mutant harboring *PBP1*Δ*LSM* Δ*LSMAD* grew at 37°C similarly to the mutant harboring empty vector, suggesting that *PBP1*Δ*LSM* Δ*LSMAD* is not functional. Interestingly, the *ccr4*Δ *khd1*Δ *pbp1*Δ mutant harboring *PBP1*Δ*LSM* or *PBP1*Δ*LSMAD* grew worse than the *ccr4*Δ *khd1*Δ *pbp1*Δ mutant harboring empty vector, suggesting that *PBP1*Δ*LSM* or *PBP1*Δ*LSMAD* is partially functional. Thus, the Rpl12-interacting domain within Pbp1, LSM, is required for the function of Pbp1.

DISCUSSION

Pbp1, a yeast ortholog of human Ataxin 2, was originally identified as a protein that interacts with the C-terminal domain of Pab1, and the *pbp1*Δ mutation is reported to suppress the lethality caused by the *pab1*Δ mutation (4). Pbp1 is also reported to interact with other factors involved in RNA processing, i.e., Fir1, Ufd3, Pbp4, and Lsm12 (5). Furthermore, Pbp1 interacts with Mkt1, and both are involved in regulation of *HO* expression (9). In this study, we found that Pbp1 interacts with ribosomal proteins Rpl12a and Rpl12b. Although it has been reported that Pbp1 cosediments with ribosomal fraction and functions in translation (5, 9, 30), it has remains unknown whether Pbp1 interacts with ribosomal proteins. This is the first report that Pbp1 interacts with ribosomal proteins. More importantly, we also found that the *rpl12a*Δ and *rpl12b*Δ mutations as well as the *pbp1*Δ mutation suppressed the growth defect caused by the *ccr4*Δ *khd1*Δ double mutation. The *lsm12*Δ, *pbp4*Δ, or *mkt1*Δ mutation did not suppress the growth defect caused by the *ccr4*Δ *khd1*Δ double mutation. Furthermore, we found that Lsm and LsmAD of Pbp1 are required for its interaction with Rpl12a and Rpl12b and that Lsm and LsmAD of Pbp1 are also required for Pbp1 function. These genetic data support that Pbp1 functions together with Rpl12a and Rpl12b.

How does the *pbp1*Δ mutation suppress the growth defect caused by the *ccr4*Δ *khd1*Δ double mutation? The *ccr4*Δ mutation 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 *khd1*Δ mutation, which confers the decreased expression of RhoGEF and Rom2, together with the *ccr4*Δ mutation (16). The *ccr4*Δ *khd1*Δ double mutant cells show a severe slow growth at room temperature and no growth with cell lysis at elevated temperature. While the *lrg11*Δ mutation suppressed the growth defect at 37°C, the *lrg1*Δ mutation only weakly suppressed the slow-growth phenotype of the *ccr4*Δ *khd1*Δ mutant on YPD plates. The *lrg1*Δ mutation did not suppress the slow-growth phenotype of the *ccr4*Δ *khd1*Δ mutant on YPD sorbitol plates. In contrast, the *pbp1*Δ 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 *ccr4*Δ *khd1*Δ double mutant. Thus, the *pbp1*Δ mutation is unlikely to act to decrease the Lrg1 level or its activity to suppress the *ccr4*Δ *khd1*Δ double mutation. Consistently, the *pbp1*Δ mutation did not decrease the *LRG1* mRNA and protein levels. Furthermore, while a deletion of *LRG1* suppresses the growth defect of a *dhh1*Δ mutant at 37°C (our unpublished data), the *pbp1*Δ mutation did not suppresses the growth defect of a *dhh1*Δ mutant at 37°C. Thus, the *pbp1*Δ-mediated suppression seems to involve genes other than *LRG1*. It has been reported that the deletion of *CRT1* and *PBP1* suppresses the HU sensitivity of the *ccr4*Δ mutant (14). Our analysis has revealed that the *pbp1*Δ mutation, but not the *crt1*Δ mutation, suppressed the growth defect caused by the *ccr4*Δ *khd1*Δ double mutation. Furthermore, we found that unlike the case for *LRG1* mRNA, the *CRT1* mRNA level was not increased in the *ccr4*Δ 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 *ccr4*Δ mutant. Thus, the *pbp1*Δ-mediated suppression seems to involve genes other than *CRT1* and *WHI5*.

The *rpl12a*Δ and *rpl12b*Δ mutations as well as the *pbp1*Δ mutation suppressed the growth defect caused by the *ccr4*Δ *khd1*Δ double mutation, indicating that the Pbp1 and Rpl12 proteins have a negative effect on the cell growth in the *ccr4*Δ *khd1*Δ double mutant cells. Since *LRG1* overexpression is toxic in the *ccr4*Δ single and the *ccr4*Δ *khd1*Δ 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 *ccr4*Δ *pbp1*Δ double mutant cells, and the *pbp1*Δ 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 multicopy *SSD1* encoding an RNA-binding protein suppressed the growth defect caused by the *ccr4*Δ *khd1*Δ 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 *ccr4*Δ *khd1*Δ 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 *rpl12a*Δ and *rpl12b*Δ mutations as well as the *pbp1*Δ 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 (CBF), Ref2, and a factor interacting with Ref2, Fir1, is required for the formation of a normal-length poly(A) tail on precleaved *CYC1* pre-mRNA (5).

While the yeast extracts from *ref2*Δ mutant cells synthesize longer poly(A) tails than those synthesized by wild-type extracts, *pbp1*Δ and *fir1*Δ extracts synthesize shorter poly(A) tails (5). Since the shorter poly(A) tails in the *pbp1*Δ 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 *ccr4*Δ and *khd1*Δ mutations by the *pbp1*Δ mutation might be due to the shorter poly(A) tails caused by the *pbp1*Δ mutation. Indeed, we have found that overexpression of *PAN2* suppressed the growth defect of the *ccr4*Δ *khd1*Δ double mutants and that the *pan2*Δ mutation prevented the efficient suppression of cell growth by the *pbp1*Δ mutation. On the other hand, we also found that the *ccr4*Δ *khd1*Δ *pbp1*Δ *pan2*Δ quadruple mutants grew better than the *ccr4*Δ *khd1*Δ *pan2*Δ triple mutants. Thus, the *pbp1*Δ mutation suppressed the growth defect of the *ccr4*Δ mutant in both a *PAN2*-dependent and -independent manner.

Since the *rpl12a*Δ and *rpl12b*Δ mutations as well as the *pbp1*Δ mutation suppressed the growth defect caused by the *ccr4*Δ *khd1*Δ double mutation, Pbp1-mediated translation with Rpl12a and Rpl12b seems to involve the *PAN2*-independent suppression of the *ccr4*Δ *khd1*Δ double mutation. The *rpl12a*Δ and *rpl12b*Δ mutant cells grow slower than wild-type cells, and the *rpl12a*Δ *rpl12b*Δ mutant cells show a more severe growth defect. However, the *pbp1*Δ mutant cells grow normally, and the *pbp1*Δ mutation does not affect the cell growth of the *rpl12a*Δ and *rpl12b*Δ 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 *rpl12a*Δ and *rpl12b*Δ mutations as well as the *pbp1*Δ mutation suppressed the growth defect caused by the *ccr4*Δ *khd1*Δ mutation, we have to further analyze the significance of the physical interaction between Pbp1 and Rpl12. On the other hand, the *pbp1*Δ *dhh1*Δ double mutant cells grew worse than the *dhh1*Δ 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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