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RNA Polymerase II Subunits Link Transcription and mRNA Decay to Translation

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SUMMARY

Little is known about crosstalk between the eukaryotic transcription and translation machineries that operate in different cell compartments. The yeast proteins Rpb4p and Rpb7p represent one such link as they form a heterodimer that shuttles between the nucleus, where it functions in transcription, and the cytoplasm, where it functions in the major mRNA decay pathways. Here we show that the Rpb4/7 heterodimer interacts physically and functionally with components of the translation initiation factor 3 (eIF3), and is required for efficient translation initiation. Efficient translation in the cytoplasm depends on association of Rpb4/7 with RNA polymerase II (Pol II) in the nucleus, leading to a model in which Pol II remotely controls translation. Hence, like in prokaryotes, the eukaryotic translation is coupled to transcription. We propose that Rpb4/7, through its interactions at each step in the mRNA lifecycle, represents a class of factors, "mRNA coordinators," which integrate the various stages of gene expression into a system.

INTRODUCTION

The production of a specific set of proteins at any given time is critical for achieving the appropriate phenotype in response to an ever changing environment. This process, carried out by the translation machinery, is therefore subject to robust and complex regulation at several levels involving various cellular compartments. Translation initiation is considered to be a key step in protein synthesis (Pestova et al., 2007; Sonenberg and Hinnebusch, 2009). Among the general initiation factors is the eukaryotic initiation factor 3 (eIF3) that, in yeast, is composed of 6 subunits, all of which, except for Hcr1p, are essential and stoichoimetric. eIF3 serves as a scaffold for the assembly of the multifactorial initiation complex by virtue of its capacity to bind many initiation factors (Hinnebusch, 2006).

Nontranslating mRNAs can accumulate in discrete complexes called processing bodies (PBs) that contain various proteins including many mRNA decay factors (Eulalio et al., 2007; Parker and Sheth, 2007). In addition, mRNPs seem to move back and forth between polyribosomes (polysomes) and PBs (Brengues et al., 2005; Coller and Parker, 2005). Consequently, any factor that can shift the equilibrium between the two movement pathways can regulate translation (and possibly also mRNA decay). The yeast mRNAs can reside in yet another type of RNP complex, called stress granule (SG), en rout from PBs to polysomes (Buchan et al., 2008).

A link between translation and mRNA decay has started to emerge in the last several years. The 5′ to 3′ decay of some yeast mRNAs is executed while these mRNAs are associated with polysomes (Hu et al., 2009). Several factors (e.g., Pat1p, Dhh1p) are known to control both processes (Coller and Parker, 2005). Moreover, decreasing translational initiation by a variety of means causes an increase in mRNA decay rate and the accumulation of PBs (Balagopal and Parker, 2009; LaGrandeur and Parker, 1999; Muhlrad and Parker, 1999; Schwartz and Parker, 1999, 2000; Teixeira et al., 2005). Conversely, inhibition of translation elongation leads to a significant decrease in both the rate of decapping (Beelman and Parker, 1994) and PB accumulation (Sheth and Parker, 2003; Teixeira et al., 2005). Unlike the coupling between translation and mRNA decay, little is known about possible cross talk between the cytoplasmic translation apparatus and Pol II in the nucleus.

Cross talk between the yeast Pol II and the cytoplasmic mRNA decay pathways has been uncovered previously. It involves the Pol II subunits Rpb4p and Rpb7p that form a heterodimer (Rpb4/7) that shuttles between the nucleus and cytoplasm (Selitrennik et al., 2006) and mediates both transcription (Choder, 2004) and the two major cytoplasmic mRNA decay pathways (Lotan et al., 2007; Lotan et al., 2005). Although Rpb4/7 binds Pol II transcripts cotranscriptionally (Ujvári and Luse, 2006) and is associated with the mRNA throughout its life (Goler-Baron et al., 2008), it is not known whether Rpb4/7 has any impact on the mRNA life in the cytoplasm, except for stimulating its demise.

Here we show that Rpb4/7 interacts with the eIF3 components Nip1p and Hcr1p and stimulates translation initiation. Provoked by the capacity of Rpb4/7 to interact with factors mediating other stages of gene expression, we propose that Rpb4/7 serves as a coordinator of all the major stages in the mRNA lifecycle. Hence, we coined the term "mRNA coordinator." As shown here, the execution of the posttranscriptional functions of the mRNA coordinator is dependent on its recruitment to Pol II in

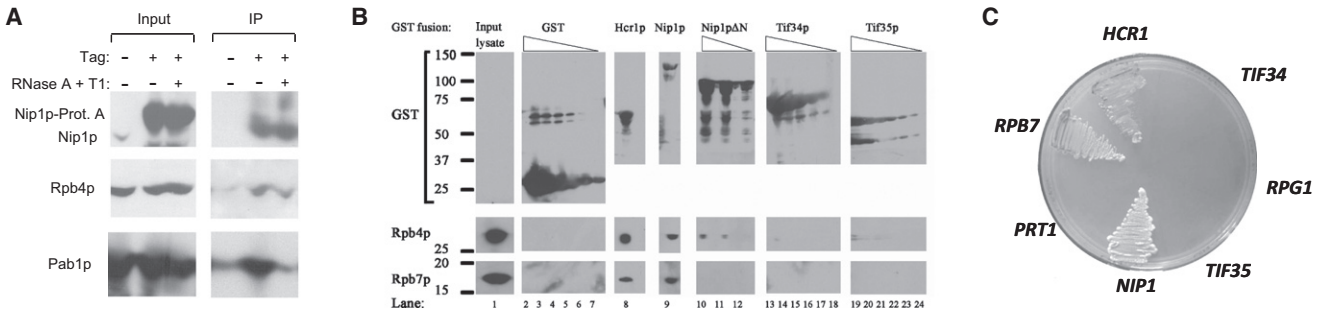

Figure 1. Rpb4/7 Physically Interacts with Components of eIF3

(A) Rpb4p interacts with Nip1p by RNase insensitive manner. Extracts from control cells or cells expressing Nip1p-Protein-A, as indicated, were immunoprecipitated (IPed) using IgG sepharose and processed for western analysis using the indicated antibodies. When indicated, a cocktail of RNase A (20 U/ml) and RNase T1 (750 U/ml) (Ambion) was added 30 min before IP begun. Input – whole cell extract. Note that Nip1p-Protein A interacts with the secondary antibodies directly, resulting in stronger signal.

(B) Rpb4p and Rpb7p are specifically pulled-down by GST-Hcr1p and GST-Nip1p. Pull down assay between the indicated GST-fusion proteins and extract of cells overexpressing both *RPB4* and *RPB7* was performed as described (Asano et al., 1998). Nip1pΔN lacks 156 AA of Nip1p N terminus (Hinnebusch B3753) (Asano et al., 1998). To obtain the full range of binding of Rpb4p and Rpb7p with the various GST-fusion proteins, decreasing amounts (in 2-fold dilutions) were analyzed, when indicated. We have similarly found interactions between purified Rpb4/7 and Nip1p-GST and Hcr1p-GST (data not shown).

(C) Two hybrid interaction assay between Rpb4p as the bait and each of the indicated eIF3 components. Rpb7p is shown as a positive control. The assay was performed as described previously (Lotan et al., 2005).

See also Figure S1.

the nucleus, placing Pol II as a key regulator of the major stages of the gene expression system.

RESULTS

Rpb4p Interacts with Components of eIF3

To examine whether Rpb4/7 functions outside the context of Pol II, we compared between proteins associated with the core Pol II subunit, Rpb3p-TAP, and Rpb4p-TAP to identify proteins specifically associated with Rpb4p-TAP (Figure S1 available online). Several bands were detected in the Rpb4p-containing complexes that were not purified by Rpb3p-TAP, among them the eIF3 components Rpg1p and Nip1p (Figure S1). Association of Rpb4p and Rpb7p with Nip1p was verified and confirmed by coimmunoprecipitation and/or GST pull-down assays (Figures 1A and 1B). Including RNases before and during the IP experiment led to the disappearance of Pab1p from Nip1p-containing complex, but not Rpb4p, indicating that the interaction between Rpb4p and Nip1p is not mediated by RNA. Interestingly, Nip1p lacking 156 residues from the N terminus, a domain that interacts with eIF1 and eIF5 (Asano et al., 2000), pulled-down Rpb4/7 poorly (Figure 1B, cf lane 9 and 12). Nip1p and Hcr1p, but no other eIF3 subunits examined here, formed a pair-wise two hybrid interaction with Rpb4p (Figure 1C). No two hybrid interactions could be detected between any of the eIF3 subunits and Rpb7p (data not shown). Finally, the interaction between Rpb4/7 and GST-Hcr1p was corroborated by GST pull-down assay (Figure 1B). Taken together, our results are consistent with a direct interaction between Rpb4p and Hcr1p and Nip1p.

rpb4Δ and rpb7-26 Cells Are Hypersensitive to Translation Inhibitors

The interaction of Rpb4/7 with components of the translation initiation complex prompted us to investigate the involvement of Rpb4/7 in translation. As a first approach, we found that *rpb4Δ* cells are hypersensitive to drugs that target translation, i.e., paromomycin and anisomycin (Figure S2A) and cycloheximide (CHX) (data not shown). These inhibitors have been previously used to identify mutants with defects in translation, and their effect is insensitive to growth rate (Gross et al., 2007; Ruiz-Echevarría et al., 1998). We next screened a collection of *rpb7* temperature-sensitive (ts) mutant alleles (Lotan et al., 2007), and found that several of them exhibited hypersensitivity to these drugs at the permissive temperature (30°C) (data not shown). One of them, *rpb7-26* (Figure 2A) was selected for further analyses because it is not defective in transcription under all tested conditions (Lotan et al., 2007) and see also Figure S4A). Moreover, this mutant exhibits WT mRNA decay rates at 30°C; only at the nonpermissive temperature ($\geq$37°C) is mRNA decay defective in this mutant (Lotan et al., 2007).

Genetic Interactions between RPB4 and Genes Encoding Translation Factors

Under optimal conditions, cells lacking both *RPB4* and *HCR1* are viable. However, upon entry into stationary phase, deletion of the two genes is deleterious ("synthetic sickness") (Figure 2B). Furthermore, overexpression of *HCR1* had little effect on the proliferation rate of WT cells. In contrast, *rpb4Δ* and *rpb7-26* cells could not proliferate under these conditions (Figures 2C and 2D). Similarly, both mutants were hypersensitive to overexpression of *CAF20* (Figures S2B and S2C), encoding a negative translation modulator (Altmann et al., 1997; de la Cruz et al., 1997; Ptushkina et al., 1998). Overexpression of *HCR1* adversely affected polysomal accumulation (Figure 2E), possibly by titrating out some limiting component(s) of the translation initiation complex. Whereas this could be tolerated by WT cells, *rpb7-26* cells exhibited very little translation (Figure 2E), which seems to be below the threshold required for cell division (Figure 2D). Collectively, these results indicate that *rpb7-26* and *rpb4*Δ cells are hypersensitive to modulations in translation.

