The Rpb7p subunit of yeast RNA polymerase II plays roles in the two major cytoplasmic mRNA decay mechanisms

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The steady-state level of mRNAs is determined by the balance between their synthesis by RNA polymerase II (Pol II) and their decay. In the cytoplasm, mRNAs are degraded by two major pathways; one requires decapping and 5′ to 3′ exonuclease activity and the other involves 3′ to 5′ degradation. Rpb7p is a Pol II subunit that shuttles between the nucleus and the cytoplasm. Here, we show that Rpb7p is involved in the two mRNA decay pathways and possibly couples them. Rpb7p stimulates the deadenylation stage required for execution of both pathways. Additionally, Rpb7p is both an active component of the P bodies, where decapping and 5′ to 3′ degradation occur, and is capable of affecting the P bodies function. Moreover, Rpb7p interacts with the decapping regulator Pat1p in a manner important for the mRNA decay machinery. Rpb7p is also involved in the second pathway, as it stimulates 3′ to 5′ degradation. Our genetic analyses suggest that Rpb7p plays two distinct roles in mRNA decay, which can both be uncoupled from Rpb7p's role in transcription. Thus, Rpb7p plays pivotal roles in determining mRNA levels.

Introduction

A balance between synthesis and decay determines the steady-state level of mRNA. For most mRNAs, synthesis and decay take place in different cellular compartments, and little is known about cross talk between these two processes.

The yeast RNA polymerase II (Pol II) is composed of 12 subunits termed Rpb1p-Rpb12p (Young, 1991). The crystal structure of yeast Pol II reveals that the enzyme comprises two distinctive parts (Armache et al., 2003; Bushnell and Kornberg, 2003); a ten-subunit core carrying the catalytic active site and a two-subunit heterodimer composed of Rpb4p and Rpb7p (for review see Choder, 2004; Sampath and Sadhale, 2005). Rpb7p is an essential Pol II subunit that is conserved from archea to humans (McKune et al., 1993; Mitsuzawa et al., 2003; Choder, 2004). It carries two highly conserved RNA binding domains (Todone et al., 2001; Armache et al., 2005; Meka et al., 2005) that likely bind RNA cooperatively (Todone et al., 2001). In vitro studies indicate that Rpb7p can bind RNA in a sequence-independent fashion (Orlicky et al., 2001; Meka et al., 2003, 2005). Importantly, Rpb7p interacts with a transcript during in vitro transcription as soon as the latter emerges from the Pol II core (Ujvari and Luse, 2006). Therefore, Rpb7p is most likely among the first proteins to interact with nascent Pol II transcripts.

Unlike Rpb7p, Rpb4p is a nonessential protein (Woychik and Young, 1989; Choder and Young, 1993; Rosenheck and Choder, 1998). Notably, Rpb4p is involved in mRNA export from the nucleus to the cytoplasm only during stress (Farago et al., 2003) and plays a direct role in cytoplasmic degradation of a specific class of mRNAs encoding protein biosynthetic factors (PBFs) (Lotan et al., 2005). PBFs include ribosomal proteins, translation initiation factors, aminoacyl tRNA synthetases, and ribosomal biosynthetic proteins. Because cells can proliferate in the absence of *RPB4*, it is clear that the indispensable Rpb7p can function independently of Rpb4p and can interact with Pol II in the absence of Rpb4p (Sheffer et al., 1999). However, it is not known whether Rpb7p plays a role outside Pol II context.

In yeast, major pathways of mRNA degradation (for review see Coller and Parker, 2004; Amrani et al., 2006; Garneau et al., 2007) initiate with shortening of the mRNA poly(A) tail. When the length of the poly(A) tail reaches 10–12 bases or less, one of two alternative pathways is initiated (or both pathways are initiated simultaneously). One pathway involves removal of the mRNA 5′ cap [m(7)GpppN] by the Dcp1p/Dcp2p heterodimer

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Abbreviations used in this paper: HS, heat shock; mRNP, mRNA-proteins complex; PB, processing body; PBF, protein biosynthetic factor; Pol II, RNA polymerase II; ts, temperature sensitive; WT, wild type.

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(Decker and Parker, 1993; Dunckley and Parker, 1999; Liu and Kiledjian, 2006). Several proteins regulate this decapping process, including Pat1/Lsm1-7 (Boeck et al., 1998; Bonnerot et al., 2000; Bouveret et al., 2000; Tharun et al., 2000). Pat1p is recruited to mRNA while it is still associated with translation factors (Tharun and Parker, 2001). Subsequently, Pat1p recruits the hepta-heterodimer Lsm1-7 complex and this event is associated with the transition from translation to decay (Tharun and Parker, 2001; Coller and Parker, 2004). In vitro, Pat1-Lsm1-7 complex binds at or near the 3′ end of the mRNA (Chowdhury et al., 2007) and is cable of protecting the 3′ ends of mRNAs in vivo from trimming (He and Parker, 2001). The deadenylated mRNA can be degraded by alternative pathway that degrades the mRNA from its 3′ terminus. A well-studied 3′ to 5′ exonuclease is a large complex known as exosome (for review see Houseley et al., 2006). The relative contribution of each mechanism remains a subject of debate. In *Saccharomyces cerevisiae*, knocking out components of either the 3′ to 5′ (Houalla et al., 2006) or the 5′ to 3′ pathway (He et al., 2003) had minimal effects on the transcriptome, which implies tight regulation of mRNA level involving cross talk between the two decay mechanisms. Nonetheless, very little is known about any possible dialogue between the two decay mechanisms.

Recently, we proposed that Rpb4p is the first mRNA decay factor to be recruited to mRNA in the nucleus. After transport of the mRNP to the cytoplasm, Rpb4p is involved in recruiting the Pat1/Lsm1-7 complex, or stimulating Pat1/Lsm1-7 function, and stimulates mRNA decay (Lotan et al., 2005). However, because Rpb4p does not have an RNA-binding domain (Todone et al., 2001; Armache et al., 2005; Meka et al., 2005), it was not clear how this protein is recruited to the mRNP.

Here, we report novel functions for Rpb7p in the two cytoplasmic mRNA decay pathways. Mutation analyses reveal the existence of temperature-sensitive (ts) Rpb7ps that are transcriptionally functional but fail to stimulate mRNA decay. We have identified mutant forms of Rpb7p that are specifically defective in only one of their three functions, raising the possibility that the three functions are distinct. Further genetic analyses suggest that Rpb7p functions in the context of Rpb4/7. Nevertheless, whereas Rpb4p plays a role in the decay of specific mRNAs, Rpb7p has a more general role in the mRNA decay pathway. Collectively, our observations suggest that the Rpb4/7 is involved in a cross talk between the two cytoplasmic mRNA decay mechanisms and between the nuclear and cytoplasmic stages of gene expression.

Results

Rpb7p is localized in cytoplasmic P bodies

Previously we have shown that Rpb7p shuttles between the nucleus and the cytoplasm by a transcription-dependent mechanism. Moreover, Rpb7p shuttles as a heterodimer together with Rpb4p (Selitrennik et al., 2006), which plays a role in the cytoplasmic mRNA decay pathway (see Introduction). In addition, we observed that Rpb7p-GFP localizes in discrete foci that resemble processing bodies (PBs) (Selitrennik et al., 2006), the site where decapping and degradation of mRNAs occurs (Ingelfinger et al., 2002; Lykke-Andersen, 2002; van Dijk et al., 2002; Sheth and Parker, 2003; Teixeira et al., 2005). These observations prompted us to investigate whether Rpb7p is involved in mRNA decay. To determine the nature of Rpb7p cytoplasmic foci, we examined whether known PB proteins colocalize in these areas. Indeed, two PB markers (Sheth and Parker, 2003; Brengues et al., 2005), RFP-tagged Dcp2p (Fig. 1 A) and Lsm1-RFP (unpublished data), colocalize with Rpb7p-GFP. Colocalization was observed in response to starvation and ranges between 10 and 60% of the cases, depending on the strain background and on the environmental conditions. Rpb7p-GFP foci were also colocalized with Rpb4-RFP foci that were recently shown to represent PBs (Lotan et al., 2005). Moreover, like genuine PBs, Rpb7p-GFP foci disappear in response to pretreatment with the translation inhibitor cyclohexamide (Fig. 1). These results confirm our suspicion that Rpb7p is localized in PBs and raise the possibility that Rpb7p is involved in the deadenylation-dependent mRNA decay that takes place there.

