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RESEARCH COMMUNICATION

Transcription in the nucleus and mRNA decay in the cytoplasm are coupled processes

Vicky Goler-Baron, Michael Selitrennik, Oren Barkai, Gal Haimovich, Rona Lotan, and Mordechai Choder[1]

Department of Molecular Microbiology, Rappaport Faculty of Medicine, Technion, Israel Institute of Technology, Haifa 31096, Israel

Maintaining appropriate mRNAs levels is vital for any living cell. mRNA synthesis in the nucleus by RNA polymerase II core enzyme (Pol II) and mRNA decay by cytoplasmic machineries determine these levels. Yet, little is known about possible cross-talk between these processes. The yeast Rpb4/7 is a nucleo-cytoplasmic shuttling heterodimer that interacts with Pol II and with mRNAs and is required for mRNA decay in the cytoplasm. Here we show that interaction of Rpb4/7 with mRNAs and eventual decay of these mRNAs in the cytoplasm depends on association of Rpb4/7 with Pol II in the nucleus. We propose that, following its interaction with Pol II, Rpb4/7 functions in transcription, interacts with the transcript cotranscriptionally and travels with it to the cytoplasm to stimulate mRNA decay. Hence, by recruiting Rpb4/7, Pol II governs not only transcription but also mRNA decay.

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mRNA levels are determined by two distinct processes: transcription, catalyzed by RNA polymerase II (Pol II), and mRNA decay. Transcription occurs in the nucleus whereas the major mRNA decay pathways operate in the cytoplasm. As synthesis and decay processes contribute equally to maintaining mRNA levels, it is quite likely that organisms have evolved a mechanism to coordinate events in the two compartments. To date, little is known about any relationships between these processes.

In yeast, the major mRNA decay pathways occur in the cytoplasm, triggered by shortening of the mRNA poly(A) tail. One pathway culminates in exonuclease digestion of the mRNA from 5′ to 3′ by Xrn1p. The second pathway culminates in exonucleolytic degradation of the mRNA from 3′ to 5′ (for recent reviews, see Coller and Parker 2004; Amrani et al. 2006; Garneau et al. 2007).

Structural studies indicate that the Pol II complex, comprising Rpb1p to Rpb12p, consists of two discrete parts (Armache et al. 2003; Bushnell and Kornberg 2003). The core comprises 10 subunits and includes the catalytic active site. The other part comprises two subunits, Rpb4p and Rpb7p, which form a heterodimer, designated Rpb4/7, that can bind RNA (for review, see Choder 2004). The interface between the two substructures is small and consists of several residues in Rpb7p, termed the "Rpb7p tip", and a small pocket in the core formed by five protein regions: three small regions from Rpb1p, one region from Rpb2p, and one region from Rpb6p (Armache et al. 2003; Bushnell and Kornberg 2003). As Rpb4/7 is present in excess over Pol II complexes (Rosenheck and Choder 1998; Choder 2004), it is possible that Pol II–free Rpb4/7 interacts independently with transcripts either in the nucleus or in the cytoplasm. Alternatively, Rpb4/7 may bind RNA only during transcription in association with Pol II.

Recently, Rpb4p and Rpb7p were found to shuttle between the nucleus and the cytoplasm by a transcription-dependent pathway (Selitrennik et al. 2006) and to play roles in the decay of mature mRNAs in the cytoplasm (Lotan et al. 2005, 2007). Importantly, genetic analyses indicate that the roles of Rpb7p in the two major mRNA decay pathways are distinct from its role in transcription (Lotan et al. 2007). Although it is conceivable that the nuclear and the cytoplasmic functions of Rpb4p and Rpb7p are mechanistically unrelated, an intriguing alternative is that their two functions are coupled, and thus Pol II influences both mRNA synthesis and decay. Here we provide evidence for the second alternative.