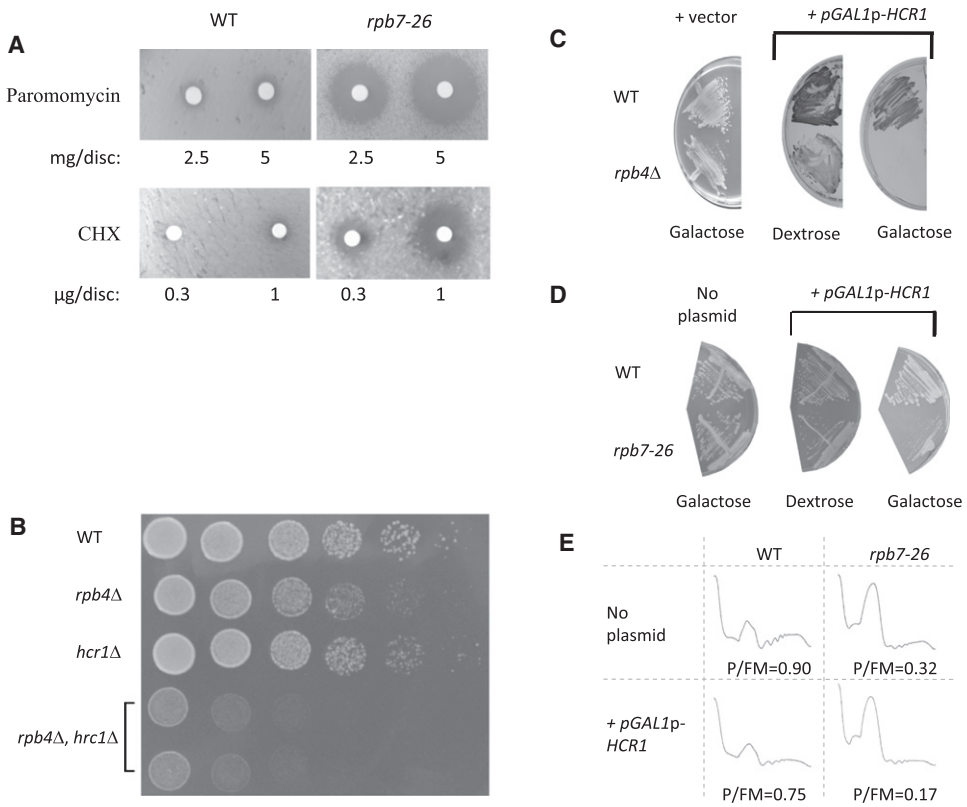

Figure 2. Cells Lacking *RPB4* or Carrying the *rpb7-26* Allele Are Hypersensitive to Drugs or Genetic Manipulations that Affect Translation

(A) Lawns of the indicated cells were grown on plates with filter disks containing the indicated translation inhibitors. The radius of the zone of inhibition reflects the drug's critical inhibitory concentration.

(B) The indicated strains were allowed to enter stationary phase in rich medium (YPD). Two weeks later, equal aliquots of cells were spotted in 5-fold serial dilutions on YPD plate, which was icubated at 24°C for 2 days. Two independent clones of the double mutant are shown.

(C and D) Optimally growing cells carrying the indicated plasmids were streaked on selective plates containing dextrose (represses expression) or galactose (induces overexpression). Cells lacking the plasmid in (D) were plated on the same selective plate supplemented with uracil. The plates were incubated at 30°C for several days.

(E) WT and *rpb7-26* cells were allowed to proliferate in dextrose-containing selective medium until midlog phase. Cultures were then washed twice with water and shifted to galactose-containing selective medium for 7 hr before polysomal profiles were determined as described in Experimental procedures. The ratio between polysomal RNA and free RNA + monosomal RNA (designated P/FM) is depicted below each profile.

See also Figure S2.

Rpb4/7 Regulates Translation

To examine more directly if Rpb4/7 is involved in translation, we compared the protein synthetic rate and polysome profiles of WT, *rpb4Δ* and *rpb7-26* cells . Importantly, under optimal conditions at 30°C, *rpb7-26* cells synthesize and degrade mRNAs normally (Lotan et al., 2007), see also Figure S4A). Figures 3A and 3C show that efficient translation is dependent on Rpb4p and Rpb7p, as evident by the abnormally slow incorporation kinetics of [^{35}S]-methionine in the mutant cells. Consistently, polysomal profiles of extracts derived from the mutant strains exhibit an abnormally low proportion of polysomes ("P/FM") (Figures 3B and 3D). Defective profile can be evaluated by the ratio between polysomal and sub-polysomal signals in the mutant divided by the same ratio in the WT (P/FM mutant:P/FM WT). By definition, the ratio of WT/WT pair is 1. These ratios were 0.18 (0.30/1.67) (Figure 3B), and 0.27 (0.17/0.63) (Figure 3D).

An additional assay to test translation efficiency is to examine the presence of P bodies (PBs) (Balagopal and Parker, 2009; Brengues et al., 2005; Coller and Parker, 2005). During proliferation under optimal conditions, PBs were not detectable in WT cells, as expected (Decker et al., 2007; Teixeira et al., 2005), whereas they were readily observed in *rpb7-26* cells (Figure 3E, and Figure 4A "Optimal conditions" panel). We argue that accumulation of PBs in the mutant cells is due to a defect in translation initiation. This is based on inverse correlation between polysomes and PBs (Brengues et al., 2005; Coller and Parker, 2005) and because accumulation of PBs in *rpb7-26* cells cannot be attributed to slow mRNA decay which is normal under the conditions used here (Lotan et al., 2007).

Consistent with its effect on the polysomal profile, Rpb4p was shown to be physically associated with polysomes. Detecting association of Rpb4p with polysomes was dependent on blocking translation elongation by CHX (Figure 3F), consistent with association of Rpb4p with translationally active ribosomes (Moldave, 1985; Ramirez et al., 1991; Wyers et al., 2000). To rule out the contribution of Pol II on Rpb4p cosedimentation with

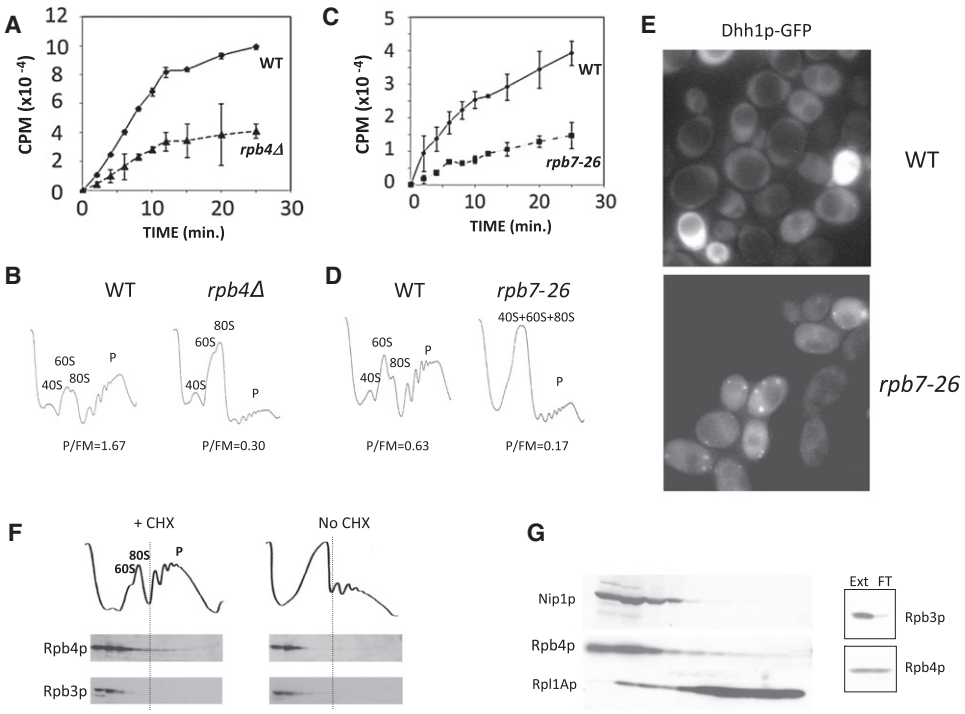

Figure 3. Rpb4p and Rpb7p Are Involved in Translation

(A and B) *RPB4* is required for efficient translation. (A) Incorporation of [^{35}S]-Methionine by optimally proliferating cells (at 24°C) was determined as described in Experimental Procedures. Data are represented as mean of two independent experiments, each one is based on triplicates, ± SD. P value was calculated for any time point, using Student's t test. P values for 2′ and 4′ are < 0.02 and the values for 6′ and later time points are < 0.0007. (B) Polysomal profile of optimally proliferating WT and *rpb4Δ* strains was performed as detailed in Experimental procedures. P/FM was determines as in Figure 2E.

(C and D) *rpb7-26* cells exhibit poor translation capacity. (C) Data are represented as mean of two independent experiments, each one is based on triplicates, ± SD. P value was calculated for any time point, using Student's t test. P values for 2′ is 0.01 and those for 6′ and later time points are < 0.0004. (D) Polysomal profile of optimally proliferating WT and *rpb7-26* strains was performed as in (B).

(E) Optimally proliferating *rpb7-26* cells accumulate abnormally high levels of PBs. Cells expressing the PB marker Dhh1p-GFP (Sheth and Parker, 2003) were allowed to proliferate under optimal conditions at 30°C for at least 18 hr until 5 × 10^6 cells/ml. Cells were collected by centrifugation, resuspended in the same medium and immediately examined by fluorescence microscopy. The same results were obtained using Pat1p-GFP as the PB marker (data not shown).

(F) Rpb4p associates with polysomes in a CHX-dependent manner. Whole cell extracts of WT cells carrying *RPB3*-TAP were prepared from optimally proliferating cells that were either treated with CHX or left untreated ("No CHX"). Polysomal profiles were obtained using gradients that contained or lacked CHX, respectively, (top panels) and the corresponding fractions (excluding fraction 1) were analyzed by western blotting with anti-Rpb4p or anti-TAP antibodies (bottom panels).

(G) Rpb4p from a Pol II-depleted extract associates with polysomes. An extract of CHX-treated Rpb3-TAP-expressing cells was depleted of Pol II complexes using an IgG sepharose column that binds Rpb3-TAP. Depletion of 90% of Rpb3-TAP was achieved (compare extract with flow-through; right panel), whereas depletion of Rpb4p was negligible (since most Rpb4p is not associated with Pol II –see text). The Pol II-depleted flow-through material was fractionated through a standard sucrose gradient (containing CHX). Fractions were analyzed by western blotting to detect the indicated proteins. Rpl1p is ribosomal protein.