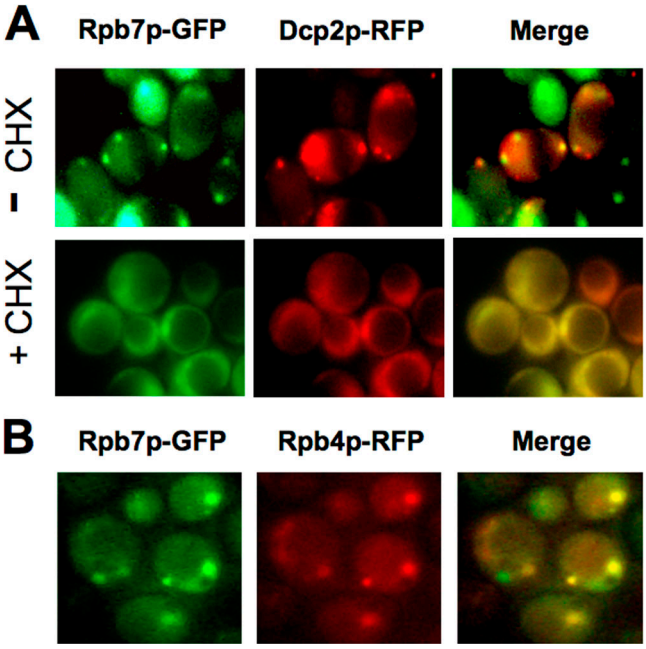

Figure 1. **Rpb7p-GFP colocalizes with Dcp2p-RFP in PBs.** Cells expressing *RPB7*-GFP and *DCP2*-RFP (yRL11) and cells expressing *RPB7*-GFP and *RPB4*-RFP (yMS8) were starved and then visualized microscopically, as described previously (Lotan et al., 2005). +CHX panels, cyclohexamide-treated cells (Lotan et al., 2005).

Mutations in RPB7 compromise the decay of PBF and non-PBF mRNAs

To determine more directly whether Rpb7p is involved in mRNA decay, we mutagenized randomly the essential *RPB7* gene and selected ts alleles (see Materials and methods). Consistent with previous results (Zaros and Thuriaux, 2005), we could not identify ts alleles that carry only one mutation. We selected 13 ts mutants that carry 2–6 point mutations (see Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb. 200701165/DC1). The mutations are scattered throughout the proteins, suggesting that the overall conformation of this small protein is important

for its function. First, we determined the transcriptional capacity of the ts mutants at their nonpermissive temperature by monitoring de novo synthesis of various heat-shock (HS) mRNAs in response to abrupt HS. As expected for an essential pol II subunit, some of the 13 mutants exhibit transcriptional defects at nonpermissive temperatures (Fig. S2; *rpb7-29*). Notably, however, some of the mutants do not exhibit diminished transcription capacity (Fig. S2; *rpb7-26* and *rpb7-28*). Consistently, levels of these mutant forms of Rpb7p, as detected by Western analysis, are comparable to that of the wild-type (WT) Rpb7p (unpublished data). Yet, these mutants are ts. These results suggest that Rpb7p has an essential posttranscriptional role(s) at 37°C. Moreover, because the levels of HS mRNAs in the transcriptionally functional ts mutants are higher than in WT, these data support the premise that the essential posttranscriptional role of Rpb7p is in mRNA decay. It is worth noting that although most mRNA decay factors are nonessential at 30°C, some of them become essential at high temperatures (Coller and Parker, 2004; Parker and Song, 2004).

Therefore, we determined whether any of the ts mutants exhibit defects in mRNA degradation. To monitor mRNA decay, transcription was blocked either synthetically by adding 1, 10, phenanthroline (Fig. 2, A and B) or naturally by shifting cells from 30°C (our standard permissive temperature) to 42°C (Fig. 2 C). The latter method relies on physiological responses to HS, which comprises aborting transcription of non-HS genes and enhancing decay of non-HS mRNAs (Lotan et al., 2005; see also Fig. 2, A and B). Using this HS assay, which simultaneously inactivates the ts Rpb7p and blocks transcription, we found that >50% of the ts mutants exhibit detectable defects in the decay of *YEF3* and *TIF1* mRNAs (unpublished data). We analyzed in more detail yRL26, yRL28, yRL29, and yRL34 strains (carrying *rpb7-26*, *rpb7-28, rpb7-29,* and *rpb7-34* ts alleles, respectively), which show the most severe defects in mRNA decay.

Next, we examined whether the mutant strains are defective in mRNA decay at 37°C, instead of 42°C. Because at 37°C transcription is not blocked, we monitored decay of Tet-Off-*MFA2pG* transcript after blocking transcription by doxycycline. As shown in Fig. S3 (available at http://www.jcb.org/cgi/content/full/jcb.200701165/DC1), the initial decay of *MFA2* mRNA was similar in both strains. However, after 25 min post-temperature shift-up, mRNA degradation was slower in the mutant as compared to its degradation in the WT. This behavior shows that, already at 37°C, mRNA decay is defective in the mutant cells, but raises the possibility that inactivation of Rpb7p ts at 37°C was delayed and requires ~25 min. In our subsequent analyses we monitored mRNA stability at 42°C, as discussed below.

To systematically examine mRNA decay in our mutant strains, we first examined mRNA turnover at the permissive temperature. At 30°C, decay kinetics in the mutants are comparable to those in WT (Fig. 2 A; unpublished data). In agreement with this, at the permissive temperature the steady-state levels of various mRNAs in all our mutant strains are comparable to those in WT. In contrast, at 42°C, the mutants exhibit defective decay (Fig. 2, B and C). This defect is observed independently of the method that was used to block transcription (compare Fig. 2 B with Fig. 2 C; and unpublished data). Importantly, at 42°C,

