

Nab2 functions in the metabolism of RNA driven by polymerases II and III

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ABSTRACT Gene expression in eukaryotes is an essential process that includes transcription, RNA processing, and export. One important player in this interface is the poly(A)⁺-RNA-binding protein Nab2, which regulates the mRNA poly(A)⁺-tail length and export. Here we show that Nab2 has additional roles during mRNA transcription, tRNA metabolism, and ribosomal subunit export. Nab2 is associated with the entire open reading frame of actively transcribed RNA polymerase (RNAP) II and III genes. As a consequence, *nab2* mutations confer translation defects that are detected by polysome profiling. Genome-wide analysis of expression of a conditional degron *nab2* mutant shows that the role of Nab2 in RNAPII transcription and RNAPIII metabolism is direct. Taken together, our results identify novel functions for Nab2 in transcription and metabolism of most types of RNAs, indicating that Nab2 function is more ubiquitous than previously anticipated, and that it is a central player in the general and coordinated control of gene expression from transcription to translation.

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INTRODUCTION

Gene expression in eukaryotes is an essential process that includes transcription, RNA processing, and RNA export to the cytoplasm, where translation into proteins takes place. The whole process is highly regulated, and its different steps are coupled temporally and spatially. Many mRNA processing factors that participate in 5'-end capping, splicing, 3'-end processing, and polyadenylation are loaded cotranscriptionally to the pre-mRNA through interactions with the carboxy-terminal domain (CTD) of the RNAPII (Kohler and Hurt, 2007; Luna *et al.*, 2008). Any failure in mRNA processing affects the other steps and could cause nuclear mRNA retention at the nuclear pore complex (NPC) or at the transcription site. The blocked defective mRNAs are then suscep-

tible to be degraded by the nuclear exosome (Schmid and Jensen, 2008).

One example of this coupling is found in the essential Nab2 protein of *Saccharomyces cerevisiae*, which is involved in several steps of mRNA metabolism. Nab2 is a poly(A)⁺-binding protein (PABP) that has been shown to physically interact with poly(A)⁺ RNA in vitro and in vivo (Hector *et al.*, 2002; Viphakone *et al.*, 2008). In contrast to other PABPs, which bind to RNA through a well-known RRM RNA-binding motif (Mandel *et al.*, 2008), Nab2 belongs to a new class of tandem zinc finger proteins that interact with poly(A)⁺ RNAs via the conserved Cys-Cys-Cys-His zinc fingers (Kelly *et al.*, 2007). Previous studies demonstrated that Nab2 regulates the poly(A)⁺-tail length of mRNAs, protecting their 3' ends and impeding an excessive polyadenylation mediated by Pap1 (Hector *et al.*, 2002; Viphakone *et al.*, 2008). In addition, Nab2 is able to shuttle between the nucleus and the cytoplasm, accompanying the messenger ribonucleoprotein (mRNP) and facilitating its export. This is supported by the observation that most *nab2* mutants accumulate poly(A)⁺ RNAs within the nucleus (Green *et al.*, 2002) and that Nab2 interacts functionally and physically with many RNA export factors such as Mex67, Yra1, and Mlp1/2 (Green *et al.*, 2003; Vinciguerra *et al.*, 2005; Fasken *et al.*, 2008). Accordingly, a recent study placed Nab2 as the adaptor for the recruitment of the mRNA export receptor Mex67-Mtr2, whereas Yra1 would act as a cofactor facilitating this interaction and acting as a part of the mRNA surveillance mechanism together with Mlp1 (Iglesias *et al.*, 2010). Finally, Nab2 reaches the cytoplasm, where it

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Abbreviations used: ChIP, chromatin immunoprecipitation; CTD, carboxy-terminal domain; GFP, green fluorescent protein; hnRNP, heterogeneous nuclear ribonucleoprotein; MPA, mycophenolic acid; mRNP, messenger ribonucleoprotein; NPC, nuclear pore complex; ORF, open reading frame; PABP, poly(A)⁺ binding protein; RNAP, RNA polymerase; UTR, untranslated region.

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is released from the mRNA by the Dbp5 RNA helicase to allow translation initiation (Tran *et al.*, 2007). Taken together, these data indicate a function of Nab2 in a central step in mRNP biogenesis.

A connection between mRNA export and transcription has been shown. Some eukaryotic genes are dynamically anchored to the nuclear periphery after transcription activation, presumably to facilitate mRNA export (Akhtar and Gasser, 2007). A functional connection between transcription and mRNA export is provided by the conserved THO and THSC (also called TREX2) complexes. The yeast THO complex, formed by Hpr1, Tho2, Mft1, and Thp2, binds to actively transcribed chromatin, playing a role during transcription elongation and mRNA metabolism and export (Luna *et al.*, 2008). It physically and functionally interacts with the Sub2 and Yra1 factors that allow the recruitment of Mex67-Mtr2 (Jimeno *et al.*, 2002; Strasser *et al.*, 2002). The THSC/TREX-2 complex (Thp1, Sac3, Sus1, Cdc31) is located at the nuclear periphery in association with the NPC and has a role in mRNA export facilitating the anchoring of Mex67 to the NPC (Kohler and Hurt, 2007; Luna *et al.*, 2008). In addition, THSC also functions during transcription elongation in connection with its role in mRNP metabolism and export (Chekanova *et al.*, 2008; González-Aguilera *et al.*, 2008; Klockner *et al.*, 2009). Of interest, Nab2 overexpression suppresses both the RNA export and the transcription defects of THSC mutants (Gallardo *et al.*, 2003).