Results and Discussion

Pol II mutant that poorly recruits Rpb4/7

To determine whether Pol II controls mRNA decay by recruiting Rpb4/7, we took advantage of a Pol II core that comprises Rpb6^{Q100R}p. This mutant core binds Rpb4/7 poorly, as the Rpb6^{Q100R}p mutation alters one of the very few residues in the core pocket responsible for direct contact with the tip of Rpb7p (Bushnell and Kornberg 2003; Tan et al. 2003; Armache et al. 2005). Consistently, tandem affinity purification (TAP)-tagged Rpb4p pulls down wild-type Pol II efficiently, whereas its capacity to pull down Rpb6^{Q100R}p-containing Pol II is compromised (Supplemental Fig. S1A). Note that the poor immunoprecipitation is observed despite wild-type level of Rpb4p in the mutant cells (Supplemental Fig. S1; see also Supplemental Fig. S4).

rpb6^{Q100R} *cells are defective in executing mRNA decay*

To determine if *rpb6*^{Q100R} cells are defective in mRNA decay, we blocked transcription using two approaches. In the first one, we monitored decay of natural mRNAs, exploiting the heat-shock response whereby the transcription of most genes is blocked rapidly in response to heat. Following transcription arrest, we monitored the decay of mRNAs that had existed in the cytoplasm prior the temperature shiftup (Lotan et al. 2005, 2007, and references therein). Because the Rpb4/7–Pol II interaction is essential for transcription at high temperatures, transcription is arrested more robustly in response to

[*Keywords*: RNA polymerase II; mRNA decay; yeast]
[1]Corresponding author.
E-MAIL choder@technion.ac.il; FAX 972-4-829-5225.
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temperature increases in *rpb6*^{Q100R} cells than in wild-type cells (Rosenheck and Choder 1998; Tan et al. 2003; Choder 2004). Using this approach, we observed that *rpb6*^{Q100R} cells are defective in the decay of various natural mRNAs (Fig. 1A,B). Notably, *RPL25* pre-mRNA disappears rapidly in both strains (Fig. 1A, "Unspliced *RPL25*"), consistent with rapid transcription arrest that occurs in both strains upon temperature shiftup.

To gain mechanistic understanding of this difference in mRNA decay kinetics, we compared deadenylation in wild-type and *rpb6*^{Q100R} cells using the PAGE-Northern technique (Sachs and Davis 1989). Deadenylation rates are slower in *rpb6*^{Q100R} cells (Fig. 1C; Supplemental Fig. S3). Moreover, the fully deadenylated mRNAs are degraded abnormally slowly in the *rpb6* mutant. Indeed, whereas 78% of deadenylated RNA was degraded in 10 min in wild-type cells, only 64% of deadenylated RNA was degraded in a much longer time (25 min) in the mutant cells (Fig. 1C). Notably, defective deadenylation and slow decay of deadenylated RNAs are characteristic phenotypes of mutations in *RPB4* and *RPB7* (Lotan et al. 2005, 2007).

Using a second approach to block transcription, we next examined decay features of the synthetic Tet-Off-*MFA2pG* transcript (Hilleren and Parker 2003) after blocking its transcription by doxycycline, a drug that has no significant effect on overall proliferation rate (Gari et al. 1997). As shown in Figure 1D, *MFA2pG* mRNA decays more slowly in *rpb6*^{Q100R} cells ($T_{1/2}$ = 25 min) than it does in wild-type cells ($T_{1/2}$ = 12 min). This occurs at 30°C. Thus, we found that degradation of both synthetic and natural transcripts, under either optimal or stress conditions, is slower in *rpb6*^{Q100R} cells than it is in wild-type cells. A degradation intermediate of *MFA2pG* mRNA, designated "Frag." in Figure 1D, accumulates in the cytoplasm due to a poly(G) tract that blocks 5′-to-3′ exonuclease activity of Xrn1p (Vreken and Raue 1992; Decker and Parker 1993). This fragment is degraded ultimately by the 3′-to-5′ pathway (Jacobs Anderson and Parker 1998); therefore, its accumulation is often due to defects in this pathway. Previously, we showed that Rpb7p stimulates both deadenylation and 3′-to-5′ degradation (Lotan et al. 2007). Consequently, *rpb7* mutants accumulate 2.9-fold more *MFA2pG* fragment than wild-type cells do (Lotan et al. 2007). As shown in Figure 1D, the *rpb6*^{Q100R} mutation also leads to abnormally high accumulation of "Frag.". We suspect that accumulation of this fragment is due to the poor capacity of *rpb6*^{Q100R} cells to execute Rpb7p-mediated 3′-to-5′ decay (see below). Summarily, although *rpb6*^{Q100R} cells carry wild-type *RPB4* and *RPB7*, this mutant strain exhibits several defective features of mRNA decay similar to those reported as characteristic of strains carrying certain *RPB7* ts alleles (Lotan et al. 2007) or lacking *RPB4* (Lotan et al. 2005). Based on all these data, we hypothesize that poor association of Rpb4/7 with Rpb6^{Q100R}-containing Pol II results in defective mRNA decay in the cytoplasm. We took various approaches to validate this premise as outlined below.