See also Figure S3.

polysomes, we cleared the extract from Pol II complex by affinity purifying Rpb3-TAP. Affinity purification of Rpb3p-TAP was very efficient, whereas the depletion of Rpb4p was negligible (Figure 3G, right panel). This is because Rpb4p is present in vast excess over other Pol II subunits (Choder, 2004; Rosenheck and Choder, 1998). For example, in stationary phase, Rpb4/7 binds Pol II in a stoichiometric manner (Choder and Young, 1993), nevertheless, most Rpb4/7 molecules are found in the cytoplasm, away from Pol II (Farago et al., 2003). Figure 3G (left panel) shows that cosedimentation of Rpb4p with polysomes was not affected by the preclearance of Pol II.

If Rpb4/7 is required for translation initiation, defects in this feature may adversely affect mRNA movement from PBs to polysomes (see Introduction). We took advantage of the observation that, in response to starvation, mRNAs are stored in PBs and excluded from polysomes (Brengues et al., 2005; Paz and Choder, 2001; Teixeira et al., 2005). When cells are refed with rich medium, mRNAs move from PBs to polysomes and translation resumes (Brengues and Parker, 2007; Brengues et al., 2005; Teixeira et al., 2005). As shown in Figure 4A, in response to refeeding WT cells with fresh medium, polysomes rapidly accumulated and PBs rapidly dissociated. In contrast, *rpb7-26* cells failed to efficiently accumulate polysomes or to disassemble PBs. Careful examination indicated that after 5′ of refeeding, the mutant cells regain ~36% of their full polysomal content (0.207/0.579), whereas WT cells regain only ~20%. We suspect that the mutants attain their (reduced) steady state level faster than WT, but additional time points are required to determine

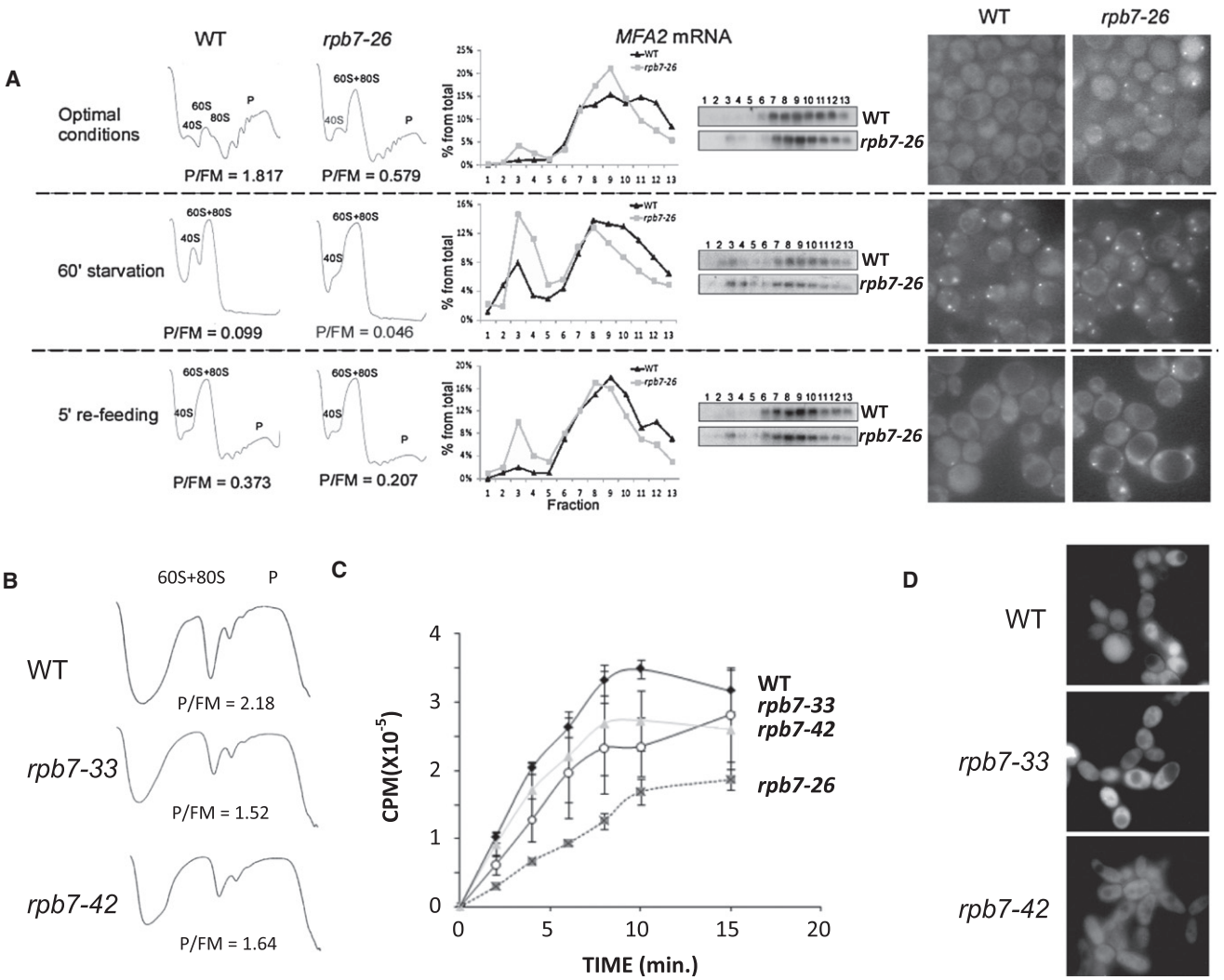

Figure 4. Rpb4/7 Is Required for Efficient Accumulation of Polysomes upon Refeeding of Starved Cells and Affects the Interplay between Polysomes and PBs

(A) *rpb7-26* cells are defective in accumulation of polysomes, association of *MFA2* mRNA with polysomes and disassembly of PBs in response to refeeding. WT and *rpb7-26* cells were allowed to proliferate in rich synthetic medium until midlog phase (1×10^7 cells/ml). Cell samples were collected from the culture ("Optimal conditions"), and the remaining culture was either starved for 1 hr, or starved for 1 hr followed by 5 min refeeding. Polysomal profiles were obtained as in Figure 3D (left panel), and *MFA2* mRNA in each fraction was detected by northern blotting and quantified by PhosphoImager (middle panel). Cells were analyzed by fluorescence microscopy to visualize Pat1p-GFP-containing PBs (right panel). Similar results were obtained using Dhh1p-GFP (data not shown).

(B–D) *rpb7* mutant strains that are not defective in translation. The indicated strains were analyzed as in panel "Optimal conditions" in (A), (B and D), or as in Figure 3A (C). Data in (C) are represented as mean of three experiments ± SD.

See also Figure S4.

the kinetics. We then examined *MFA2* mRNA because its accumulation in polysomes during refeeding is insensitive to transcription (Brengues and Parker, 2007). During optimal conditions and during starvation, *MFA2* mRNA was translated poorly in *rpb7-26* cells, as manifested by its association with fewer ribosomes and the high proportion of ribosome-free mRNAs in fraction 2 and 3 (Figure 4A). During these conditions, *rpb7-26* cells express normal levels of *MFA2* mRNA (Figure S4A), consistent with their normal capacity to synthesize and degrade mRNAs at 30°C (Lotan et al., 2007). The mutant cells failed also to efficiently assemble *MFA2* mRNA with polysomes after 5′ refeeding, as manifested by the percent shift from fractions 2–4 into the polysomes (42% in *rpb7-26* cells; 77% in WT), despite their efficient capacity to transcribe *MFA2* (Figure S4A, refeeding). Taken together, Figure 4A and S4A indicate that *MFA2* mRNA distribution represents the actual translatability of this mRNA at any given time, regardless of its level. Collectively, we conclude that *rpb7-26* cells are defective in translation initiation and in PBs disassembly in response to refeeding. Like *rpb7-26* cells, starved *rpb4Δ* cells failed to efficiently assemble polysomes in response to refeeding (Figure S3A). We argue that because translation initiation and PBs disassembly are defective in the

mutant cells, not only the kinetics of polysomes and PBs assembly/disassembly is defective; also the steady state levels (during optimal growth conditions) of both polysomes and PBs are abnormal in these cells. This steady state level is one manifestation of the balance between the rates of PBs assembly and disassembly.

We next directly determined the involvement of Rpb4/7 in the dissociation of *MFA2* mRNA from PBs. *MFA2*-U1A mRNA can be detected in vivo by U1A-GFP that binds the U1A site, placed in the 3′ untranslated region of *MFA2* mRNA (Brengues et al., 2005; Brodsky and Silver, 2000). *MFA2*-U1A mRNA accumulates in PBs during stationary phase, whereas in response to refeeding it dissociates from PBs in a transcription-independent and mRNA degradation-independent manner and assembles with polysomes (Brengues et al., 2005). We found that efficient dissociation of *MFA2*-U1A mRNA-containing PBs is dependent on Rpb4p (Figures S3B and S3C). Importantly, cells lacking *RPB4* deadenylate and further degrade *MFA2* mRNA comparably to WT cells (Lotan et al., 2005); thus, any difference between WT and *rpb4*Δ cells in PB dissociation cannot be attributed to mRNA decay.

Interestingly, we found that, also during stationary phase, mRNAs can leave PBs and associate with ribosomes despite strong translational repression. Consequently, CHX treatment, which blocks mRNA movement from polysomes to PBs (Brengues et al., 2005), led to a substantial decline in PB number (Figure S3D), albeit more slowly than that observed in proliferating cells. This relatively slow dissociation allowed us to determine possible changes between strains more precisely. Significantly, CHX-induced PB dissociation occurred more slowly in *rpb7-26* cells as compared to WT (Figure S3D). Thus, during long-term starvation, when translation is strongly repressed (Fuge et al., 1994; Paz and Choder, 2001), PBs remain relatively dynamic complexes from which mRNAs can leave by a Rpb4/7-mediated process to assemble with ribosomes. Consistently, also during stationary phase, *rpb7-26* cells incorporated [^{35}S]-Met abnormally slowly (Figure S3E). It is quite possible that mRNA association with ribosomes is more transient during stationary phase than during optimal conditions, while its association with PBs is relatively long-lived.

In summary, in the absence of *RPB4* or when *RPB7* is replaced with the *rpb7-26* mutant allele, both the dissociation of mRNAs from PBs and the assembly of mRNAs with polysomes are defective, consistent with a role for Rpb4/7 in translation initiation. A plausible scenario is that the interaction of Rpb4/7 with eIF3 stimulates mRNA release from PB and assembly of polysomes. However, in the absence of detailed kinetics, this conclusion is tentative.