For a better understanding of the interconnection between RNP biogenesis and export we have further explored the function of Nab2. Our data reveal that Nab2 is associated with the entire open reading frame (ORF) of actively transcribed RNAPII and RNAPIII genes. We show that Nab2 has a role during RNAPII transcription and is needed for proper tRNA expression and ribosomal subunit export. As a consequence, *nab2* mutations confer translation defects. Using a conditional degron allele and genome-wide analysis of expression, we demonstrate that the role of Nab2 in RNAPII transcription and RNAPIII metabolism is not the result of a secondary effect. We conclude that Nab2 functions in RNAPII transcription and has a general role in the metabolism of RNAPIII-driven RNAs.

RESULTS

Nab2 binds RNAPII and RNAPIII actively transcribed chromatin in vivo

We previously suggested that the poly(A)⁺-RNA-binding protein Nab2 could have a role during RNAPII transcription since the *nab2-1* mutant shows low levels of *LacZ* mRNA driven from the regulated *GAL1* promoter (Gallardo *et al.*, 2003). Therefore we wondered whether Nab2 is associated with chromatin in vivo. Yeast strains containing a tandem affinity purification (TAP)-tagged version of *NAB2* at the chromosomal locus were constructed. The TAP-tagged strain had the same growth and expression behavior as the otherwise

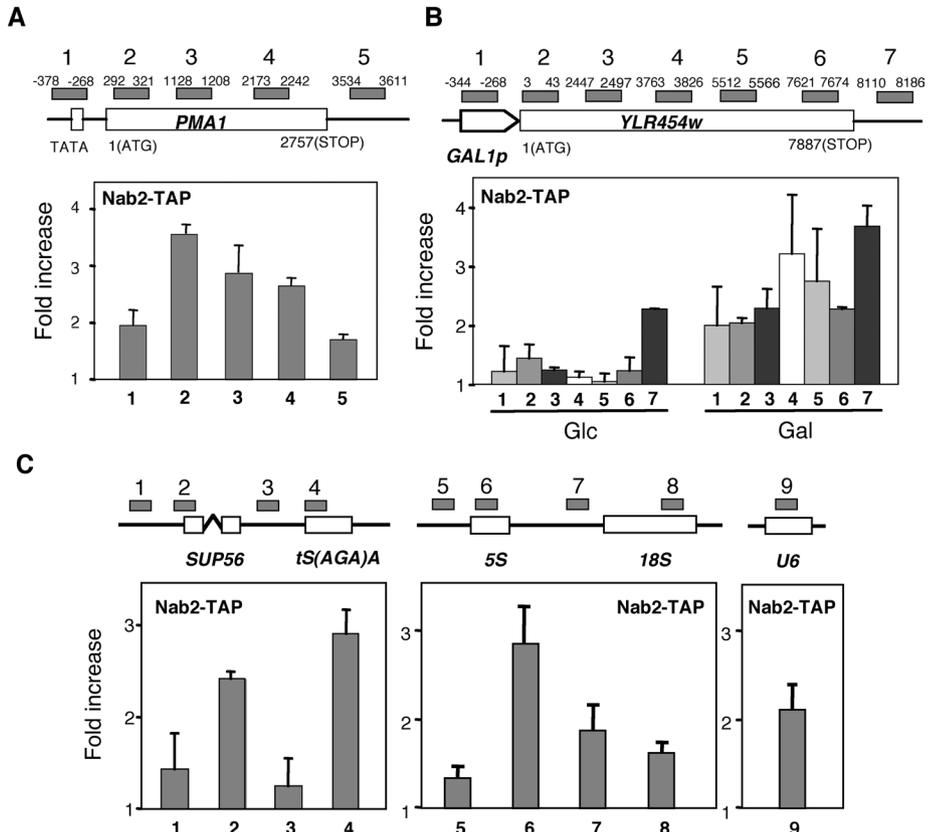


FIGURE 1: Nab2 binds to actively transcribed RNAPII and RNAPIII genes. (A) ChIP analysis of Nab2-TAP tagged at the endogenous *PMA1* gene in the wild-type strain NAT. (B) ChIP analysis of Nab2-TAP at the *GAL1p::YLR454w* fusion construct located at the endogenous *YLR454w* locus of wild-type strain NATYL-4D. (C) ChIP analysis of Nab2-TAP at *SUP56*, *tS(AGA)A*, 5S rDNA, U6 snDNA, and 18S rDNA genes and their flanking regions in the wild-type strain (NAT). The scheme of the genes and the PCR-amplified fragments are shown. Recruitment values shown were calculated from the amount of DNA present in each region, normalizing IP/input ratios, and relative to the amount of DNA of the intergenic region. Values above twofold are considered specific (see Supplemental Figure S1). Errors bars, SD.