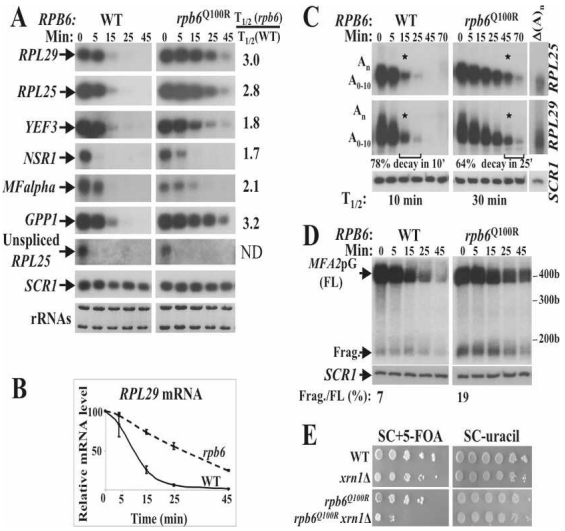

Figure 1. *rpb6*^{Q100R} cells exhibit defective mRNA decay. (*A*) Cells growing optimally at 30°C were shifted rapidly to 42°C to block transcription naturally (Lotan et al. 2005, 2007). Post-shift decay kinetics were determined by Northern analysis, using probes indicated on the *left* (see the Materials and Methods). $T_{1/2}$ were determined as in *B*. The ratio between the $T_{1/2}$ of a given mRNA in the mutant and its $T_{1/2}$ in the wild type ($T_{1/2}$ [*rpb6*]/$T_{1/2}$ [WT]) is indicated on the *right*. (*B*) Half-lives ($T_{1/2}$) were determined by plotting mRNA levels as a function of time post-transcription block. A graphic illustration of *RPL29* mRNA decay kinetics is shown as an example. To obtain this graph, the intensity of each band, determined by PhosphorImager technology, was normalized to that of *SCR1* (a Pol III transcript). The normalized band intensity at time 0 (before transcription arrest) was defined as 100% and the intensities at the other time points were calculated relative to time 0. Results were plotted as a function of time. The graph represents an average of three independent assays. Error bars indicate the standard deviation from the mean values. Half-lives were obtained from these graphs and are depicted on the *right*. Variations in the calculated half-lives were <15%. (*C*) Transcription was blocked as in *A*. RNA samples analyzed by the PAGE-Northern technique (Sachs and Davis 1989), using the probes indicated on the *right*. The positions of fully adenylated (A_n) and deadenylated (A_{0-10}) RNAs are shown on the *left*. Lane "Δ$(A)_n$" shows the position of fully deadenylated RNA (see Lotan et al. 2007). The asterisk (*) indicates the time point when deadenylation is estimated to be complete. The proportional decrease in radioactivity between this time point and the following one was assessed using PhosphorImager technology (normalized to *SCR1*) and is depicted underneath the respective lanes to estimate the stability of the deadenylated RNA. Quantitative illustration of the deadenylation kinetics is shown in Supplemental Figure S3. (*D*) *rpb6*^{Q100R} cells degrade *MFA2pG* mRNA abnormally slowly and accumulate abnormally high decay intermediate of *MFA2pG* mRNA. Wild-type and *rpb6*^{Q100R} cells expressing Tet-Off-*MFA2pG* were grown at 30°C to mid-logarithmic phase before transcription was blocked by adding doxycycline (2 µg/mL) (Hilleren and Parker 2003). Following drug addition, cultures continued to be shaken at 30°C and samples were taken at the indicated time points. RNA samples analyzed by the PAGE-Northern technique (Sachs and Davis 1989), hybridized with an *MFA2pG*-specific probe (see the Materials and Methods). The position of *MFA2pG* full-length mRNA (FL) is indicated on the *left*. "Frag." indicates a degradation intermediate that accumulates due to a poly(G) tract that blocks 5′-to-3′ exonuclease activity by Xrn1p (Vreken and Raue 1992; Decker and Parker 1993). The ratios between this fragment and *MFA2pG*, the full-length one, at 0 time points (at steady state) were determined by PhosphorImager technology (normalized to *SCR1* RNA) and are indicated at the *bottom* as percentages. Half-lives were calculated as in *B*; in wild type it was 12 min and in the mutant, 25 min. The positions of the size marker bands are shown on the *right*. (*E*) *rpb6*^{Q100R} cells are hypersensitive to deletion of *XRN1*. Strains, indicated on the *left*, carrying p*RPB4/7/URA3*/2µ (overexpressing Rpb4/7) were spotted, in 10-fold dilutions, on 5-FOA-containing plate (the drug kills cells expressing *URA3*). In parallel, cells were spotted on a synthetic medium lacking uracil that selects for cells carrying the plasmid (designated SC–uracil). Plates were incubated for 3 d at 30°C.