As indicated above, although Rpb7p is involved in transcription and mRNA degradation, Rpb7-26p is specifically defective in translation. As controls, we analyzed *rpb7* mutant strains that maintain abnormally low levels of mRNAs (Figure S4B) and proliferate slowly (Figure S4C). These mutants exhibit only a 25%–30% decrease in their overall polysomal profile and a small decrease in their capacity to incorporate ^{35}S-Met (Figures 4B and 4C), probably due their abnormally low levels of mRNAs. Moreover, during optimal proliferation, they do not accumulate PBs (Figure 4D). In contrast, optimally proliferating *rpb7-26* cells maintain high levels of mRNAs (Figure S4A), yet exhibit over 3-fold decrease in their polysomal content, poorly incorporate ^{35}S-Met and accumulate PBs (Figures 4A and 4C). Moreover, polysomal profile of *HYP2* mRNA in *rpb7-33* and *rpb7-42* strains is comparable to that of WT (Figure S4E), unlike the case of *rpb7-26* strain (Figure S4D). These results demonstrate that, by using *rpb7-26, rpb7-33*, and *rpb7-42*, mRNA translatability can be uncoupled from its level.

rpb6^{Q100R} Cells Exhibit a Defective Polysomal Profile at Optimal Conditions and during Exit from Starvation

The key question that the above findings raise is whether the function of the Rpb4/7 heterodimer in translation is mechanistically coupled to its transcriptional activities. To address this question, we asked whether recruitment of Rpb4/7 to Pol II is required for its function in translation. Previously, we demonstrated that efficient interaction of Rpb4/7 with pol II is a prerequisite for its ability to stimulate mRNA degradation (Goler-Baron et al., 2008). Since translation and mRNA decay are intimately linked (see Introduction), we applied the same approaches used in the previous study to examine whether Pol II can impact also translation. Specifically, we took advantage of a mutant Pol II core that comprises Rpb6^{Q100R}p. This mutant core binds Rpb4/7 poorly (retaining ~25% of its original binding capacity), as the Q100R substitution in Rpb6p alters one of the very few residues in the Pol II "pocket" responsible for direct contact with the "tip" of Rpb7p (Armache et al., 2005; Bushnell and Kornberg, 2003; Tan et al., 2003). *rpb6^{Q100R}* cells displayed an abnormal polysomal profile (P/FM mutant:P/FM WT = 0.47) (Figure 5A). This abnormal profile is observed despite comparable levels of mRNAs in the mutant and WT cells (Goler-Baron et al., 2008). The Q100R substitution in Rpb6p compromises association of Rpb4/7 with mRNAs (Goler-Baron et al., 2008), raising the possibility that this substitution adversely affects the association of Rpb4/7 with polysomes. To better detect Rpb4p in the polysomal fractions, cells were crosslinked with HCHO prior to lysis. Crosslinking has been demonstrated to be a reliable approach for determining the protein composition of polysomes in vivo (Valásek et al., 2007). The lysis buffer contained high salt (0.5M) to dissociate any proteins from the ribosomes that had not been cross-linked in vivo. Figure 5A (lower panel) shows that less Rpb4p associated with polysomes in the mutant cells compared to WT cells, suggesting that association of Rpb4/7 with polysomes requires its prior association with Pol II transcripts in the nucleus. Similar results were obtained using a standard procedure involving no crosslinking, except that less Rpb4p was associated with the polysomes (data not shown, see Figure 3F). The crosslinking approach reveals that about half of the Rpb4p molecules are engaged in translation. Since Rpb4/7 is present in vast excess over the other Pol II subunits (Choder, 2004), this proportion seems reasonable. Nevertheless, this proportion seems to be in contrast with the strong nuclear signal of Rpb4-GFP during optimal proliferation conditions (Choder, 2004). We argue that the strong nuclear signal, observed earlier, resulted from the higher local concentration of the nuclear Rpb4-GFP over the cytoplasmic portion due to the much smaller volume of the former compartment.

To further test the possibility that the capacity of Pol II to recruit Rpb4/7 affects translation, we stimulated translation by feeding

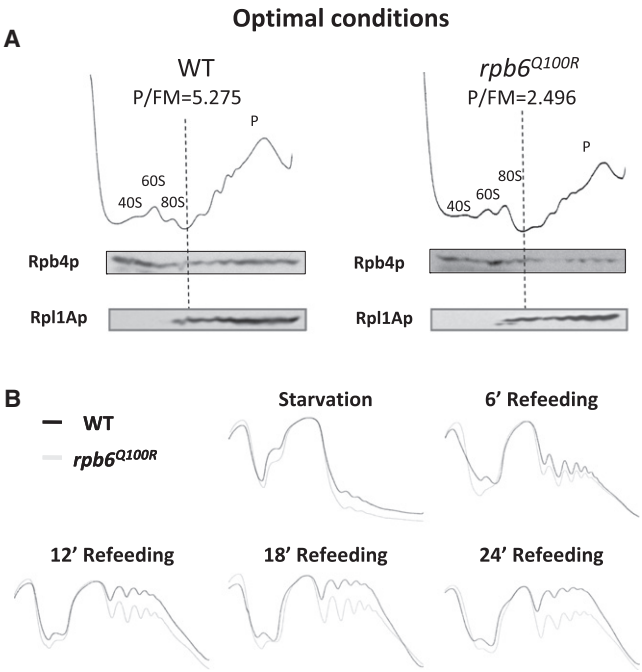

Figure 5. *rpb6*^{Q100R} Cells Are Defective in Translation

(A) Polysomal profile and association of Rpb4p with polysomes are defective in *rpb6*^{Q100R} cells. Cells were cultured in rich medium until midlog phase. Cross-linking was performed and polysomal profiles were obtained as described in Experimental Procedures (upper panels). The polysomal fractions were subjected to western analysis, using the indicated antibodies (lower panels).

(B) Cells were allowed to proliferate in rich synthetic medium until midlog phase (1×10^7 cells/ml). The cultures were either starved for 1 hr, or starved for 1 hr followed by refeeding with fresh rich medium for the indicated times. See also Figure S5.

starved cells with fresh medium. As shown in Figure 5B and Figure S5, the mutant cells accumulated polysomes more slowly than the WT cells. The slow translational response of *rpb6*^{Q100R} cells, which also characterizes *rpb4Δ* (Figure S3A) and *rpb7-26* mutant cells (Figure 4A), is consistent with the poor binding of Rpb4/7 to mRNAs (Goler-Baron et al., 2008) and to polysomes (Figure 5A). Consistently, *rpb6*^{Q100R} cells are hypersensitive to paromomycin and CHX (data not shown).

Like rpb6^{Q100R} Cells, rpb1$^{C67S;\ C70S}$ Cells Exhibit Abnormal Translation Phenotypes

Finally, we took additional approach to verify that the poor interaction of Rpb4/7 with Pol II compromises translation. We reasoned that if the inability of *rpb6*^{Q100R} cells to mount efficient translation upon refeeding is, indeed, due to the poor interaction between Rpb4/7 and Pol II, then mutations in another Pol II subunit, which also binds Rpb4/7 and affects its recruitment to Pol II, should result in a similar phenotype. Thus, we investigated the translation phenotype of a strain carrying C67S and C70S substitutions in Rpb1p, which have been reported to compromise recruitment of Rpb4/7 to Pol II (Donaldson and Friesen, 2000). The polysomal profile of an extract from optimally growing *rpb1*$^{C67S;\ C70S}$ cells, as well as the association of *MFA2* and *HYP2* mRNAs with polysomes in these cells, were defective (P/FM mutant:P/FM WT = 0.58) (Figure 6A). Moreover, upon refeeding of starved cells, the mutant cells accumulated polysomes slower than WT (Figure 6B). As a control, we used cells carrying D261N mutations in Rpb1p, located outside the pocket region in Pol II that interacts with the Rpb7p tip (Armache et al., 2003, 2005; Bushnell and Kornberg, 2003; Donaldson and Friesen, 2000). These cells, whose transcription is defective (Malagon et al., 2006), exhibited a normal polysomal profile upon refeeding (Figure 6C). Moreover, unlike *rpb1*$^{C67S;C70S}$ cells, *rpb1*^{D261N} cells are not hypersensitive to CHX (Figure 6D).

Collectively, the results shown in Figure 5 and Figure 6, together with the previously reported results that led us to propose a role for Pol II in mRNA decay (Goler-Baron et al., 2008), support our model that Pol II can regulate translation by stimulating the association of Rpb4/7 with mRNAs in the nucleus, and later with polysomes in the cytoplasm.

Cells Expressing Rpb4p Mutant Form that Is Localized Mainly in the Cytoplasm Fail to Mount Normal Translation

We propose that cotranscriptional association of Rpb4/7 with mRNAs is important for its function later in translation. Hence, failure of Rpb4/7 to import to the nucleus should compromise translation. To examine this possibility, we used Rpb4p mutant form (*rpb4-25*), carrying K80N; N200D substitutions, which is localized mainly in the cytoplasm (Figure S6A). The K80N mutation is in the middle of a basic motif. As shown in Figure S6B, this motif can function as a nuclear localization sequence (NLS), and the K to N substitution compromises this capacity. Interestingly, GFP-Rpb4-25p is localized dispersedly in the cytoplasm as well as in granules. These granules are not PBs or SGs, as they are unaffected by CHX treatment, unlike Edc3p-RFP, and are not colocalized with Edc3p-RFP (Figure S6C). The nature of these granules remains to be determined. Cells expressing this mutant, like *rpb4Δ* cells, are defective in translation, as determined by their abnormal polysomal profile, by their abnormal incorporation of ^{35}S-Met into proteins and abnormal polysomal profile of *MFA2* mRNA (Figure S6D–S6F). These results reinforce our argument that Rpb4p can function in translation initiation in the cytoplasm only if it can be imported to the nucleus.

DISCUSSION

Rpb4/7 Stimulates Translation Initiation

Here we show that Rpb4/7, a heterodimer that can be cocrystallized together with Pol II complex and is involved in transcription, mRNA export and mRNA decay, stimulates translation initiation. Our results indicate that Rpb4/7 functions, together with eIF3, in stimulating translation initiation. Importantly, this function is more apparent during starvation (e.g., see Figure 2B, Figure 4A, and Figure S3E), suggesting that the major role of Rpb4/7 in translation is to permit appropriate responses to environmental cues.