isogenic strain, indicating that the Nab2-TAP allele is fully functional. We analyzed whether Nab2 could be recruited to chromatin at five regions of the constitutively transcribed *PMA1* gene: the promoter, 5', middle, and 3' regions of the ORF and the untranslatable 3'-end region (3'-UTR) of the gene. As shown in Figure 1A and Supplemental Figure S1, Nab2 is associated with chromatin in vivo mainly along the ORF. Nab2 showed the highest recruitment at the 5' and middle regions of the gene and a low but detectable signal at the promoter region. To test whether recruitment of Nab2 was also observed in regulated genes and whether this was dependent on transcription, the chromatin immunoprecipitation (ChIP) analysis was extended to the 8-kb-long *YLR454w* gene fused to the *GAL1* promoter (Mason and Struhl, 2005). In this system, seven regions covering the entire ORF, the promoter, and the 3'-UTR regions were analyzed. As shown in Figure 1B, there was no amplification of the DNA sequences analyzed at the promoter and ORF regions when transcription was repressed (2% glucose), indicating no recruitment of Nab2 to inactive chromatin. However, recruitment of Nab2 to *GAL1p::YLR454w* was obvious when transcription was active (2% galactose). Recruitment was detectable at the promoter and all over the ORF. These results indicate that Nab2 is able to associate with chromatin in a transcription-dependent manner. Of interest, a signal at the 3'-UTR region of *YLR454w* was detected under both repressed and active transcription (Figure 1B). The level of the signal observed under active

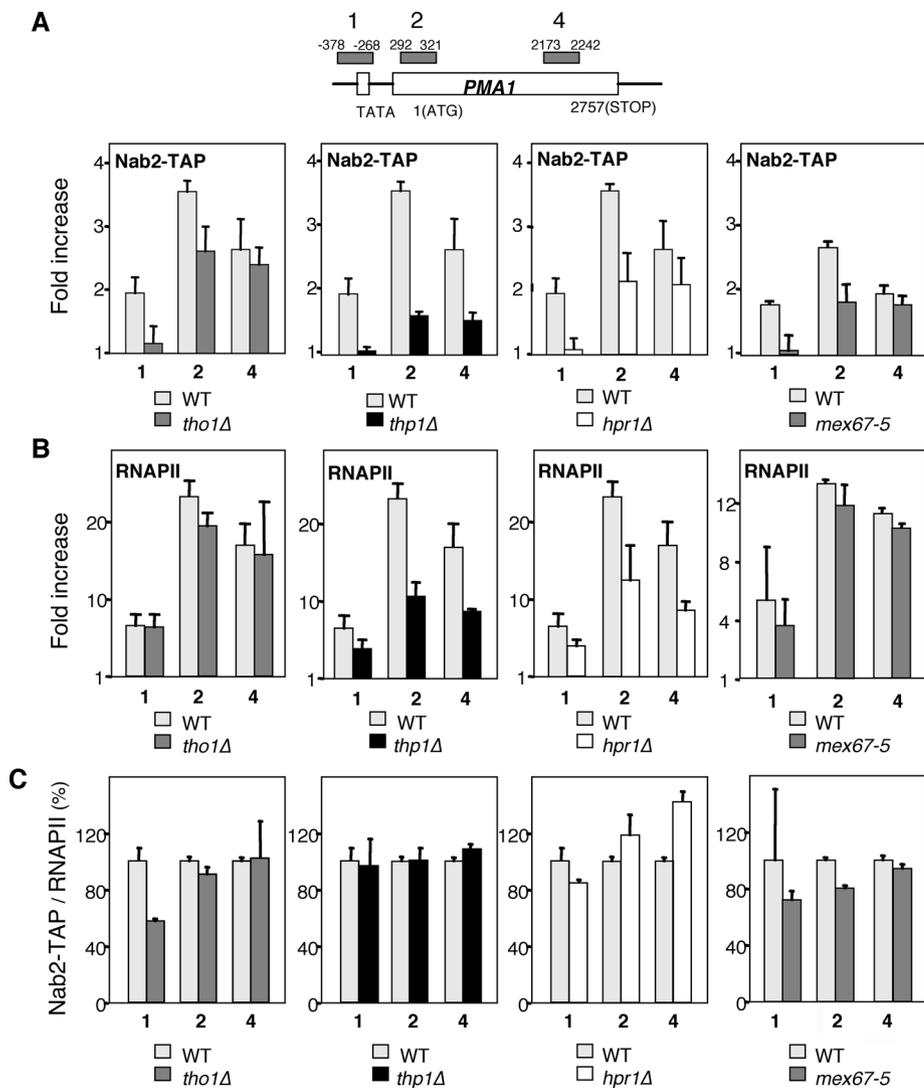


FIGURE 2: Association of Nab2 with chromatin in mRNA biogenesis mutants. (A) ChIP analyses of Nab2-TAP in wild-type (NAT), *tho1Δ* (NATT1-9D), *thp1Δ* (NATT-6A), *hpr1Δ* (NATH-7C), and *mex67-5* (NATM-1C) strains at the *PMA1* gene. (B) ChIP analyses of RNAPII at the *PMA1* gene in wild-type and the same mutant strains as in A. (C) Normalization of ChIP Nab2-TAP data with those of RNAPII. All experiments were performed at 30°C except for *mex67-5*, which was performed at 37°C. Other details in Figure 1.

transcription was higher than expected, considering the pattern observed in the *PMA1* gene (Figure 1, A and B). This suggested that it might be caused by an additive effect of the signal corresponding to the 3'-UTR region under active transcription and the one observed also in repressed conditions. Indeed, the region of the genome corresponding to the fragment amplified by PCR covers the 3'-UTR region of the *YLR454w* gene, but also the tRNA gene *tL(AAU)* *L2*. Given that tRNA genes are constitutively transcribed, consistent with the signal observed under conditions of repression of the *GAL1* promoter, these results opened the possibility that Nab2 also could be recruited to RNAPIII genes. To verify that Nab2 associates with tRNA genes, we studied recruitment of Nab2 to another region in the genome that codes for two tRNA genes, *tL(CAA)*A (*SUP56*) and *tS(AGA)*A, located more than 1.2 kb apart from the nearest RNAPII genes. In addition, we studied recruitment to other RNAPIII-driven genes, such as the 5S rRNA and U6 snRNA genes, and RNAPI-driven genes, such as the 18S rRNA gene. As can be seen in Figure 1C, Nab2 could be recruited to a different extent to tRNA, 5S rRNA, and

U6 snRNA genes, whereas a weak and non-specific signal was detected for the 18S rRNA gene as compared with the signals from upstream and downstream flanking regions.