Overexpression of RPB4/7 *in* rpb6^{Q100R} *cells increases association between Rpb4/7 and Pol II, suppresses the cells sensitivity to high temperature, and corrects their transcriptional defect*

As shown in Supplemental Figure S4B, overexpressing *RPB4* or *RPB7* alone has little or no effect on cell proliferation at high temperatures. We used a high-copy plasmid (p*RPB4/7* 2μ) that expresses both genes while maintaining the relative level of *RPB4* mRNA and *RPB7* mRNA in both the *RPB6* wild-type (Lotan et al. 2007) and *rpb6*^{Q100R} strains (Supplemental Fig. S4A). Introduction of p*RPB4/7* 2μ suppresses partially the temperature sensitivity of *rpb6*^{Q100R} cells (Supplemental Fig. S4B), encouraging us to examine the mechanistic consequences of this overexpression. First, we observed that overexpression of *RPB4/7* increases the association between the mutant Pol II and Rpb4/7 (Supplemental Fig. S1B). We then reasoned that if defective transcription and mRNA decay by the *rpb6*^{Q100R} strain are indeed due to weakened Rpb4/7–Pol II interactions, then overexpression of *RPB4/7* should correct both the transcription and the decay defects of *rpb6*^{Q100R} cells. As shown in Supplemental Figure S2, high-copy Rpb4/7 restores the transcriptional defect of the mutant cells during heat shock, suggesting that the mutation in Rpb6p affects transcription only through binding Rpb4/7.

rpb6^{Q100R} *cells exhibit hypersensitivity to deletion of* XRN1 *that can be suppressed by overexpressing* RPB4/7

As discussed above, excessive accumulation of the *MFA2pG* mRNA degradation intermediate in *rpb6*^{Q100R} cells (Fig. 1D) suggests that these cells are defective in 3′-to-5′ decay (Vreken and Raue 1992; Decker and Parker 1993). We took advantage of the observation that strains lacking both the 5′-to-3′ and 3′-to-5′ decay pathways are inviable (Johnson and Kolodner 1995; Jacobs Anderson and Parker 1998; van Hoof et al. 2000). If our interpretation that the fragment accumulates because of defective 3′-to-5′ decay is correct, then the *rpb6*^{Q100R} allele should be synthetically lethal, or synthetically sick, with deletion of *XRN1*. Moreover, deletion of *XRN1* should not be lethal in mutant cells that overexpress *RPB4/7* if such an overexpression indeed suppresses the 3′-to-5′ decay defect. We therefore deleted *XRN1* from *rpb6*^{Q100R} cells that also carry p*RPB4/7* 2μ. Creation of *rpb6*^{Q100R} *xrn1*Δ cells overproducing Rpb4/7 (Fig. 1E, right panel) indicates that these cells have the capacity to execute mRNA degradation using the 3′-to-5′ pathway. However, removal of the high-copy *RPB4/7* plasmid by 5-FOA uncovers the predicted synthetic sickness phenotype of *rpb6*^{Q100R} *xrn1*Δ cells, as their proliferation slows or stops (Fig. 1E, left panel). In contrast, cells lacking *XRN1* but carrying wild-type *RPB6* proliferate well irrespective of whether or not they bear p*RPB4/7* 2μ. These results support our conjecture that *rpb6*^{Q100R} cells are defective in executing the 3′-to-5′ pathway, much like cells carrying some mutations in *RPB7* (Lotan et al. 2007). More importantly, this mRNA decay defect is not a direct effect of the mutation in *RPB6* per se, as *RPB4/7* overexpression can suppress this defect (Fig. 1E, right panel).