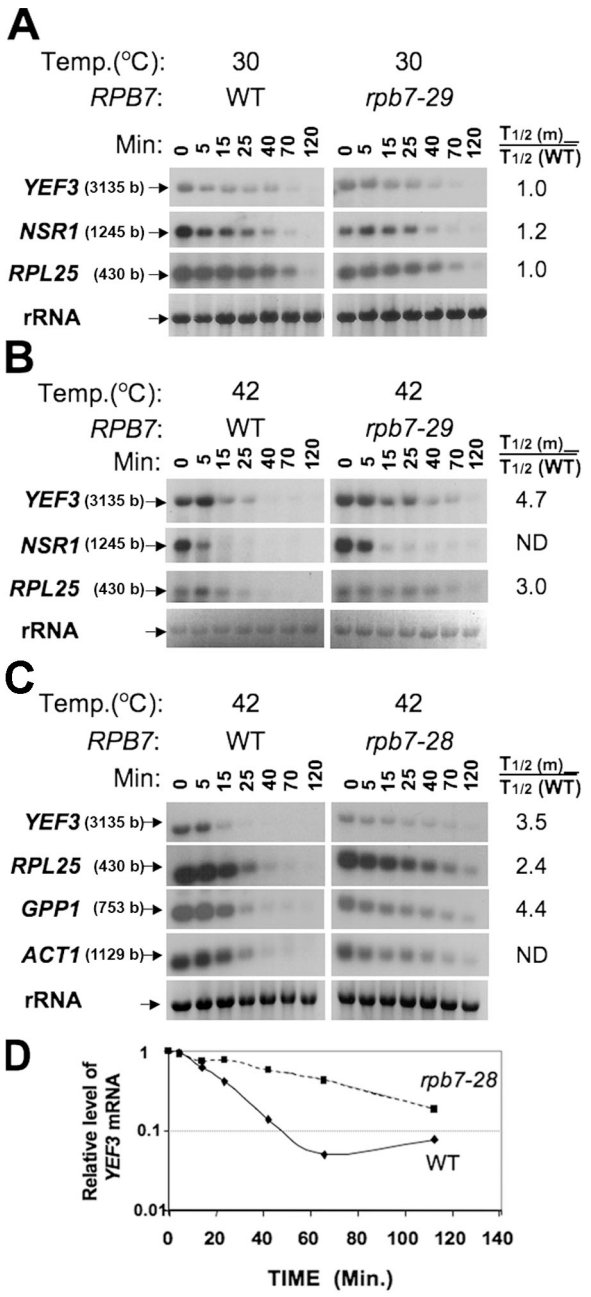

Figure 2. **PBF and non-PBF mRNAs decay abnormally slowly at the nonpermissive temperature in *rpb7* ts mutants.** (A) At the permissive temperature, *rpb7-29* and WT exhibit similar mRNA decay kinetics. The indicated strains were proliferated at 30°C until mid-log phase. 1, 10, phenanthroline was added to block transcription (Grigull et al., 2004; Lotan et al., 2005). Decay kinetics was determined by monitoring mRNA levels at the indicated time points post-transcription block, using Northern analysis with the indicated probes. rRNA serves to demonstrate equal loading. Half-lives (designated $T_{1/2}$) were determined as described previously (Lotan et al., 2005) and the ratios between $T_{1/2}$ of the mutant and that of the wild-type [$T_{1/2}$ (m)/$T_{1/2}$ (WT)] are indicated on the right. Lengths of the open reading frames are indicated in parentheses next to the gene name on the left. (B) At the nonpermissive temperature, *rpb7-29* cells exhibit defective mRNA decay. Cells were challenged simultaneously with 1, 10, phenanthroline and shifted to 42°C and mRNA decay was determined as in A. (C) *rpb7-28* cells are defective in mRNA decay. Cells were proliferated at 30°C till mid-log phase and then shifted abruptly to 42°C (no drug was added). The high temperature led both to inactivation of Rpb7-28p ts and to transcription arrest (Lotan et al., 2005). mRNA decay was determined as in A. (D) Decay kinetics of *YEF3* mRNA in WT and *rpb7-28* cells was determined as described previously (Lotan et al., 2005).

the mutants exhibited defective decay as soon as cells were shifted to the high temperature (Fig. 2, B and C). A good example for rapid inactivation of Rpb7 ts is the decay of *NSR1* mRNA, which becomes very unstable at 42°C (Lotan et al., 2005). Already 5 min after the temperature shift, more mRNA is observed in the mutant than in the WT (Fig. 2 B, *NSR1*). A quantitative example is the decay of *YEF3* (Fig. 2, C and D).

Rpb4p is specifically involved in the decay of PBF mRNAs (see Introduction). Unlike Rpb4p, mutations in Rpb7p affect the decay of both PBF and non-PBF mRNAs. Among the non-PBF mRNAs are short-lived mRNAs, such as *MFA2* and *ACB1* (see Fig. 3) and *GPP1* mRNAs, as well as relatively stable mRNAs, such as *ACT1* mRNA (Fig. 2 C). Thus, it appears that the role of Rpb7p in mRNA decay is more general than that of Rpb4p (see Discussion).

Rpb7p stimulates deadenylation

To gain insight into the mechanism by which Rpb7p functions in the major mRNA decay, we examined what step(s) in mRNAs turnover is/are stimulated by Rpb7p. Cells were shifted to 42°C to inactivate both transcription and the function of Rpb7p ts form, and then the levels and poly(A) tail lengths of *RPL29* and *RPL25* mRNAs were monitored using the polyacrylamide gel electrophoresis Northern (PAGEN) technique (Sachs and Davis, 1989), as shown in Fig. 3. These mRNAs were chosen because their short length enables detection of small differences in the size of their poly(A) tail. Three conclusions can be made concerning the results shown in Fig. 3. First, the decay kinetics of these mRNAs is fast in WT cells and slower in mutant cells, consistent with our results obtained by standard Northern analysis shown in Fig. 2. Second, deadenylation is abnormally slow in the mutant cells. For example, whereas complete deadenylation of *RPL29* mRNA is observed in WT after 45 min (Fig. 3, lane 5), it takes 70 min for this to occur in *rpb7-28* (Fig. 3, lane 13). We conclude that Rpb7-28p is defective in stimulating rapid deadenylation. As shown in Fig. 3, after its complete deadenylation 25 min post-transcription arrest, it is only a further 20 min before *RPL25* mRNA is almost completely degraded in WT (between 25 to 45 min post-transcription arrest; see lanes 4 and 5). In contrast, to degrade comparably the deadenylated *RPL25* mRNA in *rpb7-28* takes 75 min (lanes 12–14). A third conclusion, then, is that Rpb7p stimulates not only deadenylation, but also post-deadenylation step.

In summary, our data support a dual function for Rpb7p in mRNA decay. Rpb7p both accelerates deadenylation and stimulates a post-deadenylation step. The nature of the post-deadenylation step is discussed in the next section.

Mutations in RPB7 can lead to defective execution of the 3′ to 5′ pathway of mRNA degradation

The major post-deadenylation step that is defective in *rpb7-28* cells might be either decapping or 5′ to 3′ degradation or 3′ to 5′ degradation of the decaying mRNA (Coller and Parker, 2004; Amrani et al., 2006; Garneau et al., 2007). To distinguish between these processes we took advantage of a construct designed to identify degradation intermediates. Specifically,

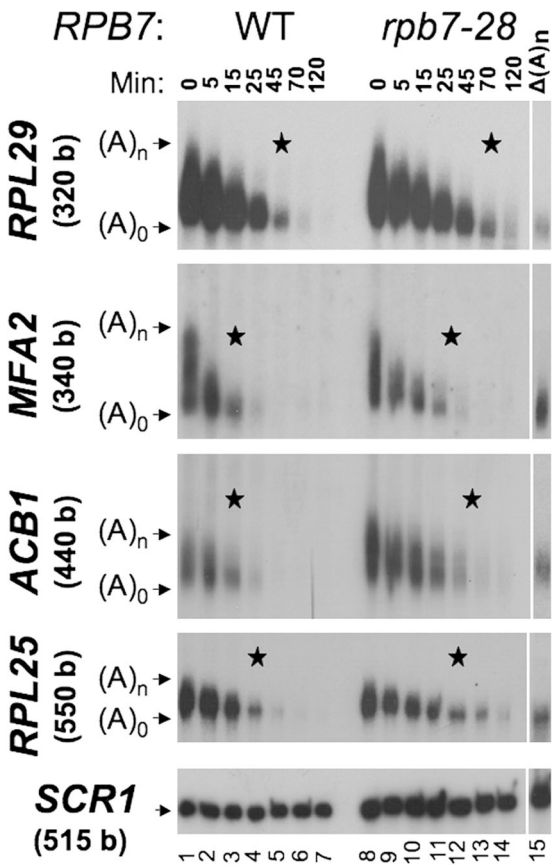

Figure 3. **Rpb7p is required for efficient deadenylation and subsequent decay of both PBF and non-PBF mRNA.** Cells were shifted rapidly to 42°C to block transcription and to inactivate the ts Rpb7-28p. RNA was extracted at the indicated time points post-shift and analyzed by the polyacrylamide Northern technique (Sachs and Davis, 1989). The same membrane was hybridized sequentially with the indicated probes. Actual lengths (relative to size marker) of the indicated mRNAs (without the poly(A) tail) are indicated in parentheses next to the gene name on the left. Lane "Δ(A)n" shows the position of fully deadenylated RNA. This RNA was obtained by hybridizing RNA sample from time point 0 with oligo(dT), followed by digestion of the poly(A) tail by RNase H. The star indicates the time point when deadenylation seems to be complete. We suspect that in some cases complete deadenylation occurred in between two time points. In these particular cases, the star is placed between lanes. *SCR1* RNA (a Pol III transcript) serves to demonstrate equal loading.