Nab2 recruitment to transcribed genes is altered in the absence of mRNP biogenesis factors

Next we tested whether Nab2 recruitment to transcribed chromatin depended on mRNP biogenesis factors. For this, ChIP experiments were performed with the Nab2-TAP tagged protein in mutants of the THO complex (*hpr1Δ* mutant), the Tho1 hnRNP (*tho1Δ*), and the mRNA export factor Mex67-Mtr2 (*mex67-5* thermosensitive mutant), which are all known to be recruited to actively transcribed ORFs, and in mutants of the THSC complex (*thp1Δ*) (Strasser *et al.*, 2002; Gwizdek *et al.*, 2006; Jimeno *et al.*, 2006). We first determined that the cellular level of the Nab2-TAP protein was not decreased in any of the mutants tested (Supplemental Figure S2). Then we analyzed by ChIP the recruitment of Nab2-TAP to the 5'-end, 3'-end, and promoter regions of the *PMA1* gene. Figure 2A shows that recruitment of Nab2 was reduced at different degrees in all mutants tested, the strongest reduction being observed in the *thp1Δ* and *hpr1Δ* mutants. To know whether this reduction was due to a reduced transcription of *PMA1*, we determined the levels of RNAPII in this gene by ChIP experiments in all mutants. As can be seen in Figure 2B, RNAPII recruitment was also reduced in THO and THSC mutants. Normalization of the Nab2-TAP levels with respect to RNAPII revealed that most of the reduction in Nab2-TAP recruitment to chromatin could be explained by a decrease in transcription (Figure 2C). In *tho1Δ* mutants, in which transcription is not affected, Nab2 was reduced at the promoter, but the signals are too low to allow conclusions (see Supplemental Figure S1).

As performed with the RNAPII-driven *PMA1* gene, we analyzed whether the association of Nab2 with the *SUP56*, 5S rRNA, and U6 snRNA RNAPIII genes was dependent on THO and THSC complexes and the Tho1 hnRNP. Nab2 recruitment to the genes analyzed was almost abolished in *thp1Δ*, largely reduced in the 5S rDNA in *hpr1Δ*, and practically unchanged in *tho1Δ* mutants (Figure 3A). These results indicate that recruitment of Nab2 to RNAPIII genes depends on the THSC complex and to a lesser extent on the THO complex. This result opened the possibility that THSC and THO were also recruited to RNAPIII genes. Consequently, ChIP was performed in strains containing a TAP-tagged version of proteins of the THO complex (Hpr1-TAP), the Tho1 hnRNP (Tho1-TAP), and the THSC complex (Thp1-TAP). Figure 3B shows that whereas Tho1 was not recruited to the RNAPIII genes tested, recruitment could be seen for Hpr1 and Thp1, in particular at the 5S rRNA gene. Thus we conclude that Nab2 is associated with RNAPIII genes and that this association

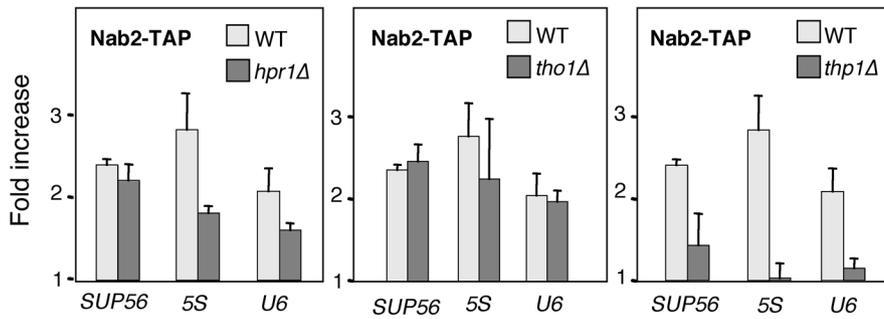
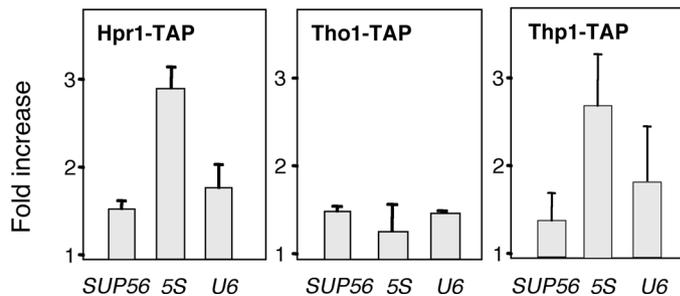
A**B**

FIGURE 3: Association of Nab2 with RNAPIII genes in mRNA biogenesis mutants. (A) ChIP analyses of Nab2-TAP in the wild-type (NAT), *hpr1Δ* (NATH-7C), *tho1Δ* (NATT1-9D), and *thp1Δ* (NATT-6A) strains at the *SUP56*, 5S rDNA, and U6 snDNA genes. (B) ChIP analysis in Hpr1-TAP (Hpr1-TAP), Tho1-TAP (WWT1T), and Thp1-TAP (THP1-TAP) at the *SUP56*, 5S rDNA, and U6 snRNA genes. Other details in Figure 1.

might depend on THSC and THO, which are also recruited to RNAPIII genes.