Overexpression of RPB4/7 *in* rpb6^{Q100R} *cells suppresses partially the defect in mRNA decay*

Next we examined directly the effect of overexpressing Rpb4/7 on mRNA decay. Rpb4/7 overexpression in wild-type cells does not cause detectable changes in mRNA decay or in deadenylation kinetics (Fig. 2A; Supplemental Fig. S5, lanes 1–10). These results suggest that the heterodimer is not a limiting factor in the wild-type decay process. In contrast, Rpb4/7 overexpression in *rpb6*^{Q100R} cells suppresses partially their defect in deadenylation (Supplemental Fig. S5, lanes 11–20) and in overall mRNA decay (Fig. 2B). This partial suppression conferred by exogenous Rpb4/7 argues against a direct and Rpb4/7-independent role for Rpb6p in mRNA decay. Together with the genetic data (Fig. 1E), these results indicate that the defective capacity of *rpb6*^{Q100R} cells to degrade mRNAs and their dependence on *XRN1* are related to the poor interaction of Rpb6^{Q100R} p with Rpb4/7.

The capacity of Rpb4/7 to associate with mRNAs is smaller in rpb6^{Q100R} *cells than it is in wild-type cells*

Rpb7p was shown to interact with the emerging transcript during in vitro transcription (Ujvari and Luse 2006) and in vivo (Lotan et al. 2005), using at least one of its two RNA-binding domains (Todone et al. 2001; Meka et al. 2003, 2005; Choder 2004). In order to determine if

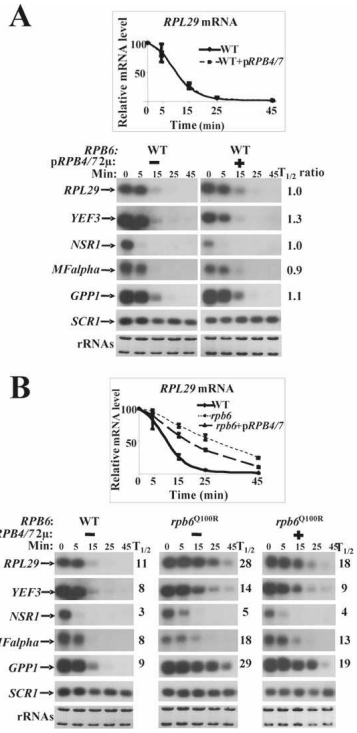

Figure 2. Overexpression of *RPB4/7* in *rpb6*^{Q100R} cells suppresses partially the defect in mRNA decay. mRNA decay was monitored as in Figure 1B. (*Top* panels) Graphic representation of *RPL29* mRNA decay kinetics in the indicated isogenic strains was generated as in Figure 1B. (*Bottom* panels) Autoradiograms showing the decay kinetics of several mRNAs (indicated on the *left*). The presence (+) or absence (–) of the high-copy plasmid overexpressing *RPB4/7* is indicated *above* the autoradiograms. Half-lives ($T_{1/2}$) were determined as in Figure 1B. (*A*) Overexpression of *RPB4/7* does not change mRNA decay kinetics in wild-type cells. The ratios between the $T_{1/2}$ in wild-type cells expressing normal levels of Rpb4/7 and the $T_{1/2}$ in wild-type cells overexpressing Rpb4/7 ("$T_{1/2}$ ratio") are indicated on the *right*. Variations were <20%. (*B*) Overexpression of *RPB4/7* accelerates mRNA decay in *rpb6*^{Q100R} cells. $T_{1/2}$ are indicated on the *right*. Variations were <20%.