a previous study has revealed that a (G)18 tract, placed in the 3′ UTR of *MFA2* mRNA, serves as a barrier for exonuclease activity. Consequently, 5′ to 3′ degradation of *MFA2*pG mRNA by Xrn1p produces a ∼190- base degradation intermediate fragment (Fig. 4, "Frag.") that stretches from the poly(G) tract to the 3′ end (Vreken and Raue, 1992; Decker and Parker, 1993). This fragment can be degraded by the 3′ to 5′ pathway (Anderson and Parker, 1998). Because Xrn1p degrades only uncapped RNA, accumulation of this intermediate is usually indicative of efficient decapping (Hatfield et al., 1996; Dunckley and Parker, 1999; Tharun et al., 2000, 2005). We introduced Tet-Off-*MFA2pG* in WT and *rpb7-28* cells and used this assay to determine which post-deadenylation step is defective in the mutant cells. As shown in Fig. 4 A, fragment level relative to the full-length mRNA is threefold higher in *rpb7-28* cells than it is in wild-type cells. Because the fragment is degraded by 3′ to 5′

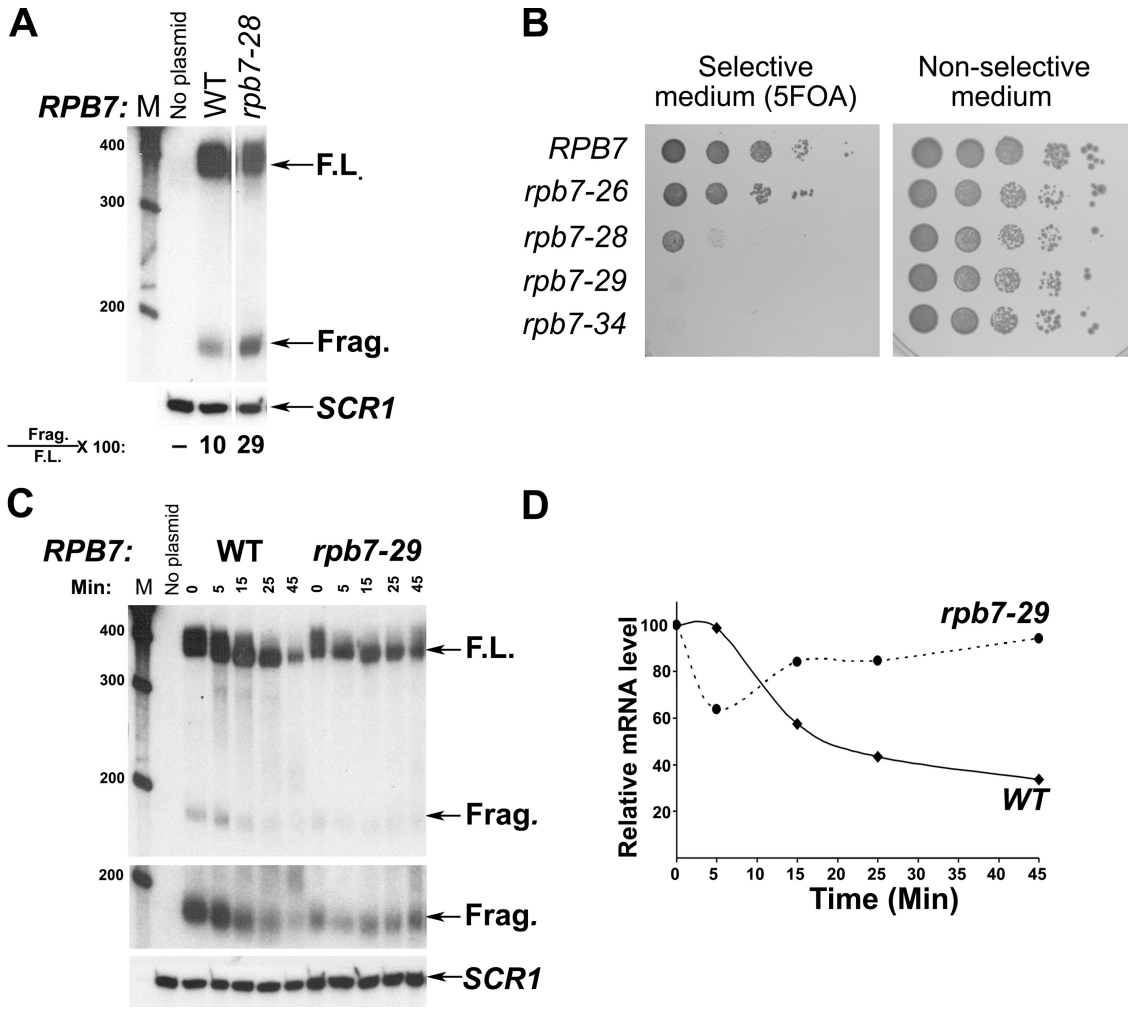

Figure 4. **Rpb7p is required for efficient execution of the 3′ to 5′ degradation of MFA2pG mRNAs.** (A) *rpb7*-28 cells contain high level of *MFA2pG* degradation intermediate. WT and *rpb7*-28 cells expressing Tet-Off-*MFA2pG* (yVB32 and yVB34) were harvested in mid-log phase. *MFA2pG* mRNA (designated F.L.) and its degradation intermediate fragment (designated Frag.) were examined using PAGE Northern technique as in Fig. 3, except that electrophoresis was shorter to allow detection of the fragment. *MFA2pG*-specific probe was used (see Materials and methods). As a control for the probe specificity, RNA from cells lacking the plasmid was also analyzed ("No plasmid" lane). *SCR1* RNA (a Pol III transcript) serves to demonstrate equal loading. The ratio between the fragment radioactivity and that of the full length was obtained by PhosphorImager technology and is indicated at the bottom. (B) Deletion of *XRN1* displays synthetic lethality in combination with *rpb7-29 and rpb7-34*, synthetic sickness in combination with *rpb7-28*, but not with *rpb7-26*. A strain lacking *XRN1* and carrying p*RPB7::URA3* in lieu of *RPB7* (yMC414) was transformed with *HIS3* plasmid carrying *RPB7* or *rpb7* mutants as indicated on the *left*. Synthetic lethality assay was performed as described in Materials and methods. In parallel, cells were spotted on a plate lacking 5-FOA (SC lacking histidine), designated "non-selective medium", to demonstrate spotting of equal number of cells. (C) *rpb7*-29 cells are defective in 3′ to 5′ exonuclease activity. WT and *rpb7*-29 cells were grown to mid-log phase at 30°C. 2 μg/ml doxycycline was added to block transcription of Tet-Off-*MFA2pG* mRNA. Immediately thereafter, 100 μg/ml cyclohexamide was added in order to block decapping and consequently block Xrn1p activity. Immediately thereafter, cells were shifted to 37°C and samples were harvested at the indicated time points and their RNAs were analyzed by PAGE Northern as in A. Lane designated "No plasmid" used as a control as in A. *SCR1* RNA was used for normalization. Short exposure of the membrane is shown in the top autoradiogram. Longer exposure of the fragment is shown in the bottom autoradiogram, for better detection. (D) 3′ to 5′ decay kinetics of the fragment. Fragment level was monitored by PhosphorImager, normalized to that of *SCR1* RNA, and plotted as a function of time post-transcription and decapping block. A band intensity at time 0 was defined as 100%, and intensities at other time points were calculated relative to time 0. Similar results were obtained by four hybridization analyses. Variations were <15%.

exonuclease (Anderson and Parker, 1998), high fragment level is often indicative of efficient decapping and poor 3′ to 5′ exonuclease activity.

To examine if 3′ to 5′ decay is defective in *rpb7* mutants, we first took advantage of a genetic assay based on the observation that strains lacking both the major deadenylation and the alternative 3′ to 5′ are inviable (Anderson and Parker, 1998; van Hoof et al., 2000). Thus, any double-mutant that contains a block to the 5′ decay pathway (*xrn1*Δ) with a block to the 3′ decay pathway (e.g., *ski2*Δ, *ski3*Δ) is synthetically lethal (Anderson and Parker, 1998). The synthetic relationship between *XRN1* and *rpb7* ts mutants is shown in Fig. 4 B. *rpb7-26* can replace *RPB7* in *xrn1*Δ cells. This result indicates that cells lacking *XRN1* and carrying *rpb7-26* as the only source of Rpb7p can support 3′ to 5′ decay. In contrast, *rpb7-29* cells and *rpb7-34* cells cannot survive in the absence of *XRN1*. *rpb7-28* cells proliferate very slowly without *XRN1* (Fig. 4 B), consistent with the abnormal accumulation of the *MFA2pG* fragment (Fig. 4 A).