Transcription defects in *nab2-1* in vivo

We previously showed that multicopy *NAB2* suppressed the *thp1Δ* mutant (Gallardo *et al.*, 2003). Thp1 is a component of the THSC complex, which has a role in both mRNA export and transcription elongation of long or G+C-rich genes (Gallardo *et al.*, 2003; González-Aguilera *et al.*, 2008). This, together with the transcription-dependent recruitment of Nab2 to active chromatin at ORFs, led to the hypothesis that Nab2 could also have a role in RNAPIII transcription. To address this, genetic and molecular biology analyses were performed with the *nab2-1* mutant, which grows well at 30°C but shows a severe growth defect at 16°C. This allele produces an N-terminal deletion from residues 4–97 resulting in a protein (Nab2ΔN) defective in poly(A)⁺-mRNA export and *lacZ* mRNA expression (Gallardo *et al.*, 2003; Marfatia *et al.*, 2003). First, an analysis was done on the sensitivity of *nab2-1* to mycophenolic acid (MPA), a drug that reduces the intracellular ribonucleotide pools, resulting in slow growth that is exacerbated in mutants impaired in transcription elongation. Figure 4A shows a clear growth inhibition of *nab2-1* in the MPA plate. As a control, *rpb9Δ* was used, a mutant of the Rpb9 subunit of RNAPIII that has a critical role in transcription elongation by virtue of its interaction with the transcription elongation factor TFIIS (Hemming *et al.*, 2000; Tous *et al.* 2011). Second, *nab2-1* was crossed with *rpb9Δ* and *spt4Δ* mutants, which affects transcription elongation, to test whether the double mutants showed synergistic phenotypes. We were not able to recover any double mutants from 24 and 36 tetrads analyzed, respectively, indicating that *nab2-1* was synthetically lethal with *rpb9Δ* and *spt4Δ* (unpublished data). These

results indicate a genetic interaction between Nab2 and transcription elongation factors.

To further assess the role of Nab2 in transcription, we used an in vitro system that contains two G-less cassettes in which transcription-elongation efficiency is determined in whole-cells extracts by the ratio of the levels of the downstream (376-nucleotide [nt]-long) versus the upstream (84-nt-long) G-less RNA fragments (Figure 4B). As shown in Figure 4B, the transcription elongation efficiency of *nab2-1* was similar to that of the wild type, and thus Nab2 seems not to be required for optimal in vitro transcription elongation. Because transcription is coupled with mRNA export, we wondered whether the effect of Nab2 on transcription elongation could be relevant in vivo when coupled with RNA export through the NPC and not in cell extracts in which transcription-RNA export coupling was disrupted, as previously shown for the THSC factors (González-Aguilera *et al.*, 2008). To test this possibility, transcription elongation was analyzed by determining the in vivo distribution of RNAPIII by ChIP analysis. RNAPIII recruitment was assayed at the chromosomal *GAL1pr::YLR454w* fusion construct in the five aforementioned regions covering the entire ORF. Although the amount of total RNAPIII in the *nab2-1* mutant was similar to that in the wild type in

the 5' region (Supplemental Figure S3), the levels of RNAPIII were reduced from the 5' region to the 3' region, showing only 58% of the wild-type RNAPIII levels at the 3' end of the gene. (Figure 4C). A similar reduction in RNAPIII recruitment was previously found in the mutants of *SPT4*, *THO*, or *THSC* defective in transcription elongation (Mason and Struhl, 2005; González-Aguilera *et al.*, 2008). In addition, Northern analysis of the same construct revealed that the *nab2-1* mutant showed a similar reduction in the levels of *YLR454w* mRNA (Figure 4D). These results suggest that RNAPIII transcription elongation might be impaired in the *nab2-1* mutant in vivo.

Analysis of mRNA transcription in *nab2* cells

To assay whether the transcription defect observed in *nab2-1* mutant was a direct consequence of the lack of Nab2 activity rather than an indirect pleiotropic effect or the result of a cell adaptation to survival in conditions in which the essential Nab2 protein is not available, a "heat-inducible N-degron" of Nab2 (*nab2-td*) was constructed. Degron alleles allow a specific degradation of the protein of interest at 37°C in a short period of time (Dohmen *et al.*, 1994). Therefore we fused the degron extension to the N-terminus of Nab2 in a yeast strain background able to overexpress the ubiquitin ligase *UBR1* to improve degradation of the fusion protein (Figure 5A) (Labib *et al.*, 2000). As shown in Figure 5B, *nab2-td* was able to grow at 26°C but not at the restrictive temperature of 37°C, at which Nab2 is degraded, consistent with the essential role of Nab2 in the cell. Western blot analysis revealed that the *nab2-td* fusion protein was rapidly degraded upon shifting to 37°C and had already disappeared in 30 min (Figure 5C). We first checked whether the *nab2-td* showed any of the phenotypes observed in *nab2-1*, such as defective mRNA-export. Figure 5D shows that the bulk of