A direct role of Rpb4/7 in translation initiation is supported by the following key findings: (1) Rpb4p interacts with eIF3 components physically (Figure S1 and Figures 1A and 1B), genetically, and functionally (Figures 2B–2E). Functional interaction between

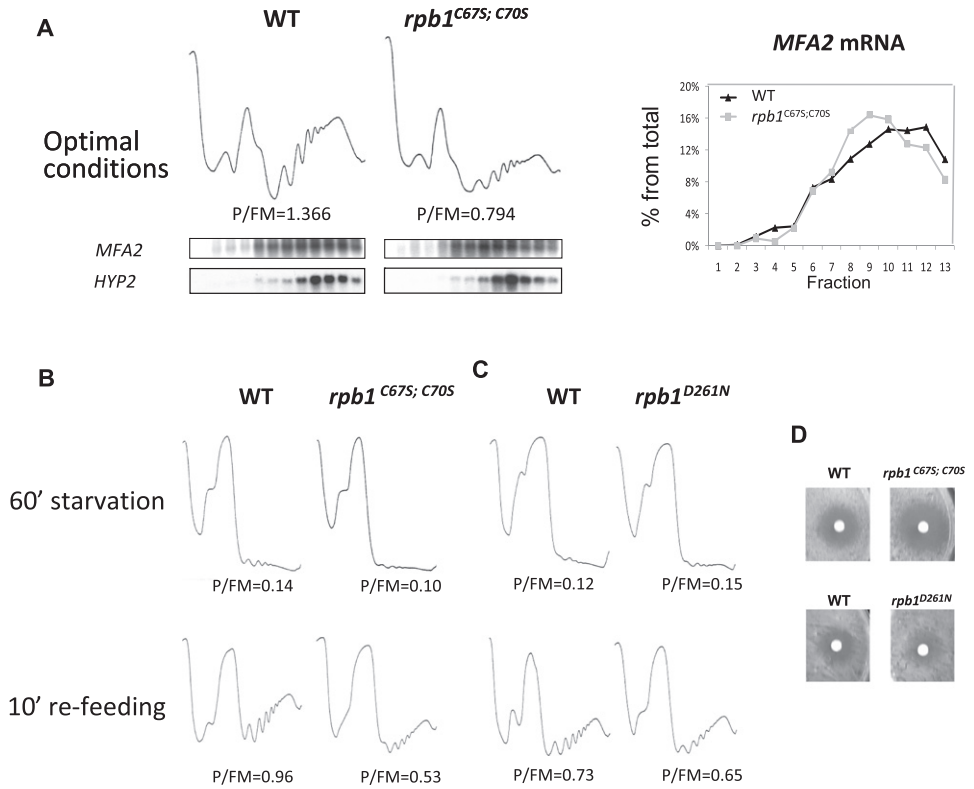

Figure 6. *rpb1*$^{C67S;\ C70S}$ Cells Are Defective in Translation

(A) Optimally proliferating *rpb1*$^{C67S;\ C70S}$ cells accumulate abnormally low levels of polysomes. Polysomal profiles were obtained from WT and *rpb1*$^{C67S;\ C70S}$ cells and the level of *MFA2* and *HYP2* mRNAs in each fraction was assessed by northern blotting (left panel) and quantified (right panel).

(B and C) *rpb1*$^{C67S;\ C70S}$ cells, but not *rpb1*^{D261N} cells, are defective in accumulation of polysomes upon refeeding of starved cells. Starved cells (1 hr) were refed with fresh medium for 5 min followed by polysomal fractionation as described in Experimental Procedures.

(D) *rpb1*$^{C67S;\ C70S}$ but not *rpb1*^{D261N} cells are hypersensitive to cycloheximide (see Figure 2A).

See also Figure S6.

Hcr1p and Rpb4/7 is demonstrated by the synergistic effect of overexpression of *HCR1* in combination with the *rpb7-26* allele (Figures 2D and 2E) or deletion of *RPB4* (Figure 2C), or by the synthetic sickness of *hcr1Δ* and *rpb4Δ* cells as they enter stationary phase; (2) Rpb4p cosediments with polysomes (Figures 3F and 3G and Figure 5A) in a CHX-dependent (i.e., translation-dependent) manner (Figure 3F); (3) *rpb4Δ* and *rpb7-26* cells cannot tolerate high levels of Caf20p, which represses translational initiation (Figures S2B and S2C); (4) The overall ratio between polysomal and subpolysomal fractions is abnormally low in *rpb4Δ* or *rpb7-26* cells (Figures 3B and 3D, and Figure 4A); (5) Under optimal conditions, abnormally high levels of PBs are detected in *rpb7-26* (Figure 3E and Figure 4A) and *rpb4Δ* mutant cells (Lotan et al., 2005). This feature also characterizes *prt1-1* cells (eIF3 subunit) at the nonpermissive temperature (Teixeira et al., 2005) ; (6) During exit from stationary phase, *MFA2*-containing PBs disappear abnormally slowly in *rpb4Δ* cells (Figures S3B and S3C); (7) During exit from stationary phase or from short sugar starvation, efficient methionine incorporation (data not shown), assembly of polysomes as well as assembly of *MFA2* mRNA with polysomes is dependent on WT Rpb4/7 (Figure 4A); (8) *rpb4Δ* or *rpb7-26* cells are hypersensitive to drugs that target the translation apparatus (Figure 2A and Figure S2A).

Rpb4/7 can modulate translation by various possible mechanisms that are not mutually exclusive. First, Rpb4/7 can stabilize the association of mRNP with eIF3. This might contribute to the stabilization of the complex that links the 5′ and 3′ ends of the mRNA (see Figure 7). Second, Rpb4p can stabilize association of the substoichiometric eIF3 component Hcr1p with the eIF3 core, by virtue of its capacity to bind both Hcr1p and Nip1p. Third, being a constituent of both PBs (Lotan et al., 2007, 2005) and the translation apparatus (this work), Rpb4/7 can stimulate the movement of mRNA from PBs to polysomes. Consistently, more PBs and less polysomes are observed in *rpb7-26* mutant cells relative to WT cells (Figures 3B, 3D, and 3E, and Figure 4A), and efficient movement of mRNA from PB to polysome during exit from starvation is dependent on *RPB4* (Figure S3). Last, by virtue of its capacity to interact with Nip1p N-terminal domain, which also interacts with eIF1 and eIF5 (Asano et al., 2000), Rpb4/7 might modulate recruitment or release of eIF1 and eIF5 to or from the translation apparatus.

RNA Polymerase II Controls the Performance of Its Products in the Cytoplasm

Although Rpb4/7 is present in excess over Pol II molecules, the interaction of Rpb4/7 with the mRNA occurs only in the context

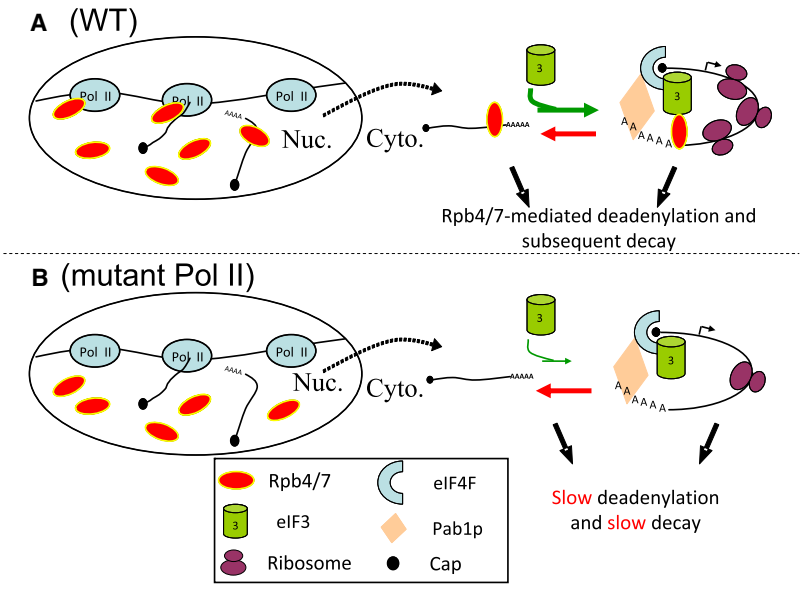

Figure 7. Model Illustrating How Pol II Controls Translation via Rpb4/7

(A) A role proposed for WT Pol II. Only a small portion of the nuclear Rpb4/7 is recruited to Pol II and is involved in transcription initiation (Choder, 2004), elongation (Verma-Gaur et al., 2008), and polyadenylation (Runner et al., 2008). At some stage during transcription, Rpb4/7 interacts with the transcript. This conditional interaction is dependent upon its proper interaction with Pol II (Goler-Baron et al., 2008). Following transcription, the Rpb4/7–RNA complex is exported out of the nucleus in an Rpb4p-mediated manner (this feature is apparent only during stress) (Farago et al., 2003). Consistently, Rpb4/7 export is dependent on transcription (Selitrennik et al., 2006). In the cytoplasm, Rpb4/7 interacts with eIF3 (probably via Hcr1p and the N terminus of Nip1p), thus stimulating translation initiation, mediated by additional factors (some are shown). In addition to its role in translation, Rpb4/7 also stimulates shortening of the poly(A) tail and subsequently the two major mRNA degradation pathways (Lotan et al., 2007, 2005).

(B) Compromised function of Rpb4/7 in case of a defective Pol II. The interaction of Rpb4/7 with mRNAs (Goler-Baron et al., 2008) and polysomes (Figure 5A) is dependent on its binding to Pol II. Consequently, every role of Rpb4/7 in translation and mRNA decay is adversely affected. Nuc, nucleus; Cyto, cytoplasm.

of Pol II (Goler-Baron et al., 2008; Ujvári and Luse, 2006). This transcription-dependent interaction is then required for Rpb4/7 capacity to stimulate both translation (this work) and mRNA decay (Goler-Baron et al., 2008). We therefore propose that Pol II can remotely control the major posttranscriptional stages via Rpb4/7.

Rpb4/7 Coordinates the mRNA Lifecycle

The involvement of Rpb4/7 in transcription (Choder, 2004), mRNA export (Farago et al., 2003), translation (this study) and the two major mRNA decay pathways (Goler-Baron et al., 2008; Lotan et al., 2007, 2005) raises the possibility that Rpb4/7 coordinates the various stages of gene expression. As proposed in the model shown in Figure 7, following transcription, Rpb4/7 remains associated with the transcript throughout its life. Rpb4/7 is capable of switching interacting partners, e.g., Pol II, Rna14p, Fcp1, components of the Pat1/Lsm1-7 complex and components of eIF3 (Choder, 2004; Kamenski et al., 2004; Lotan et al., 2007, 2005; Runner et al., 2008; and Figure 1 and Figure S1), thus exerting its impact on the different processes temporarily. Rpb4/7 seems to be located at the 3′ end of the 3′- UTR (unpublished data) in complex with Pat1/Lsm1-7 (Lotan et al., 2007, 2005) which is also located there (Chowdhury et al., 2007). As most ribosomes dissociate from the mRNA at the stop codon and do not migrate into the 3′-UTR (Eldad et al., 2008), Rpb4/7/Pat1/Lsm1-7 complex cannot be displaced by ribosomes. This location seems to permit the continuous association of Rpb4/7 with the mRNA throughout their lives in the cytoplasm. Following mRNA decay, Rpb4/7 returns to the nucleus for an additional round (Selitrennik et al., 2006). Breaking this circle, by mutating either Rpb4p NLS or the Pol II "pocket" or by specific mutations in Rpb7p, compromises translation. We propose that the mRNA coordinator integrates all stages of the mRNA lifecycle into a system.