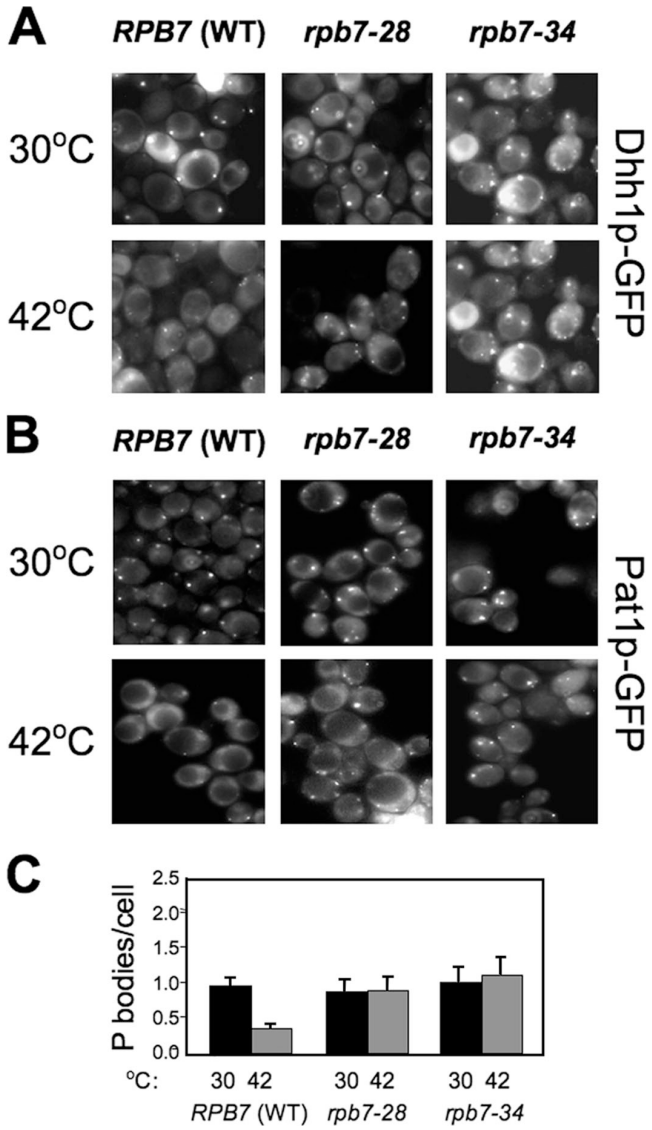

Figure 5. ***RPB7* affects the P bodies' number.** Wild-type cells and their isogenic *rpb7* ts derivatives, expressing *DHH1*-GFP (A and C) or *PAT1*-GFP (B) (see Table I), were allowed to proliferate at 30°C in a selective medium until late-log phase. Cultures were then shifted to 42°C for 45 min (A) or 60 min (B). Cells were washed 3× with water before they were visualized by fluorescent microscopy. (C) Average number of *DHH1*-GFP PBs per cell. Error bars indicate the SD from the mean values.

These results suggest that, indeed, Rpb7p is required for execution of the 3′ to 5′ decay pathway, and that some of our mutants are defective in this function. Interestingly, the synthetic lethality was observed at 30°C, suggesting that the mutants are defective in 3′ to 5′ decay at the permissive temperature.

To corroborate the synthetic lethality results biochemically, we introduced pTet-Off-*MFA2pG* in *rpb7-29* cells, which showed the most severe synthetic lethality, and determined the fragment stability. To this end, we blocked transcription of Tet-Off-*MFA2pG* by doxycycline and simultaneously blocked decapping (and hence Xrn1p capacity to act) by cyclohexamide (Beelman and Parker, 1994) while shifting cells to the nonpermissive temperature. These two inhibitors blocked supply of new fragments, permitting us to follow 3′ to 5′ decay of the fragment that had preexisted in the cytoplasm (because it is a product of Xrn1p that acts in the cytoplasm) prior drugs addition (Anderson and Parker, 1998; Boeck et al., 1998; Araki et al., 2001). Whereas in WT cells the fragment is rapidly degraded ($t_{1/2}$ = 30 min), no degradation can be detected throughout the experiment duration (Fig. 4 C and D). Thus, the 3′ to 5′ decay mechanism is defective in the mutant cells. In *rpb7-26* cells, which do not show synthetic lethality with *XRN1* (Fig. 4 B), the fragment half-life was 30 min (unpublished data). Note that in *rpb7-29* cells, the steady-state level before the temperature shift (time 0) is not higher than it is in WT cells (unlike the case of *rpb7-28* cells). It is possible that Rpb7-29 is also defective in decapping. Collectively, the results shown in Fig. 4 indicate that Rpb7p is required for efficient 3′ to 5′ degradation of mRNAs.

Rpb7p is involved in PB function

Previously it was shown that mutations in some mRNA decay factors impact the number of PBs (Sheth and Parker, 2003; Cougot et al., 2004; Kshirsagar and Parker, 2004; Coller and Parker, 2005; Anderson and Kedersha, 2006). The novel roles of Rpb7p in mRNA decay and its localization to PBs (Fig. 1) prompted us to examine whether mutations in *RPB7* similarly affect PB number, using Dhh1p-GFP or Pat1p-GFP as the PB marker (Sheth and Parker, 2003). As expected, at the permissive temperature when Rpb7p ts forms function normally (see Fig. 2), PBs are readily detected in both WT and mutant cells (Fig. 5; 30°C). To rapidly inactivate the ts Rpb7p, we exposed cells to nonpermissive temperatures (42°C) for 45–60 min before visualizing them microscopically. In response to temperature increase of WT cells, the number (and size) of PBs decreases (Fig. 5; WT). Thus, PBs respond to HS differently than they respond to some other stress conditions. Specifically, during starvation and other stresses, the number (and size) of PBs increases because mRNA decay is repressed (Teixeira et al., 2005). However, during HS, mRNA decay is not repressed (see Fig. 2 C). Moreover, because both transcription initiation (see Fig. 2 C) and translation initiation (Holcik and Sonenberg, 2005) are repressed at high temperatures, there is no new supply of mRNPs to PBs. Consequently, at least one mechanism for disassembly of PBs during HS is mRNA degradation. In contrast with WT, no decrease in the number (and size) of PBs is observed in the *rpb7* ts mutant in this time frame (Fig. 5). These results indicate that Rpb7p is an active component of PBs. They are consistent with the defective mRNA degradation that characterizes *rpb7* mutants at nonpermissive temperatures, as such a defect is likely to result in more mRNA molecules remaining in PBs after the temperature increase and hence less dissociation of PBs.

Genetic and two-hybrid interactions between RPB7 and genes encoding components of the Pat1/Lsm1-7p complex

We have shown previously that Pat1p can be immunoprecipitated with Rpb4p and Rpb7p (Lotan et al., 2005). To corroborate the interaction between Rpb7p and Pat1p by other means, we used both genetic and two-hybrid approaches. Taking advantage of the temperature sensitivity (ts) of *pat1Δ* cells (Bonnerot et al., 2000; He and Parker, 2001), we determined whether overexpression of