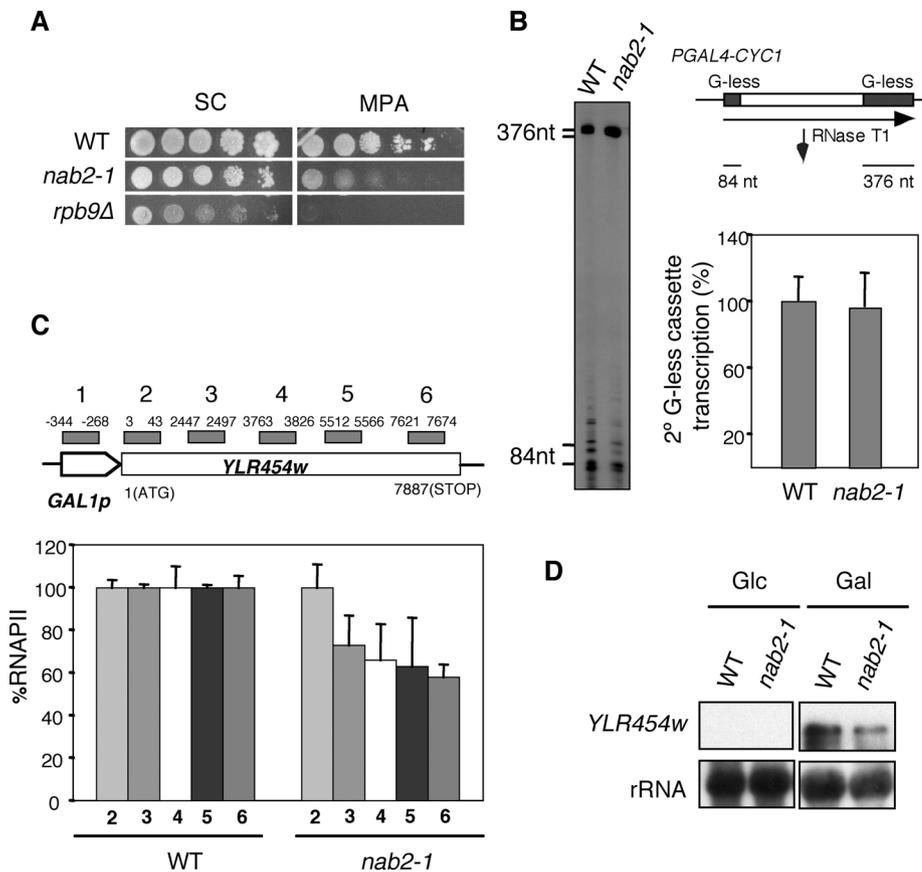


FIGURE 4: *nab2-1* is affected in transcription in vivo. (A) Tenfold serial dilutions of wild-type (WT) (ACY429), *nab2-1* (*nab2-1*), and *rpb9Δ* (RPB9-1C) strains on SC plates containing MPA (50 μg/ml). Photographs were taken after 5 d of growth at 30°C. (B) In vitro transcription-elongation analysis of WT (ACY429) and *nab2-1* (*nab2-1*) strains. The scheme of the two G-less cassette system of plasmid pGCYC1-402 is shown. Efficiency of transcription elongation was determined as the percentage of total transcripts that reached the 376-nt G-less cassette with respect to the transcripts that covered the 84-nt cassette. The mean value and SD of three independent experiments are shown. (C) ChIP analyses of RNAPII at the *GAL1p::YLR454w* fusion construct in WT (WT-YLR454) and *nab2-1* (NAYL-3D) strains. The scheme of the gene and the PCR-amplified fragments is shown. Values were first normalized to the amount of DNA in region 2 and then with respect to the WT value, taken as 100%. Other details are in Figure 1. (D) Northern blot analysis of the expression of *GAL1p::YLR454w* in WT (WT-YLR454) and *nab2-1* (NAYL-3D) strains. RNA was isolated from cultures grown at 30°C in 3% glycerol–2% lactate up to an OD₆₀₀ of 0.5 and induced for 3 h in galactose. The ³²P-labeled DNA probes used are described in Supplemental Table S3.

poly(A)⁺ RNAs were retained in the nucleus in *nab2-td* both 30 and 75 min after the shift to 37°C.

Having confirmed that the *nab2-td* was a conditional allele in which Nab2 could be inactivated in <30 min, we analyzed gene expression in yeast cells right after Nab2 inactivation. Northern analysis of the *nab2-td* strain carrying the *GAL1p::lacZ* expression system revealed that after 30 min of shifting cells to 37°C the *lacZ* mRNA levels were reduced to 43% of the wild-type levels and to 7% after 60 min (Figure 5E). This quick decrease in *lacZ* mRNA, as well as the RNA export defect, indicates that this effect is direct and rules out the possibility that it could be caused by the effect of secondary proteins or by an adaptation phenomenon.

To directly assess the role of Nab2 in transcription elongation in vivo, we performed a direct run-on–based assay (GLRO) that allows pre-mRNA quantification without any need of hybridization or PCR amplification (Tous et al., 2011) in the *nab2-td* allele. The GLRO constructs are based on the plasmid CYCds (Steinmetz and Brow,

2003) with two G-less cassettes of 262 and 132 nt separated by a 243-nt *CYC1* spacer sequence (GLRO-short) or a 2-kb fragment of the *LacZ* gene (GLRO-long), which are transcribed from the strong constitutive *TDH3* promoter. Transcription elongation efficiency was measured as the ratio of ³²P incorporated into the 132-nt G-less cassette versus the 262-nt G-less cassette for each construct. As can be seen in Figure 5F, transcription elongation is not affected in any of the systems. It seems, therefore, that a putative transcription-elongation defect is not a major factor responsible for the low mRNA levels observed. It is possible that other RNA biogenesis steps, such as transcription initiation, termination, or RNA surveillance could be the responsible for this gene expression defect.