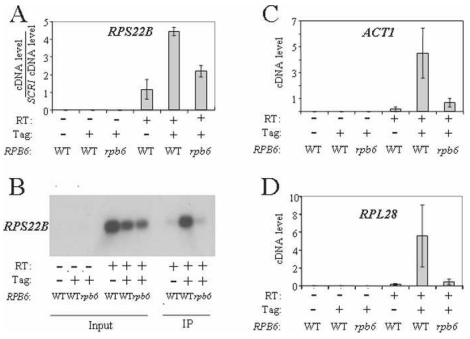

Figure 3. Efficient association of Rpb4/7 with mRNAs is dependent on Rpb6p. Equal amounts of cell extracts from wild-type or from *rpb6*^{Q100R} cells carrying or lacking TAP-tagged *RPB4*, as indicated, were subjected to RNA immunoprecipitation (RIP) as detailed in the Materials and Methods. Prior to the RIP procedure, 5% of each extract was removed to determine the levels of the indicated mRNAs in the input material. cDNA was synthesized using either random hexamer (*A*) or oligo(dT) (*B–D*) as the primer, in the presence (+) or absence (–) of reverse transcriptase (RT). cDNA levels were determined by qPCR (see the Materials and Methods). The level of each cDNA produced from the IP material was normalized to that produced from the input RNA, diluted 1:1500-fold. Each bar represents average of three experiments; each one was done in triplicate. Error bars indicate the standard deviation. (*A*) Relative cDNA levels obtained using the random primers. Each bar represents the ratio of cDNA level of *RPS22B* mRNA (normalized to that of the input) divided by that of *SCR1* cDNA (normalized to that of the input). (*B*) Levels of PCR-amplified *RPS22B* cDNA, obtained using oligo(dT), determined by Southern analysis. PCR was carried out by the real time machine. At late-exponsntial phase (cycle 31), the sample harvested and electrophoresed in 2% gel followed by Southern analysis using *RPS22B* probe. (*C,D*) Levels of the indicated cDNAs obtained using the oligo(dT) primer.

recruitment of Rpb4/7 to Pol II is required for its association with mRNAs in vivo, we immunoprecipitated TAP-tagged Rpb4p from wild-type or *rpb6*^{Q100R} cells, or from cells lacking the tag as a control, and examined how much RNA was coimmunoprecipitated with it, using reverse transcription (RT) followed by qPCR. We first used a random hexamer as the primer in the RT reaction, which allowed us to synthesize cDNA using both Pol II and Pol III transcripts as the templates. This permitted us to normalize *RPS22B* signal with that of *SCR1*. As shown in Figure 3A, *RPS22B* mRNA is coimmunoprecipitated together with Rpb4p more efficiently in wild-type cells than it is in *rpb6*^{Q100R} cells. To obtain cDNA from mature poly(A)$^+$ mRNAs, we used oligo(dT) as the primer. Also in this case, more mRNAs are associated with Rpb4p-TAP in wild-type cells than they do in *rpb6*^{Q100R} cells (Fig. 3C,D). To verify production of correct PCR fragment and to visually demonstrate the quantitative results, the PCR product of one experiment was analyzed by Southern blot hybridization technique (Fig. 3B). Taken together, the results in Figure 3A–D argue against the possibility that Rpb4/7 interacts with mRNA independently of Pol II, as the levels of Rpb4/7 are identical in both strains (see Supplemental Figs. S1, S4A). Instead, the results are in accord with "conditional interaction" between Rpb4/7 and mRNAs, whereby the interaction occurs in the context of Pol II.

Like rpb6^{Q100R} *strain,* rpb1$^{C67S;\ C70S}$ *strain is defective in mRNA decay as well*

Finally, we took a last approach to corroborate that the poor interaction of Rpb6^{Q100R} p with Rpb4/7 determines the defective mRNA decay phenotype of *rpb6*^{Q100R} cells. We reasoned that if the inability of *rpb6*^{Q100R} cells to degrade efficiently mRNAs is indeed due to the poor interaction between Rpb4/7 and Pol II, then mutations in another Pol II subunit that also binds Rpb4/7 and affects its recruitment to Pol II should result in a similar phenotype. Thus, we investigated the mRNA decay phenotype of a strain carrying C67S and C70S substitutions in Rpb1p, which (like the mutation in Rpb6p) have been reported to compromise recruitment of Rpb4/7 to Pol II (Donaldson and Friesen 2000). As controls, we examined mRNA decay phenotype in cells carrying mutations in Rpb1p outside the pocket region in Pol II that interacts with Rpb7p tip (Donaldson and Friesen 2000; Armache et al. 2003, 2005; Bushnell and Kornberg 2003). Like *rpb4*Δ cells (Lotan et al. 2005), *rpb7* mutant cells (Lotan et al. 2007), and *rpb6*^{Q100R} cells (e.g., Fig. 1), *rpb1*$^{C67S;\ C70S}$ cells are defective in both deadenylation and subsequent mRNA degradation (Fig. 4A,B). However, high-copy Rpb4/7 cannot correct the defective phenotypes of *rpb1*$^{C67S;\ C70S}$ cells as it does in *rpb6*^{Q100R} cells (data not shown). Perhaps, these specific mutations in Rpb1p compromise more severely the capacity of Pol II pocket to recruit Rpb4/7 than the Q100R substitution in Rpb6p does. In contrast with *rpb1*$^{C67S;\ C70S}$ cells, the control mutant cells exhibit normal, or nearly normal, mRNAs decay kinetics (Fig. 4A). The control mutants are defective in transcription, in vitro (Donaldson and Friesen 2000) and in vivo, as determined by the poor transcriptional induction of *HSP104* in response to HS (Fig. 4A) or by the low steady-state levels of *YEF3* (33% compared with wild type) and *TEF4* (42%) mRNAs. Thus, poor transcription per se is not responsible for poor mRNA decay.