Consistent with its role as an mRNA coordinator, disruption of Rpb4/7 function results in pleiotropic effects. Genetic analyses have indicated that the role of Rpb7p in transcription (Choder, 2004) and mRNA degradation (Lotan et al., 2007) are essential. However, cells lacking *RPB4* can proliferate under optimal conditions, albeit poorly. This suggests that Rpb4p is required for appropriate regulation of the essential functions of Rpb4/7 (e.g., by binding Nip1p and Hcr1p). Consistently, deletion of *RPB4* affects degradation of a class of mRNAs, but has little effect on Rpb7p-dependent decay of others (Lotan et al., 2007, 2005). Moreover, overexpression of *RPB7* can partially rescue the transcriptional defects associated with *RPB4* deletion (Sheffer et al., 1999). Cell size of the *rpb4Δ* strain is highly variable, suggesting a defect in the linkage between cell growth and division (M.C., unpublished data). Moreover, *rpb4Δ* cells respond abnormally to various environmental conditions (Choder, 2004), are defective in sporulation and tend to undergo a transition to pseudohyphal growth that normally occurs when cells forage for nutrients (Pillai et al., 2003). Likewise, *rpb7-26* cells, which are specifically defective in translation (this study), abnormally form pseudohyphae when cultured on raffinose as the main carbon source (data not shown), suggesting that the Rpb4/7-mediated translational response to nutrients is involved in this morphogenic transition. Interestingly, the balance between Rpb4p and Rpb7p levels in the cells is critical for normal responses to the environment (Singh et al., 2007; Choder, 1993). Collectively, all these observations are consistent with a key role for Rpb4/7 in integrating many aspects of cellular responses to the environment with mRNA synthesis, translation and decay.

Translation Is Mechanistically Coupled to Transcription

Involvement of a given factor in two different processes does not necessarily signify that they are mechanistically linked. It is possible that, during evolution, Rpb4/7 has acquired more than

one unrelated function. However, our results demonstrate that the translational role of Rpb4/7 can be executed only if Rpb4/7 is first assembled correctly with the Pol II core. Hence, Pol II affects translation by recruiting Rpb4/7 and permitting it to interact with the emerging transcripts (Goler-Baron et al., 2008; Ujvári and Luse, 2006). Recently, it was shown that dissociation of Rpb4/7 from the Pol II core can be regulated by ubiquitination (Daulny et al., 2008). This observation raises the possibility that coupling between transcription and translation can be modulated at the level of Rpb4/7 release.

It is commonly presumed that one of the main differences between prokaryotes and eukaryotes is the mechanistic separation between transcription and translation in the latter organisms. The function of the mRNA coordinator described here challenges the long-standing dogma of functional separation between transcription and translation in eukaryotes. The capacity of Pol II to regulate translation is in accordance with the notion that gene expression functions as a system, whereby mRNA synthetic machinery cross talks with the postsynthetic stages. Whether translation can, conversely, impact transcription directly (via Rpb4/7 or other messengers) remains an interesting issue for future works.

EXPERIMENTAL PROCEDURES

Yeast Strains

Table S1 depicts the yeast strains used in this study.

Tandem Affinity Purification

Purification was performed essentially as described previously (Gavin et al., 2002) except that NaCl was replaced with KAc (140 mM).

Polysomal Fractionation of Optimally Growing, Starved, and Refed Cells

Cells were allowed to proliferate in rich synthetic medium until midlog phase (1×10^7cells/ml). A portion of the culture was supplemented with 100μg/ml cycloheximide (CHX) (unless otherwise indicated) and immediately harvested and frozen at −80°C in the presence of CHX-containing 17% glycerol. The remaining culture was collected by centrifugation, washed twice with water and resuspended in starvation medium lacking sugar and amino acids for 1 hr. One half of the culture was supplemented with CHX and frozen as above and the other half was collected by centrifugation and resuspended in rich synthetic medium. The cultures were shaken at 30°C for the indicated time before adding CHX and harvesting as above. Extracts (containing 1.5–2.5 mg protein) were loaded onto 10%–50% sucrose gradients and centrifuged at 35K rpm at 4°C using a SW41 rotor, as detailed in (Eldad et al., 2008). Fractions (0.7ml) were collected while scanning continuously at A254 using an ISCO gradient fraction collector. To analyze the distribution of *MFA2* mRNA, RNA from each fraction was extracted and analyzed by northern blot hybridization, followed by quantification using PhosphorImager technology, as detailed previously (Eldad et al., 2008). Polysomal fractionation of HCHO crosslinked extracts was performed as described (Valásek et al., 2007), except that NaCl concentration in the extraction buffer was 0.5M.

In Vitro Binding Assay, GST Pulldown

Preparation of GST fusion proteins and in vitro binding assay were carried out as described previously (Asano et al., 1998).

Incorporation of ^{35}S-Methoinine into TCA Precipitable Material

Cell were allowed to proliferate in a medium lacking methionine. Equal amount of cells (5×10^6 cells) were harvested and resuspended in 50 μl of medium lacking methionine. Incorporation kinetics was determined as described previously (Paz and Choder, 2001).

Fluorescent Microscopy

Images of fluorescently labeled cells were acquired as described previously (Lotan et al., 2005).

Statistical Analysis

When indicated, statistical analysis was performed using Student's t test and "Fit Model" of the JMP program. P values are indicated as detailed in the figure legends. Error bars in figures represent SD.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, six figures, and one table and can be found with this article online at doi:10.1016/j.cell.2010.10.033.

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EXTENDED EXPERIMENTAL PROCEDURES

Tandem Affinity Purification

Purification was performed essentially as described previously (Gavin et al., 2002) with the following modifications. NaCl was replaced with KAc (140 mM). To obtain results shown in Figure S1, proteins were extracted from 6 L of culture approaching stationary phase. Extraction was done by grinding a mixture of dry ice and frozen cells using a coffee grinder. Following purification, proteins were separated by PAGE, detected by silver staining and identified by mass spectrometry.

Constructing Plasmids Expressing Rpb4p-NLS-GFP-GFP Fusions

Plasmids expressing green fluorescent protein Rpb4p NLS-GFP-GFP fusions were constructed by using pKW431 (Stade et al., 1997). This plasmid encodes fusion proteins consisting of the simian virus 40 NLS (and the nonfunctional mutant protein kinase inhibitor [PKI] NES) and two GFP molecules. The HindIII-EcoRI SV40 NLS fragment of pKW431 was replaced with the *RPB4* NLS by homologous recombination in vivo. The HindIII-RPB4 NLS-EcoRI fragment was produced by 5 cycles of primer extension (20″ at 94°C, 10″ at 60°C and 10″ at 73°C) of the following primers, whose 3′ portion that contain the RPB4 sequences aneal: Forward primer 5′-ATACAATCTGCACAATATTTCAAGCTATACCAAGCATACAATAAGCTTATGAAAAAACACAAGAAGAAGCATTTGAAGCAC 3′ and the reverse primer 5′-TTGTTGATATCAAGACCTGCTAATTTCAAGGCTAATTCATTGAATTCGTGCTTCAAATGCTTCTTCTTGTGTTT TTTC-3′. The translation start site is underlined. The mutant NLS-GFP-GFP was similarly constructed except that the two primers were: Forward primer 5′-ATACAATCTGCACAATATTTCAAGCTATACCAAGCATACAATAAGCTTATGAAAAAACACAAGAAGAATCA TTTGAAGCAC 3′, and the reverse primer 5′- TTGTTGATATCAAGACCTGCTAATTTCAAGGCTAATTCATTGAATTCGTGCTTCAAATG ATTCTTCTTGTGTTTTTTC-3′. The mutant nucleotide is underlined.

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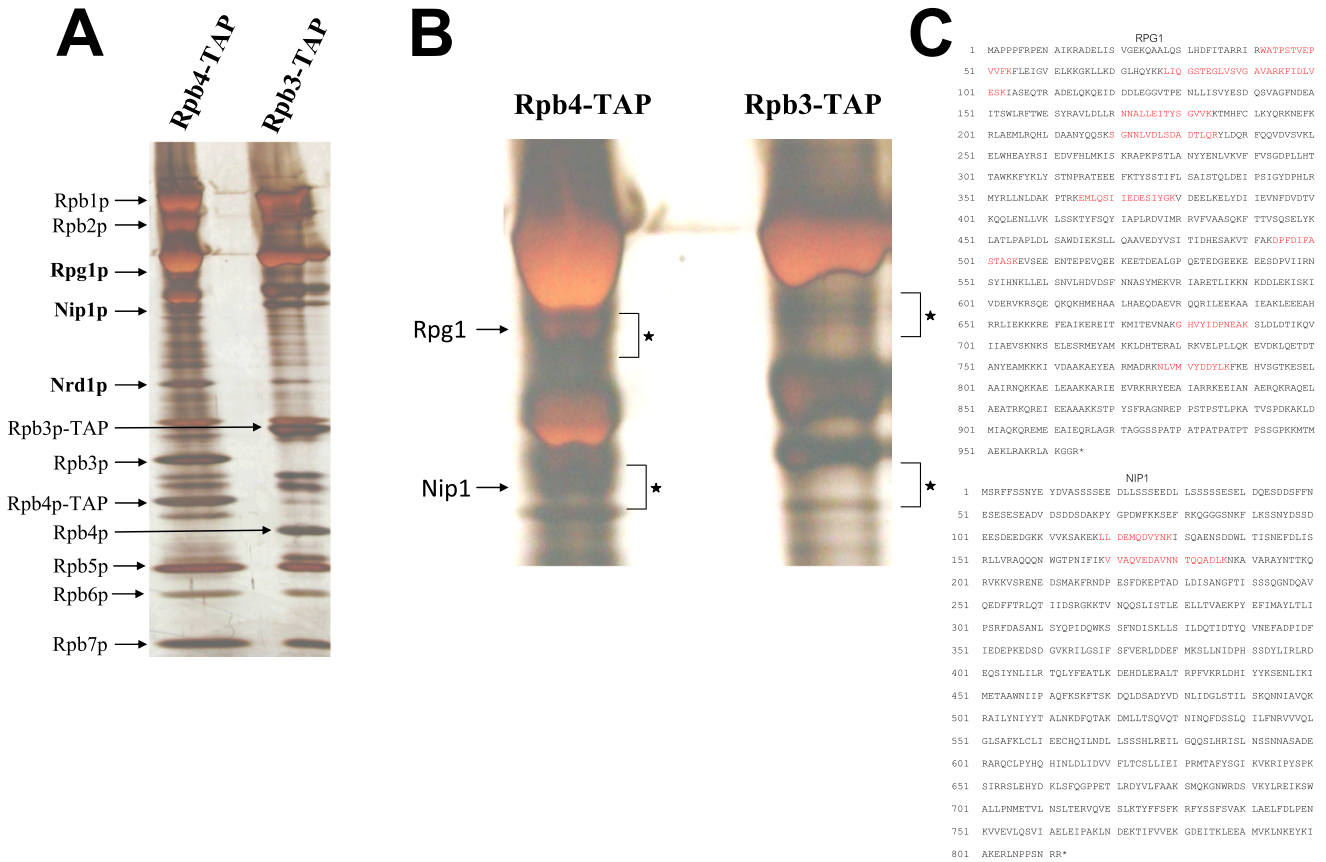

Figure S1. Components of eIF3 Copurify with Rpb4-TAP, Related to Figure 1

Protein complexes were purified from extracts (600 mg of protein extracted from 10^{12} cells) prepared from cells carrying the indicated TAP-tagged genes (Gavin et al., 2002). Following tandem affinity purification (see Experimental Procedures), the proteins were separated on 5%–15% SDS-PAGE and were visualized by silver staining. Known proteins that associate with both complexes are marked on the left. The proteins indicated in bold are unique to the Rpb4-TAP-containing complex or are found in a much higher amount in the Rpb4-TAP containing complex. They were identified by mass spectrometry. The band below Rpb4p-TAP is a degradation product of this tagged protein. (B) A close up view of the region in the gel shown in (A). Mass spectrometry was used to identify proteins located in the regions marked by asterisks. Gel slices from the two lanes (marked by asterisks) were taken for mass spec, and identified using Pep-Miner program (Beer et al., 2004). Rpg1p and Nip1p were identified only in the Rpb4p-TAP complex and not in the Rpb3-TAP complex. (C) The sequences of Rpg1p and Nip1p are shown. Peptides identified by the mass spectrometry whose score were above 85 (see Beer et al., 2004) are indicated in red.