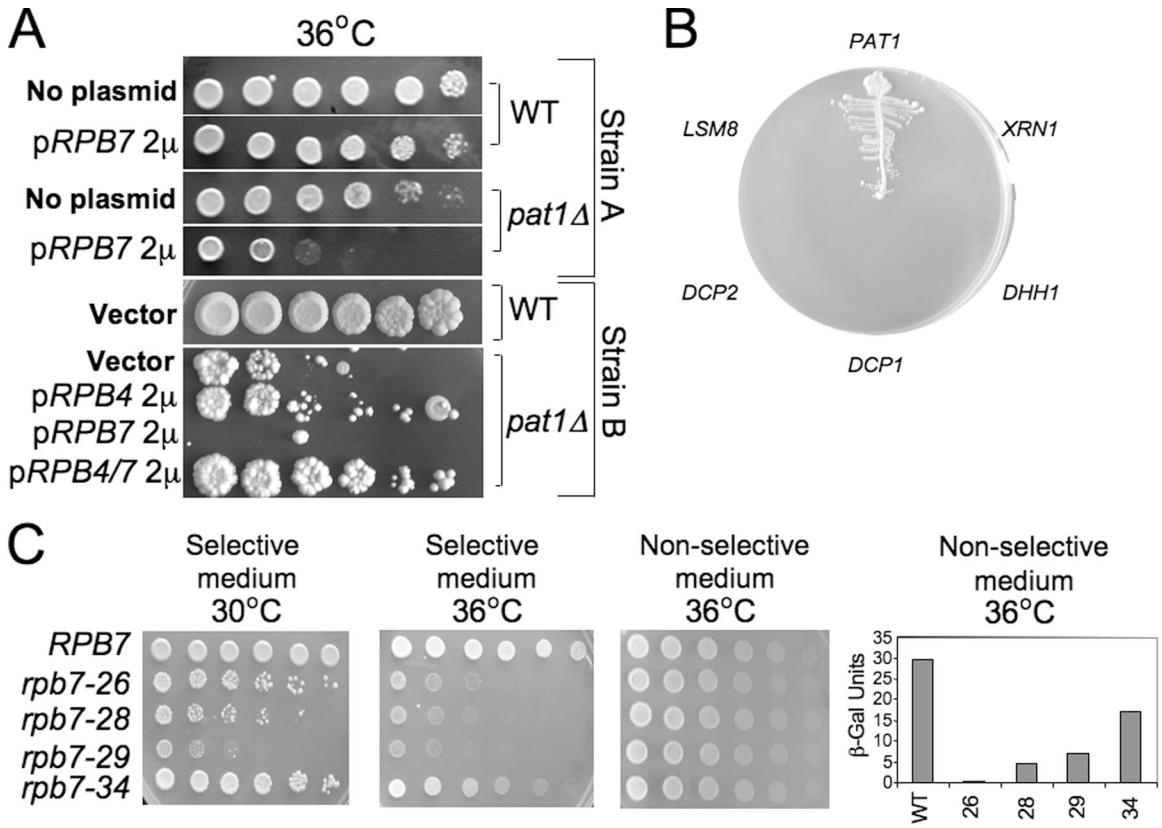

Figure 6. **Rpb7p interacts with Pat1p; the extent of interaction correlates with the capacity of Rpb7p to stimulate mRNA decay.** (A) High copy effect of the indicated genes on the proliferation capacity of *pat1*Δ cells or their WT counterparts. Two strain backgrounds were examined. WT strain (yMC229) and its isogenic *pat1*Δ strain (yMC363) are designated "Strain A"; WT (yMC269) or its *pat1*Δ derivative yMC272 are designated "Strain B". Cells carrying high copy plasmid expressing the indicated genes were spotted in fivefold serial dilutions onto selective plates. Cells were allowed to grow at the non-permissive temperature for *pat1*Δ cells, 36°C. (B) Two-hybrid interactions between Rpb7p as the bait and genes whose products are involved in mRNA decay as prey were performed as described in Materials and methods. Only 6 of 20 genes tested are shown as indicated around the plate. The other genes that did not exhibit interactions with the Rpb7p-DBD and are not shown here are Lsm1p, Lsm3p–Lsm7p, Ccr4p, Not1p, Caf1p, Pan2p, Pan3p, Pop2p, Edc1p, Edc2p, Edc3p, and Pab1p. Lsm2p showed weak interaction (not depicted). Equal amount of cells, carrying the indicated prey plasmids, were streaked onto an indicator plate as described in Materials and methods. We then verified that the growth on the indicator plates was dependent on both plasmids by evicting one plasmid at a time from the positive clones (not depicted). (C) Two-hybrid interaction between various mutant forms of Rpb7p-DBD and Pat1p-AD. Cells were spotted in threefold serial dilutions, starting with 10^6 cells/spot, on an indicator plate as in B (selective conditions). Plates were incubated for 3 d at the indicated temperatures. To demonstrate spotting of equal amounts of cells, cells were spotted on a nonselective plate that allowed growth of cells that carry both the bait and prey plasmids independently of the two-hybrid interactions (using medium lacking only leucine and tryptophane). β-galactosidase (β-Gal) values, quantitative means to determine interactions (Uetz et al., 2000), are indicated on the right. They reflect the average values determined from two independent experiments (variations were <15%). Background values obtained with cells expressing only the bait plasmid were subtracted. Levels of the various mutant forms of Rpb7p-DBD were found to be comparable to that of the WT Rpb7-DBD (not depicted).

RPB7 and *RPB4* could suppress this phenotype. Overexpression of *RPB4* has a small, yet significant, suppression effect on the ts phenotype of *pat1*Δ (Lotan et al., 2005). In contrast, overexpression of *RPB7* enhances the proliferation defect at of *pat1*Δ cells 36°C, but has little effect on proliferation of WT cells (Fig. 6 A). We also evaluated the consequence of co-overexpressing both *RPB4* and *RPB7*, including their own 5′ and 3′ noncoding regulatory sequences, on the same high copy plasmid. Overexpressing both genes in this way suppresses the proliferation defect of *pat1*Δ more substantially than overexpressing *RPB4* alone (Fig. 6 A). The genetic interaction between *RPB7* and *PAT1* supports our conjecture that their products share a similar biochemical pathway and that Rpb7p plays a direct role in the major mRNA decay pathway that involves Pat1p. The synergistic suppression effect of simultaneously over-producing both Rpb4p and Rpb7p suggests that they interact optimally with Pat1p in the context of the Rpb4/7 heterodimer. Consistent with this possibility, we found that *rpb7* ts alleles (including those that are defective only in mRNA decay), but not WT *RPB7*, are synthetically lethal with deletion of *RPB4* (Fig. S4, available at http://www.jcb.org/cgi/content/full/jcb.200701165/DC1). These results indicate that the mutant forms of Rpb7p require Rpb4p to carry out their essential function.

Two-hybrid analysis suggests that Rpb7p forms a direct contact with Pat1p (Fig. 6 B). In addition we examined two-hybrid interaction between Rpb7p as the bait and, as prey, each of 20 proteins known to be involved in the mRNA decay pathway (listed in the legend to Fig. 6 B). Only Pat1p exhibits a strong and reproducible interaction with Rpb7p. Lsm2p showed weak interaction as well (unpublished data).

To further study the mechanism by which Rpb7p functions in mRNA decay, we evaluated the capacity of our Rpb7p ts mutants to interact with Pat1, using the two-hybrid assay. As shown in Fig. 6 C, the various mutant forms of Rpb7p that

are defective in stimulating mRNA decay are also defective in interacting with Pat1p. This defect is more severe at 36°C than at 30°C. The liquid β-gal test, which examines an additional reporter of the two-hybrid interaction (Uetz et al., 2000), yields similar results (Fig. 6 C). These data suggest that the defective capacity of Rpb7p mutant forms to stimulate mRNA decay is related to their poor binding of Pat1p.

Discussion

Rpb7p plays a direct role in two stages of the cytoplasmic mRNA decay process

RNA polymerase II is composed of two distinct structural domains that can be readily dissociated from each other; one of them is the Rpb4/7 heterodimer. Thus, within the context of pol II, Rpb7p functions in concert with Rpb4p (Choder, 2004). Previously, we discovered that Rpb4p is involved in mRNA decay in the cytoplasm. Overexpression of Rpb7p could not suppress the defective capacity of *rpb4Δ* cells to support mRNA decay (Lotan et al., 2005), suggesting that Rpb4p has a distinct role in the decay of these mRNAs, which cannot be replaced by Rpb7p. Thus, an unresolved issue has been whether Rpb7p has any role in the decay pathway. Here, we show that Rpb7p plays key roles in mRNA decay.

We have uncovered two novel roles for Rpb7p in two major mRNA decay pathways. Thus, Rpb7p stimulates the deadenylation stage required for execution of both pathways. Rpb7p is required for the major pathway via its interaction with Pat1/Lsm1-7 complex (see below) and for efficient execution of the second pathway. These two functions can be uncoupled genetically as some Rpb7p mutant forms are defective and some are not defective in stimulating 3′ to 5′ decay (Fig. 4 B). We also identified a ts mutant that is not defective in stimulating deadenylation and is defective in supporting decay after deadenylation (unpublished data). Thus, Rpb7p plays distinct roles in either pathway and is probably involved in a cross talk between the two. Although it is quite possible that the two major decay pathways are linked, detailed mechanistic understanding of this possible cross talk remains to be determined.