To study the possibility that part of the reduction of mRNA levels of *nab2* mutants was due to mRNA decay, we performed Northern analysis in double mutants of *NAB2* and the exoribonuclease subunit of the nuclear exosome *RRP6* carrying the chromosomal *GAL1p::YLR454w* fusion and the *GAL1p::LacZ* system. Unexpectedly, the genetic cross revealed that *nab2Δ rrp6Δ* double mutants were viable. Thus the *rrp6Δ* mutation suppressed the lethality of *nab2Δ* (Supplemental Figure S4A). The double mutant grew poorly at 30°C, but did not grow either at 37°C (as *rrp6Δ*) or 16°C (as *nab2-1*) (Supplemental Figure S4B). Of interest, the levels of *lacZ* and *YLR454w* mRNAs in *nab2Δ rrp6Δ* and *nab2-1 rrp6Δ* double mutants were lower than in the *nab2-1* single mutant (Supplemental Figure S4, C and D). This suggests that mRNA degradation mediated by the nuclear exosome is not the main cause for the mRNA reduction of *nab2-1* cells. However, we cannot rule out the possibility that other RNA surveillance systems could affect mRNA stability.

Analysis of tRNA and rRNA metabolism in *nab2* mutants

The observed recruitment of Nab2 to RNAPIII genes opens the possibility that Nab2 could also be involved in both tRNA and rRNA metabolism. To address this question, a search was carried out to find genetic interactions of *NAB2* with tRNA and rRNA metabolism mutants. We used the multicopy plasmid YEpNAB2 carrying the *NAB2* gene (Gallardo et al., 2003) and transformed a number of tRNA biogenesis and export mutants (*cex1Δ*, *msn5Δ*, *los1Δ*, *lhp1Δ*), rRNA export mutants (*arx1Δ* and *nmd3*), and general RNA export mutants (*utp8* and *crm1*). An effect in growth was observed only with conditional mutants in which essential genes were under the doxycycline-repressible *tet* promoter. As shown in Figure 6A, *NAB2* overexpression was toxic for *Crm1*- and *Utp8*-depleted cells. In the case of *utp8*, *NAB2* overexpression was toxic only at 37°C and even in the absence of doxycycline, in which *tet::UTP8* was expressed, although probably not at wild-type levels. The rest of the mutants tested