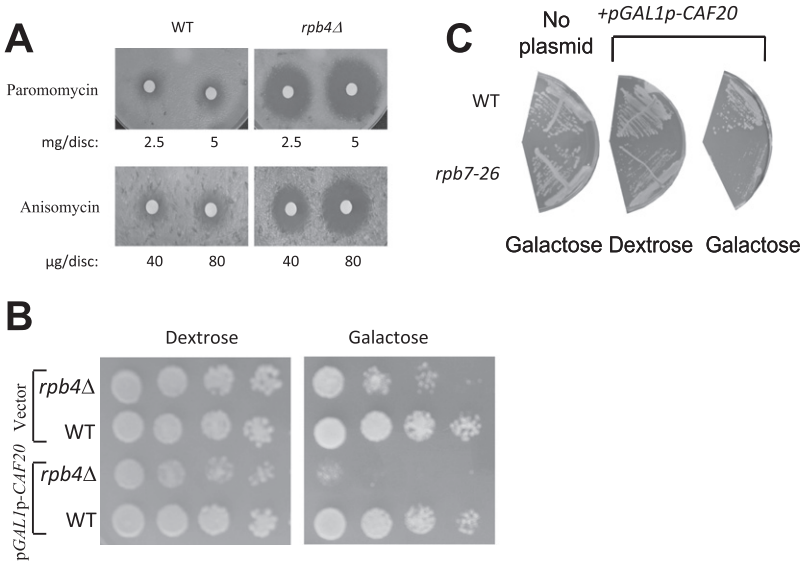

Figure S2. Sensitivity of *rpb7-26* and *rpb4Δ* Cells to Drugs that Target Translation and/or to Overexpression of *CAF20*, Related to Figure 2

(A) Lawns of the indicated cells were grown on plates with filter disks containing the indicated translation inhibitors. The radius of the zone of inhibition reflects the drug's critical inhibitory concentration. (B) WT (yMC274) and *rpb4Δ* (yMC275) cells carrying either vector alone or p*GAL1*p-*CAF20* were allowed to proliferate in dextrose-containing selective medium (lacking uracil) to midlog phase. Equal aliquots of cells were spotted in 5-fold serial dilutions on selective plates containing either dextrose (repressing conditions) or galactose (inducing conditions). The plates were incubated at 24°C for several days. (C) WT (yRL53) and *rpb7-26* (yRL26) cells carrying p*GAL1*p-*CAF20::URA3* were proliferated in dextrose-containing selective medium (lacking uracil) to midlog phase, and then streaked on selective plates supplemented with the indicated sugars (galactose induces overexpression). Cells lacking the plasmid were plated on the same selective plate supplemented with uracil. The plates were incubated at 30°C for several days.

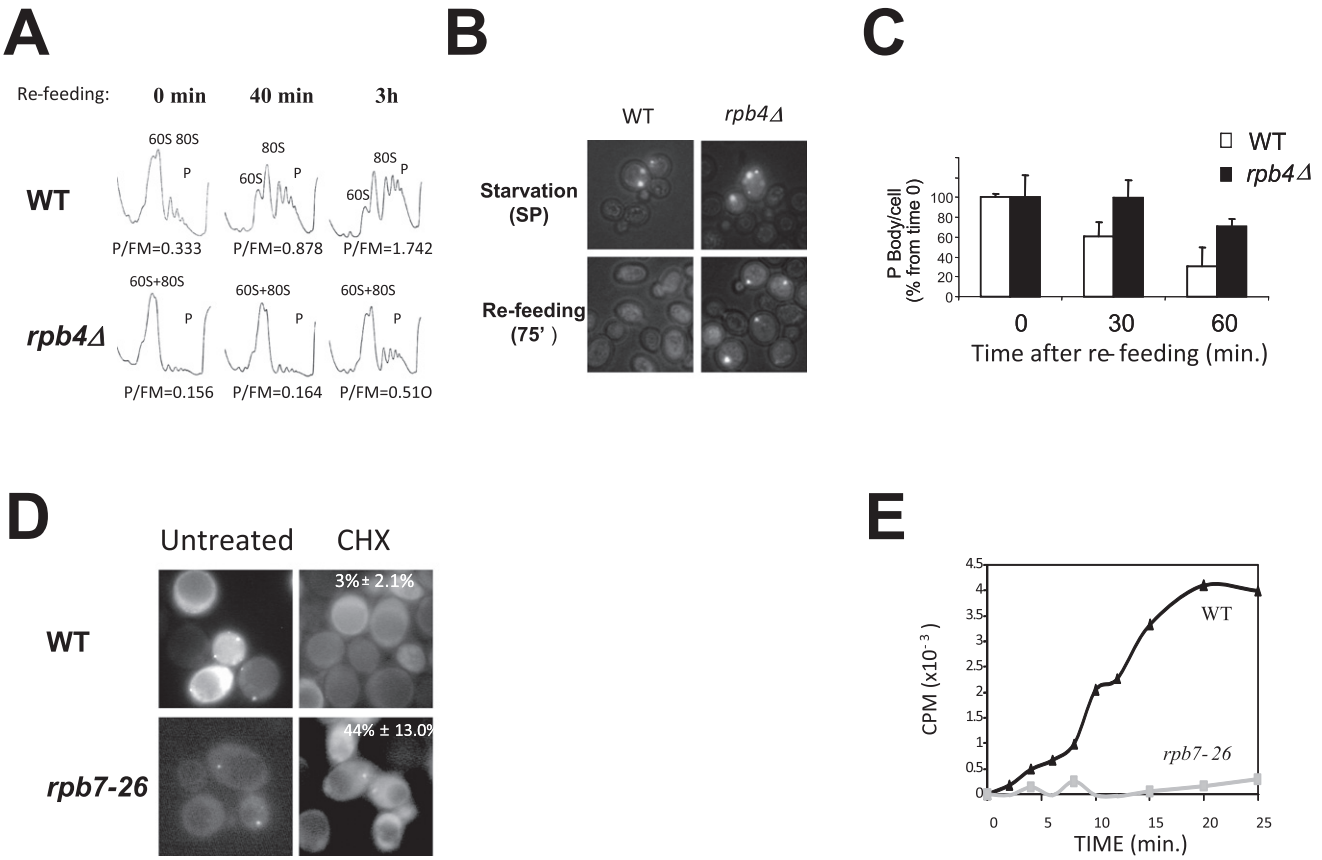

Figure S3. Rpb4/7 Is Required for Efficient Accumulation of Polysomes and Efficient Dissociation of PBs during Exit from Stationary Phase, Related to Figure 3

(A) Rpb4p is required for the limited accumulation of polysomes during SP and for efficient accumulation of polysomes during exit from SP. Cells were allowed to proliferate in synthetic medium at 24°C until stationary phase. Two days later, cells were collected by centrifugation and resuspended in 2 volumes of fresh medium, shaken at 24°C for the indicated period of time, and then processed for polysomal fractionation.

(B and C) Rpb4p is required for efficient disassembly of *MFA2* mRNA-containing PBs following refeeding. WT and *rpb4Δ* strains coexpressing U1A-GFP with *MFA2*-U1A were allowed to proliferate in SC media at 24°C for 3 days until they entered stationary phase. Cells were then shifted to fresh medium. (B) Photos were taken at 0′ and 75′ following refeeding. (C) To quantify the dissociation kinetics, images were taken 30 and 60 min after refeeding and the number of PBs per cell was counted. A minimum of 250 cells from 3 different experiments were counted. The average number of PB/cell at time 0 was defined as 100%. Error bars indicate the standard deviation from the mean values.

(D) CHX-induced PB dissociation in stationary phase cells is dependent on *RPB7*. WT (yRL93) and *rpb7-26* cells (yRL83) expressing *DHH1*-GFP were allowed to proliferate in selective medium until stationary phase. Four days later, cells were either left untreated or treated with 100μg/ml of CHX for 20 min before being assessed by fluorescence microscopy. Number of PBs/cell in untreated cells was regarded as 100%. (E) Starved *rpb7-26* cells incorporate [^{35}S]-Methionine into proteins less efficiently than WT. Cells were allowed to proliferate at 30°C in synthetic complete medium lacking Methionine until midlog phase. Cultures were then harvested, washed twice with water and resuspended in the same volume of medium lacking carbon source and lacking amino acids. After 1 hr of starvation in this medium, incorporation of [^{35}S]-Methionine was determined as in Figure 3A, except that more radioactivity was introduced (Paz and Choder, 2001).

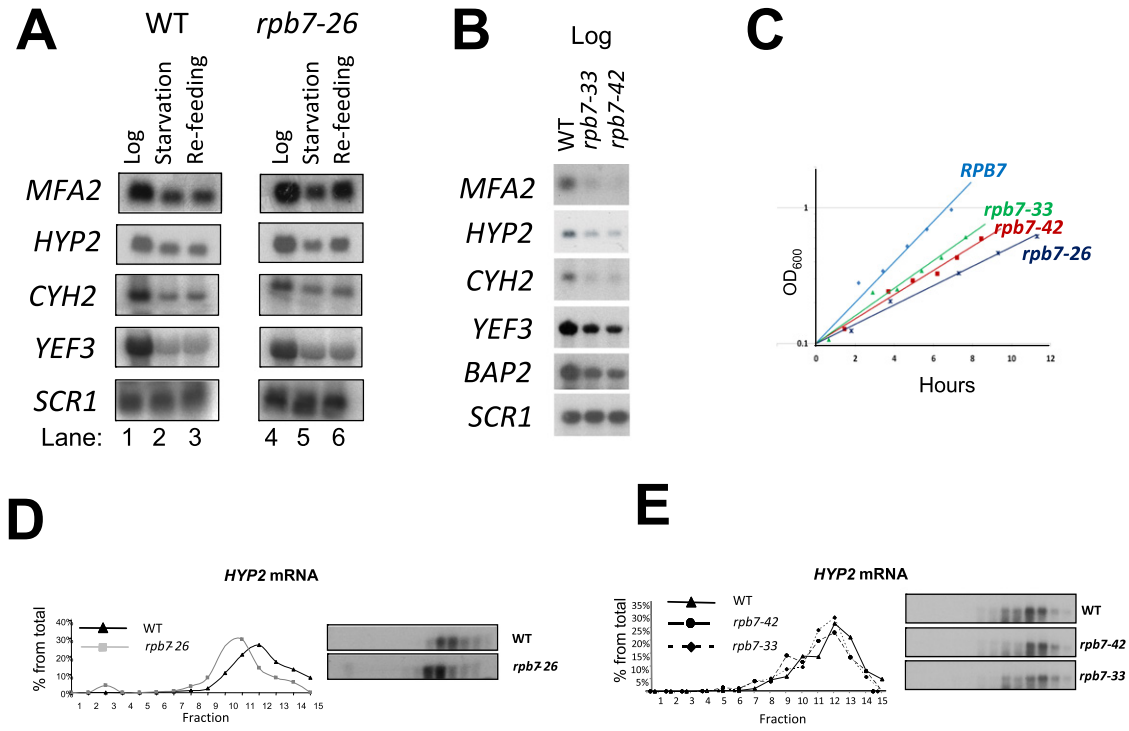

Figure S4. Transcription, Translation, and Proliferation Capabilities of Isogenic Strains Carrying Different *rpb7* Mutant Alleles, Related to Figure 4