Taken together, the results of both genetic and bio-

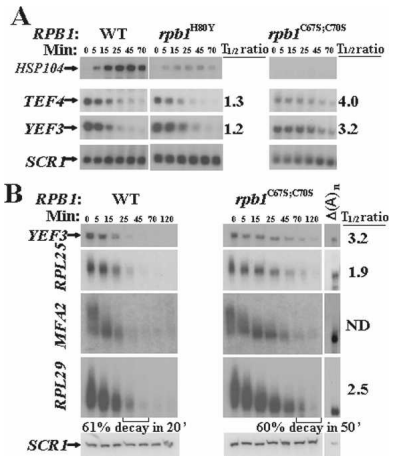

Figure 4. Cells carrying *rpb1*$^{C67S;\ C70S}$, but not another ts allele of *RPB1*, exhibit defective mRNA decay. (*A*) Decay kinetics in various mutant cells carrying transcriptionally defective *RPB1* allele. Decay kinetics was determined as in Figure 1A. Strains, indicated on *top* of the autoradiograms are described in Donaldson and Friesen (2000). Half-lives were determined as in Figure 1C. The ratios between the $T_{1/2}$ values in the mutant cells and those in the wild-type cells ("$T_{1/2}$ ratio") are indicated on the *right*. (*B*) *rpb1*$^{C67S;\ C70S}$ cells exhibit defective mRNA deadenylation and subsequent decay. Deadenylation kinetics was determined by PAGE-Northern as in Figure 1C. Half-lives were determined using Northern blot hybridization (not shown), as in *A*. Stability of the deadenylated *RPL29* RNA was estimated as described in Figure 1C.