Association of Rpb7p with the decaying mRNP might be important to maintain an appropriate RNP conformation, which is necessary for efficient execution of both pathways. Alternatively, Rpb7p might play more direct roles in mRNA decay pathways. We maintain that, at least in the major pathway, Rpb7 plays a direct role via its interaction with Pat1p and Lsm2p and its interaction with PBs (see below).

Consistent with its role in the major decay pathway, Rpb7p is a constituent of PBs. Moreover, defects in Rpb7p can affect the capacity of PBs to dissociate when cells are exposed to HS (which is also a nonpermissive temperature for *rpb7* ts alleles). Thus, like some other known mRNA decay factors (Sheth and Parker 2003; Kshirsagar and Parker 2004), Rpb7p seems to be an active PB effector. In addition, we show here that Rpb7p interacts with components of the Pat1/Lsm1-7 complex. Moreover, mutations in Rpb7p that compromise the capacity of Rpb7p to function in mRNA decay also diminish its capacity to interact with these components. All these data strongly suggest that Rpb7p plays a direct role in the major mRNA decay pathway.

As Rpb7p is both a nuclear and cytoplasmic protein, it is important to discern whether its role in mRNA decay is performed in the nucleus or in the cytoplasm. We contend that Rpb7p is involved in the cytoplasmic decay pathways for the following reasons. First, Rpb7p is involved in the major deadenylation-dependent decay pathways; these pathways operate in the cytoplasm (Coller and Parker, 2004; Amrani et al., 2006; Garneau et al., 2007). Second, Rpb7p is a constituent of PBs, which are cytoplasmic loci where mRNA decapping and degradation are performed, and Rpb7p is a functional effector of PBs. Third, Rpb7p interacts with components of the Pat1p/Lsm1-7 complex, known to be involved in the cytoplasmic decay pathway (Coller and Parker, 2004; Amrani et al., 2006; Garneau et al., 2007). Last, Rpb7p is required for 3′ to 5′ degradation of *MFA2pG* fragment. Because this fragment is the product of Xrn1p that acts in the cytoplasm (Coller and Parker, 2004; Amrani et al., 2006; Garneau et al., 2007), the stimulation of the 3′ to 5′ fragment degradation by Rpb7p must occur in the cytoplasm.

Rpb7p functions in mRNA decay in the context of the Rpb4/7 heterodimer, but performs a role distinct from Rpb4p

Several observations have led us to propose that Rpb7p role in mRNA decay, at least in the decay of some mRNAs, is performed in the context of Rpb4/7. First, Rpb4p and Rpb7p shuttle between the nucleus and the cytoplasm as a heterodimer (Selitrennik et al., 2006), suggesting that their role in the cytoplasm is also performed in the context of the heterodimer. Compromising this interaction by mutating Rpb7p (e.g., in *rpb7-29* cells) slows down import of both partners to the nucleus (Selitrennik et al., 2006). Second, both proteins interact with Pat1p and Lsm2p, but not with other known decay factors. Third, overexpression of both *RPB4* and *RPB7*, but not one of them at a time, has a strong suppressive effect on the ts phenotype of a *pat1Δ* mutant. Fourth, both proteins are colocalized in the same PBs. It is worth noting, however, that we have observed cases in which Rpb4p-containing foci were not colocalized with Rpb7p-containing ones, and vice versa. Last, *RPB4*, which is otherwise a nonessential gene, is synthetically lethal with the *rpb7* alleles whose products are specifically defective in mRNA decay (Fig. S4).

Unlike the influence of Rpb4p on mRNA decay that is limited to PBF mRNAs (Lotan et al., 2005), Rpb7p appears to play a more general role in mRNA decay. It is important to emphasize that Rpb7p can function normally in the decay of several non-PBF mRNAs (e.g., *MFA2, ACT1* mRNAs) in the absence of Rpb4p (Lotan et al., 2005). We propose that Rpb7p plays at least two roles in mRNA decay, only one of which (and not necessarily in all cases) involves recruiting Rpb4p. Furthermore, only in the case of PBF mRNA degradation, is this recruitment functionally important. Thus, each partner contributes differently to the mRNA decay pathway. We find that both proteins interact with the basal decay factor Pat1p/Lsm1-7p via direct interactions with Pat1p and Lsm2p. However, the two-hybrid approach cannot indicate the relative contribution of each partner to these interactions. Nevertheless, as overexpression of

both proteins has a synergistic effect on genetic interactions with *PAT1*, it is likely that each protein contributes to the capacity of the heterodimer to recruit the Pat1p/Lsm1-7p complex. Thus, it is not likely that the interaction of the heterodimer with Pat1p/Lsm1-7p accounts for the specific role of Rpb4p in the decay pathway of PBF mRNAs. Therefore, we posit that there may exist other partners that interact specifically with Rpb4p or with Rpb7p that are required for modulating mRNA decay. For example, we propose that Rpb4p specifically interacts with a factor that modulates the decay of the very unstable PBF mRNAs. This scenario can explain why overexpression of *RPB7* cannot correct the abnormally slow decay kinetics of PBF in *rpb4Δ* cells (Lotan et al., 2005), reinforcing the different functions of the two partners in mRNA decay. A search for these putative factors is underway.

The role of the Rpb7p–Pat1/Lsm1-7 interaction

Rpb7p interacts with Pat1p and (to a lesser extent) with Lsm2p, two subunits of a key factor in the 5′ to 3′ mRNA decay pathway, the Pat1/Lsm1-7 complex. It is likely that this interaction represents one way that Rpb7p functions in mRNA decay, as defects in this interaction correlate with defective mRNA decay. The Rpb7p–Pat1p interaction might be important for recruiting the Pat1/Lsm1-7 complex to the mRNP, as Rpb7p is among the first proteins that interact with Pol II transcripts (Orlicky et al., 2001; Meka et al., 2003, 2005; Ujvari and Luse, 2006). Alternatively, interaction of Pat1p with Rpb7p might be required for subsequent Pat1p activity. Rpb7p may activate the capacity of Pat1/Lsm1-7 to stimulate decapping or may direct the complex to appropriate locations within the mRNP complex or the mRNA sequence. Further experiments are required to obtain detailed mechanistic understanding of the role of the Rpb7p-Pat1/Lsm1-7 interaction in mRNA decay.

rpb7 ts mutant cells efficiently recruit Pat1p to PBs at the permissive temperatures and this Pat1p does not dissociate from PBs after shifting to the nonpermissive temperature. We interpret these results to indicate that once bound to the mRNP at the permissive temperature, Pat1p does not dissociate from mRNP even if its association with Rpb7p is diminished. Like Dhh1p-GFP–containing PBs, Pat1p-GFP–containing PBs do not disappear rapidly during HS in the mutant cells, probably because mRNAs are degraded slowly.

Possible cross talk between mRNA degradation and transcription

The transcription and mRNA decay machineries are equally responsible for setting the mRNA steady-state levels; however, probable communication between these two processes has yet to be characterized. Being both Pol II subunits and mRNA decay factors, Rpb4p and Rpb7p represent promising candidates for mediators of such cross talk. Two of the four Rpb7p mutants that we analyzed in detail are not defective in transcription at the nonpermissive temperature (see Fig. S2). This suggests that the functions of Rpb7p in transcription and in mRNA decay can be uncoupled. It remains to be determined whether the nuclear and the cytoplasmic roles of Rpb4p and Rpb7p are related, and whether the two proteins are involved in any dialogue between mRNA synthetic and decay machineries.

Materials and methods

Yeast strains and plasmids

Yeast strains are listed in Table I. pMC116 is a high copy (2μ) plasmid carrying *RPB7* under its own natural promoter bordered by NotI–HapI sites. pMC257 is a *HIS3 CEN* derivative of pMC116. p*RPB4* 2μ is a high copy plasmid and was described previously as pMC4 2μ (Choder, 1993). To construct p*RPB4/7* 2μ, a fragment containing *RPB7* including 428 bp of the 5′ noncoding region and 436 bp of the 3′ noncodong region was inserted into SacII–BamHI sites of p*RPB4* 2μ.