(A) Steady state mRNA levels are not affected adversely by substituting *RPB7* with *rpb7-26*. WT (yRL53) and *rpb7-26* (yRL26) cells were allowed to proliferate in synthetic medium at 30°C. Cell samples were harvested at midlog phase. The remaining cultures were washed twice with starvation medium (lacking both sugar and amino acids) and incubated in this medium for an additional hour. Cell samples were harvested and the remaining cultures were shifted to rich medium for an additional 5 min before cells were harvested again. Samples of equal amount of RNA were analyzed by northern blot hybridization, using the indicated probes, as described previously (Lotan et al., 2005). The level of *SCR1*, a Pol III transcript, is sown to demonstrate equal loading. Note that at 30°C the mutant cells are not defective in mRNA decay (Lotan et al., 2007); hence, mRNA levels reflect the transcriptional capacity of the cells. (B) Steady state mRNA levels are adversely affected by substituting *RPB7* with *rpb7-33* or with *rpb7-42* alleles. WT (yRL53), *rpb7-33* (yRL33) or *rpb7*-42 (yRL42) cells were allowed to proliferate in synthetic medium at 30°C. Cell samples were harvested at midlog phase and analyzed as in lanes 1 and 4 of (A). (C) Proliferation rate of the indicated strains in synthetic medium at 30°C, as determined by OD_{600} measurements. (D) WT (yRL53) and *rpb7-26* (yRL26) cells were allowed to proliferate in synthetic medium at 30°C. Cell samples were harvested at midlog phase and cell extracts were subjected to polysomal fractionation followed by northern analysis, using *HYP2* as the probe, as described in Figure 4A ("Optimal conditions"). (E) WT (yRL53) and *rpb7-33* (yRL33) and *rpb7-42* (yRL42) cells were analyzed as in D.

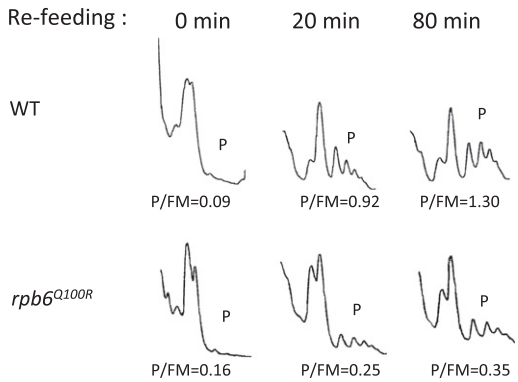

Figure S5. *rpb6*^{Q100R} cells Are Defective in Accumulation of Polysomes in Response to Refeeding of Stationary Cells, Related to Figure 5

WT and *rpb6*^{Q100R} cells were cultured in rich medium (YPD) for 5 days (stationary phase was reached after 2 days). Cell samples were collected ("0 min"). For refeeding, stationary cells were collected by centrifugation and resuspended in 3 volumes of fresh rich medium and collected at the indicated time points, as described in Experimental procedures. Polysomal profiles and P/FM values were obtained for each time point, as in Figure 3B.

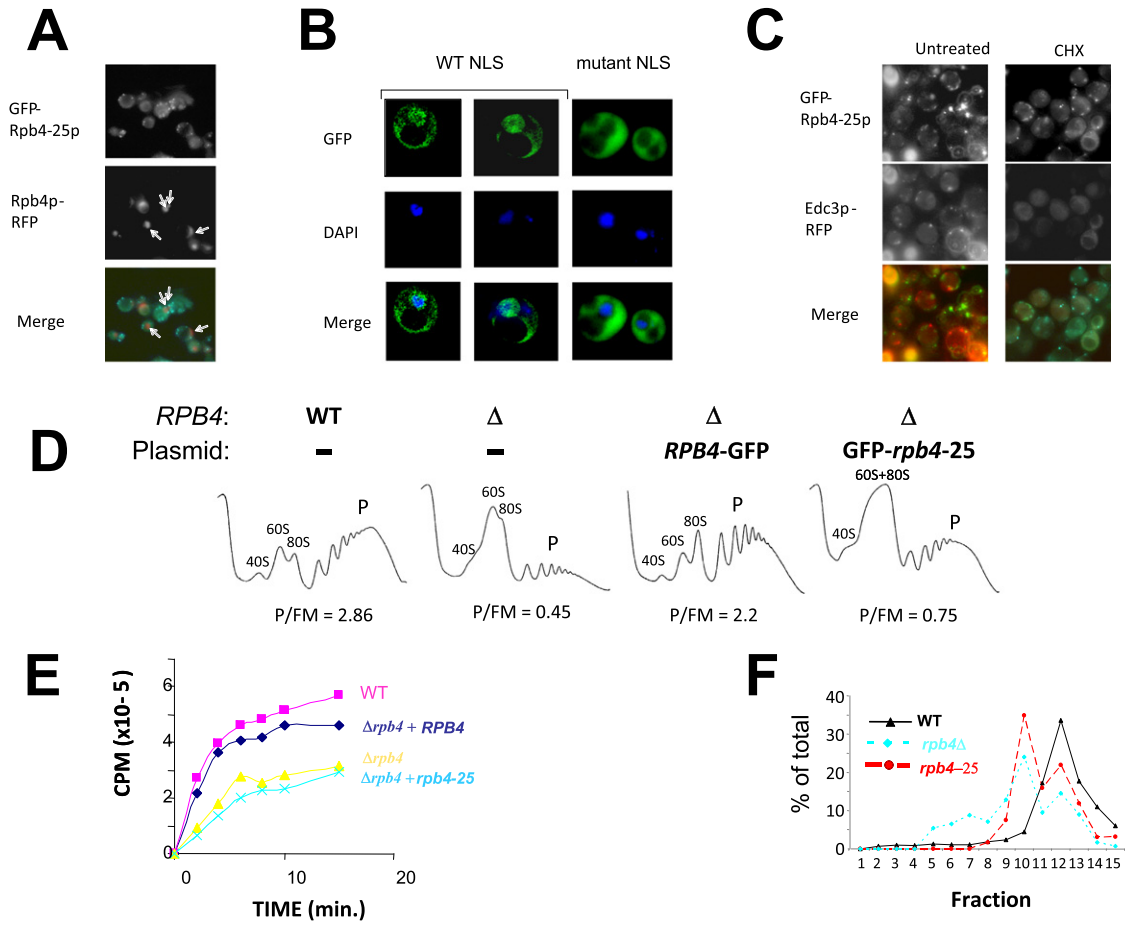

Figure S6. GFP-Rpb4-25p that Is Localized Mainly in the Cytoplasm Fails to Efficiently Support Translation, Related to Figure 6

(A) Cellular localization of GFP-Rpb4-25p and Rpb4p-RFP. Optimally proliferating yMS141 cells coexpressing GFP-*rpb4-25* and *RPB4*-RFP were harvested by centrifugation, resuspended in the same growth medium and immediately inspected under the fluorescent microscope. Photos were taken at either the green channel (upper panel) or the red one (middle panel). The bottom panel shows the merge generated by Adobe Photoshop. Arrows point at example cases of nuclei that accumulated Rpb4p-RFP, but not GFP-Rpb4-25p. GFP-Rpb4-25p localization was unaffected by the presence of the RFP-*RPB4* plasmid (data not shown). (B) Rpb4p K80N resides in the middle of a basic motif and compromises its capacity to function as a nuclear localization sequence (NLS). Rpb4p K80 resides in the following motif ·····74 KKHKKKHLKH 85····· (K80 is underlined). This motif, or its mutant derivative KKHKKNHLKH, was placed in the N terminus of GFP dimer (Stade et al., 1997), as described in the supplemental Experimental procedures. Cells, expressing either the WT Rpb4p motif-GFP-GFP (designated "WT NLS") or the mutant motif-GFP-GFP, were harvested in midlogarithmic phase, stained with 1 μM of DAPI for 5 min to detect the nuclei (and the mitochondria), and inspected under fluorescent microscope. Photos were taken at either the green channel (upper panel) or the blue one (middle panel). Image processing was performed using MetaMorph software (Molecular Devices). The bottom panel shows the merge generated by Adobe Photoshop. The black areas in the cells shown in the left panels are vacuoles, wheras those in the cells shown in the right panel are both vacuoles and nuclei (as the GFP was excluded from these nuclei – see merge panel). Nuclear accumulation of the WT NLS-containing GFP-GFP was observed in ~7% of the cells. (C) GFP-Rpb4-25p granules are not PBs or SGs. Optimally proliferating yMS142 cells coexpressing GFP-*rpb4-25* and *EDC3*-RFP were washed x3 with water and inspected under the fluorescent microscope ("Untreated" panels), or treated with 100 μg/ml cycloheximide (CHX) for 90 min before harvest, followed by x3 washes in CHX-containing water. After 10 or 30 min of CHX treatment, Edc3-RFP granules were still detected (data not shown). A few hours later, no change could be observed in the green foci (data not shown). Green (upper), red (middle) and merge panels were obtained as in A. Note that the green spots are not colocalized with the red spots. (D) Polysomal profiles of optimally proliferating cells. The presence or absence (Δ) of the chromosomal *RPB4*, as well the plasmid born GFP-*RPB4* fusion allele are indicated above the profile. Profiles and P/FM values were obtained as in Figure 3B. (E) Incorporation kinetics of the indicated strains was done as described in Figure 3A. (F) *MFA2* mRNA in each polysomal fraction shown in (D) was detected by northern blotting and quantified by PhosphoImager, as in Figure 4A. Note that this series of isogentic strains has different genetic background than the isogentic series shown in Figure 4 (see in Table S1 the genotypes of yMC122 and its derivatives yMC142, yMS141 and yMC142; versus yRL53 and yRL26); hence the polysomal profiles and *MFA2* mRNA profile of the different series are not identical.