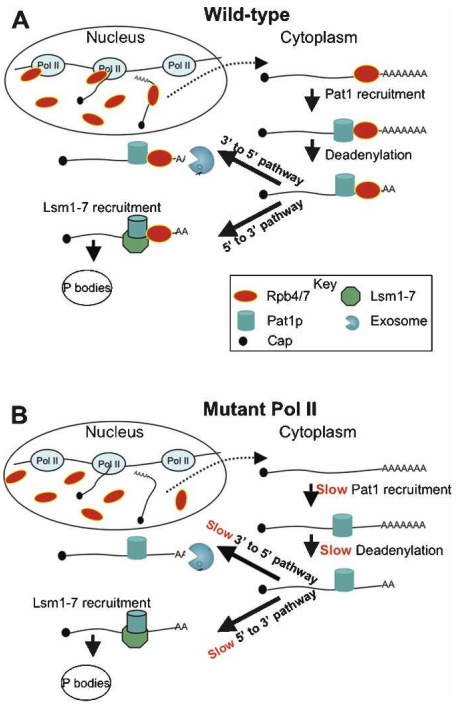

Figure 5. Pol II controls the two major mRNA decays in the cytoplasm via Rpb4/7 that serves as a mediator. (*A*) Maintaining proper levels of mRNA: schematic representation of the roles played by Pol II and Rpb4/7 in a wild-type cell. Only a small portion of the nuclear Rpb4/7 is recruited to Pol II and is involved in transcription initiation (Choder 2004), elongation (Verma-Gaur et al. 2008), and termination (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 (Fig. 3). Following transcription, the Rpb4/7–RNA complex is exported out of the nucleus by 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). At an undefined stage, Rpb4/7 helps recruiting Pat1p to the mRNA, by virtue of its capacity to interact with both Pat1p and the mRNA (Lotan et al. 2005, 2007). Rpb4/7 then stimulates shortening of the poly(A) tail by an unknown mechanism (Figs. 1C, 4B; Supplemental Fig. S3; Lotan et al. 2005, 2007). Following this stage, Rpb4/7 is involved in stimulating both major pathways of mRNA degradation (Lotan et al. 2005, 2007). The role of Rpb4/7 in the 5′-to-3′ pathway involves its interaction with Pat1p (Lotan et al. 2005, 2007) and probably stabilizing Lsm1–7 complex interaction with the mRNP (Lotan et al. 2005). The Rpb4/7–mRNP complex enters a P body (Lotan et al. 2005, 2007), where mRNA degradation is executed (association with P bodies is apparent mainly during starvation). Rpb4/7 also plays a role in the 3′-to-5′ degradation pathway. This role seems to be important as viability of mutant cells carrying some *rpb7* alleles, unlike that of wild-type cells, is dependent on the 5′-to-3′ pathway (Lotan et al. 2007). (*B*) Effect of defect in Pol II capacity to recruit Rpb4/7. Since the interaction of Rpb4/7 with mRNAs is dependent on its binding with Pol II (Fig. 3), there is little interaction in the mutant cells. Consequently, every Rpb4/7-mediated stage is adversely affected.

chemical approaches indicate that the defective capacity of *rpb6*^{Q100R} cells to degrade mRNAs is related to the poor interaction of Rpb6^{Q100R} p with Rpb4/7. We conclude that Rpb6p does not play a direct role in mRNA decay. Instead, being a Rpb4/7-interacting protein, Rpb6p governs mRNA decay by recruiting Rpb4/7 to the transcription apparatus, thus mediating the association of Rpb4/7 with mRNAs.

Pol II controls transcription via a shuttling factor, Rpb4/7, which serves as a mediator

Involvement of a given factor in two machineries 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, the data presented here contradict the "null" hypothesis that the dual functions of Rpb4/7 are unrelated. Our results demonstrate that the cytoplasmic roles of Rpb4/7 in mRNA decay can be executed only if Rpb4/7 is first assembled correctly with the Pol II core. Hence, Rpb4/7 roles in transcription and in mRNA decay are connected.

Rpb4p and Rpb7p bind mRNAs and function in their decay as a heterodimer. Whereas Rpb7p can function in the decay of many mRNAs independently of Rpb4p (Lotan et al. 2007), deletion of or mutation in *RPB4* affects class-specific mRNAs (Lotan et al. 2005). We propose that only Rpb4p can recruit a class-specific factor that modulates the decay of this class of mRNAs, explaining *rpb4*Δ phenotype.

A model proposed based on this work and previous publications is depicted in Figure 5. This study provides an example for "conditional interaction" between two interacting partners that occurs only within specific molecular context. Such kind of interaction might be the basis for other cases of coupling between two processes.

Summarily, conditional interaction between Rpb4/7 and mRNAs allows Pol II to impact not only transcription but also the fate of its products after they left the nucleus. This is the first indication that Pol II can affect mRNA decay in the cytoplasm and the first evidence for a direct mechanistic coupling between transcription in the nucleus and the two major mRNA decay processes in the cytoplasm.

Materials and methods

Yeast strains and plasmids

Yeast strains and plasmids are described in the Supplemental Material.

Determining mRNA levels and mRNA degradation profile

Two methods were used to inactivate transcription. In case of Tet-Off-*MFA2pG*, we used doxycycline (2 µg/mL). The transcription of natural non-HS genes was blocked by shifting cells rapidly to 42°C. Half-lives were determined as described previously (Lotan et al. 2005). For details see the Supplemental Material.

RNA immunoprecipitation (RIP) followed by qRT–PCR

RIP was performed essentially as described previously (Gilbert and Svejstrup 2006), with modifications as described in the Supplemental Material. RT performed using Verso^{TM} cDNA Kit (Thermo Scientific). Input RNA was diluted 1:1500 before RT. Real-time PCR was performed by Rotor-Gene 6000 (Corbett Lifesciences), as instructed by the manufacturer. For details and primers' sequences see the Supplemental Material.

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