Creating temperature-sensitive (ts) *rpb7* mutants

RPB7 was mutagenized randomly, using a PCR mutagenesis scheme as described previously (Farago et al., 2003), using oMC367 (5′-GCATGCATGTGCTCTGTATG-3′) and oMC368 (5′-GTTACATGCGTACACGCG-3′) with pMC116 as the template. Both primers recognize vector sequences flanking *RPB7*, which are identical in pMC116 and pMC257. The mutagenized *rpb7*-containing fragment was inserted into NotI–HapI-linearized pMC257 by cotransformation in yMC140. *pRPB7::URA3* present in yMC140 was evicted using 5-fluoroorotic acid (5-FOA) to select for cells that lose *URA3*, resulting in cells that carry only the mutagenized *rpb7* whose transcription is controlled by *RPB7* promoter. 5-FOA selection was done twice. 5-FOA–resistant strains were allowed to form colonies at 30 and 37°C. Mutants unable to form colonies at 37°C were selected.

Determining synthetic lethality between *rpb7* ts and *XRN1*

XRN1 was deleted from a strain whose sole Rpb7p was expressed from a *RPB7::URA3* plasmid to create yMC414. Next, yMC414 was transformed with *HIS3* plasmid carrying *RPB7* or mutant *rpb7*. Transformants were spotted in a tenfold serial dilution (starting with 10^5 cells per spot) on a 5-FOA–containing plate (this drug kills cells expressing *URA3*). Plates were incubated at 30°C for 3 d. In case of synthetic lethality, cells could not lose p*RPB7::URA3* and died. Note that all our *rpb7* mutants can proliferate well at 30°C if *XRN1* is present (see previous section).

Determining mRNA levels and mRNA degradation profile

To rapidly inactivate Rpb7p ts forms and simultaneously block transcription of non-HS genes, we shifted cells from 30°C (the permissive temperature) to 42°C. Northern analysis was done as described previously (Choder, 1991). mRNA decay assays were done as described previously (Lotan et al., 2005). The specific probe of *MFA2pG* mRNA was prepared as follows: MFA2pG was cut with BamH1 (56 bp upstream of (G)18). oMC609 (AATGAAAGGGTAGATATTGATT) was used in a primer extension reaction in the presence of 20 μCi of radiolabled dATP. The resulting fragment was 108 bases (the poly(G) was in the middle). This fragment was hybridized at high stringency as previously published (Choder, 1991), except that the hybridization temperature was 72°C. Membrane was then washed at 75°C. Under these conditions, the natural *MFA2* mRNA is undetected.

Fluorescent microscopy

Images of GFP-containing cells suspended in water were acquired with Nikon ACT-1, using a Nikon Eclipse E400, with Nikon Plan Apo 100×/1.40 oil objective. They were collected at room temperature as 1280 × 1024-pixel files with a digital camera (DXM 1200F; Nikon).

Two-hybrid assay

The bait *RPB7*-AD or AD tagged *rpb7* mutants were constructed according to Uetz et al. (2000). A two-hybrid interaction was determined by growth on plates lacking leucine, tryptophane, adenine, and histidine, supplemented with 5 mM 3-amino-1,2,4-triazole and also by β-gal liquid test, as reported previously (Uetz et al., 2000).

Online supplemental material

Fig. S1 shows the sequence of the Rpb7p mutant forms. Fig. S2, transcription capacity of *rpb7* mutants at 42°C. Fig. S3, decay of Tet-Off-MFA2 at 37°C. Fig. S4, deletion of *RPB4* displays synthetic lethality in combination with *rpb7* ts alleles. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200701165/DC1.

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Table I. **Yeast strains used in this study**

Strain	Genotype	Origin
yMC140	*MAT***a** *rpb7Δ::LEU2 ura3-52 his3Δ200 leu2-3,112 lys2Δ201 ade2* p*URA3/CEN/RPB7*	(McKune et al., 1993)
yMC229	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	EUROSCARF
yMC269	*MATa, can1-100 ade2 his3-11,15 leu2-3,112 trp1-1 ura3-1*	This work
yMC272	*MATα, pat1::TRP lys2 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	This work
yMC363	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δpat1*::KanMX4	EUROSCARF
yMC414	MATa, *rpb7Δ::LEU2 ura3, his3Δ200, leu2-3,112, lys Δ201, ade2, xrn1Δ*::KanMX4 p*URA3/CEN/RPB7*	This work
yMC436	*MAT***a** *rpb7Δ::LEU2 rpb4Δ::KAN ura3-52 his3Δ200 leu2-3,112 lys2Δ201 ade2* p*URA3/CEN/RPB7*	This work
yMS8	MATa, *his3, leu2, ura3 rpb4Δ::HIS3 RPB7*-GFP*::HIS3X6* p*URA3/CEN/RPB4*-RFP	(Selitrennik et al., 2006)
yRL11	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0 RPB7-GFP::HIS3X6* p*URA3/CEN/DCP2*-RFP	This work
yRL26	MATa, *rpb7Δ::LEU2 ura3-52, his3Δ200, leu3-3,112, lys2Δ201, ade2,* p*HIS3/CEN/rpb7-26*	This work
yRL28	MATa, *rpb7Δ::LEU2 ura3-52, his3Δ200, leu3-3,112, lys2Δ201, ade2, pHIS3/CEN/rpb7-28*	This work
yRL29	MATa, *rpb7Δ::LEU2 ura3-52, his3Δ200, leu3-3,112, lys2Δ201, ade2, pHIS3/CEN/rpb7-29*	This work
yRL34	MATa, *rpb7Δ::LEU2 ura3-52, his3Δ200, leu3-3,112, lys2Δ201, ade2, pHIS3/CEN/rpb7-34*	This work
yRL53	MATa, *rpb7Δ::LEU2 ura3-52, his3Δ200, leu3-3,112, lys2Δ201, ade2, pHIS3/CEN/RPB7*	This work
yRL72	*MAT***a** *rpb7Δ::LEU2 ura3-52 his3Δ200 leu2-3,112 lys2Δ201 ade2 PAT1*-GFP*:: HIS3X6* p*HIS3/CEN/rpb7-34*-RFP	This work
yRL74	*MAT***a** *rpb7Δ::LEU2 ura3-52 his3Δ200 leu2-3,112 lys2Δ201 ade2 PAT1*-GFP*:: HIS3X6* p*HIS3/CEN/rpb7-28*-RFP	This work
yRL78	*MAT***a** *rpb7Δ::LEU2 ura3-52 his3Δ200 leu2-3,112 lys2Δ201 ade2 PAT1*-GFP*:: HIS3X6* p*HIS3/CEN/RPB7*-RFP	This work
yRL93	*MAT***a** *rpb7Δ::LEU2 ura3-52 his3Δ200 leu2-3,112 lys2Δ201 ade2* p*HIS3/CEN/RPB7*-RFP p*URA3/CEN/DHH1*-GFP	This work
yRL95	*MAT***a** *rpb7Δ::LEU2 ura3-52 his3Δ200 leu2-3,112 lys2Δ201 ade2* p*HIS3/CEN/rpb7-28*-RFP p*URA3/CEN/DHH1*-GFP	This work
yRL97	*MAT***a** *rpb7Δ::LEU2 ura3-52 his3Δ200 leu2-3,112 lys2Δ201 ade2* p*HIS3/CEN/rpb7-34*-RFP p*URA3/CEN/DHH1*-GFP	This work
yVB32	MATa, *rpb7Δ::LEU2 ura3-52, his3Δ200, leu3-3,112, lys2Δ201, ade2, pHIS3/CEN/RPB7*, pTet-Off-*MFA2pG*	This work
yVB34	MATa, *rpb7Δ::LEU2 ura3-52, his3Δ200, leu3-3,112, lys2Δ201, ade2, pHIS3/CEN/rpb7-28*, p*URA3/CEN*/Tet-Off-*MFA2pG*	This work
yVB35	MATa, *rpb7Δ::LEU2 ura3-52, his3Δ200, leu3-3,112, lys2Δ201, ade2, pHIS3/CEN/rpb7-29*, p*URA3/CEN*/Tet-Off-*MFA2pG*	This work

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