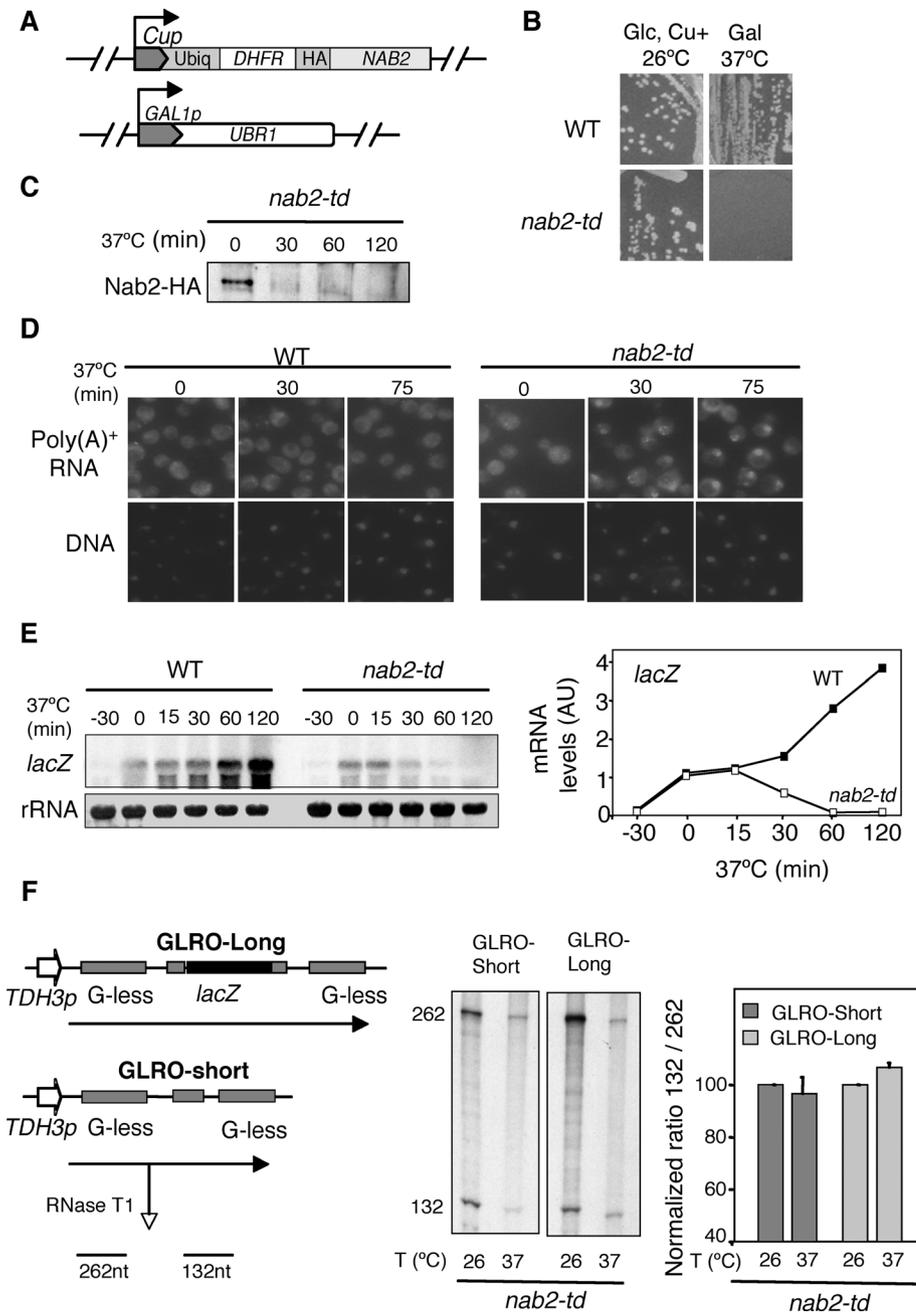


FIGURE 5: Construction and characterization of transcription and RNA export in a conditional Nab2-degron mutant. (A) Scheme of the thermosensitive degron allele. Nab2 is expressed under the control of the *CUP1* promoter and tagged with an HA epitope and a DHFR domain in the N-terminal region of the protein. The DHFR domain has an ubiquitination motif recognized by the ubiquitin ligase Ubr1 expressed under the *GAL1* promoter. (B) Growth of wild-type and *nab2-td* (*NTD1*) strains in permissive conditions (glucose and CuSO_4 at 26°C) or restrictive conditions (galactose without CuSO_4 at 37°C). Photographs were taken after 3 d of growth. (C) Western analyses of total cell extracts from *nab2-td* strain after different times of degron activation using an anti-HA antibody. Cells were cultured in permissive conditions up to the exponential phase and then transferred to SC 2%Gal without CuSO_4 at 26°C for 30 min to allow the expression of Ubr1 ($t = 0$), after which the temperature was shifted to 37°C for degron activation. (D) Subcellular localization of poly(A)⁺ RNA analyzed by in situ hybridization with digoxigenin-labeled oligo(dT) probe in wild-type and *nab2-td* strains at 0, 30, and 75 min after degron activation. Cells were grown as in C and then subjected to fluorescence microscopy. Nuclear DNA was stained with 10 g/ml 4,6-diamidino-2-phenylindole. (E) Northern blot analysis of wild-type and *nab2-td* strains transformed with the plasmid pGLL carrying the Gal-LacZ expression system. RNA was isolated from cells cultured in permissive conditions up to the exponential phase ($t = -30$) and then transferred to SGal without copper at 26°C for 30 min to allow the expression of *lacZ* ($t = 0$), followed by a shift to 37°C to induce the degron. mRNA

did not show any growth inhibition when transformed with multicopy *NAB2* (Supplemental Figure S5). Utp8 works in both rRNA metabolism and tRNA export (Dragon *et al.*, 2002; Strub *et al.*, 2007), and Crm1 participates in transport of rRNAs (Stade *et al.*, 1997; Johnson *et al.*, 2002). Because overexpression of a gene can produce toxicity in functionally related mutants, as is the case for *SUB2* and other mRNA processing factors (González-Aguilera *et al.*, 2008), this opens the possibility that Nab2 could affect tRNA and rRNA metabolism. To assay the putative role of Nab2 in tRNA metabolism, we took advantage of the *SUP11* (*tY(GUA)F1*) ochre suppressor that codifies a tRNA^{ochre} able to suppress the premature stop codon mutation of *ade2-1^o* conferring adenine auxotrophy (Figure 6B). Considering that in *nab2-1* background the expression of *ADE2* was not affected (Supplemental Table S1), we transformed an *ade2-1^o nab2-1* strain with the centromeric plasmid pUN60 harboring the *SUP11* gene and measured the ability of the mutant to grow in medium lacking adenine. Figure 6B shows that growth of *nab2-1* but not of *hpr1Δ* or *thp1Δ* was clearly reduced, suggesting that Nab2 is needed for the proper expression of the *SUP11* tRNA. To know which step in tRNA and rRNA metabolism could be affected in *nab2* mutants, we performed Northern analysis of RNAPIII (5S, *SUP56*, tM(CAU)O1, and *SCR1*) and RNAPI (5.8S) genes. Figure 6C showed that *nab2-1*, *hpr1Δ*, and *thp1Δ* mutants were not affected in the levels of 5.8S, 5S, and *SCR1* RNAs. However, tRNA levels seemed to be slightly increased in *thp1Δ* and *nab2-1* mutants at 30°C compared with wild type (Figure 6C). To analyze this effect in more detail, we quantified the levels of precursor and mature *SUP56* tRNAs. In contrast to *thp1Δ*, in which pre-tRNAs increased with respect to mature tRNAs, in *nab2-1* cells all *SUP56* tRNA species

quantifications were normalized with respect to the rRNA levels of each sample. AU, Arbitrary units. (F) GLRO analysis of *nab2-td* strain transformed with GLRO-short and GLRO-long constructs. The run-on products of noninduced (26°C) and induced degron strains (37°C) were resolved in a 6% PAGE. For each sample the ratio of the distal vs. the proximal G-less cassette was normalized respect to the noninduced degron control. A representative acrylamide gel is shown. The mean value and SD of three independent experiments are shown.

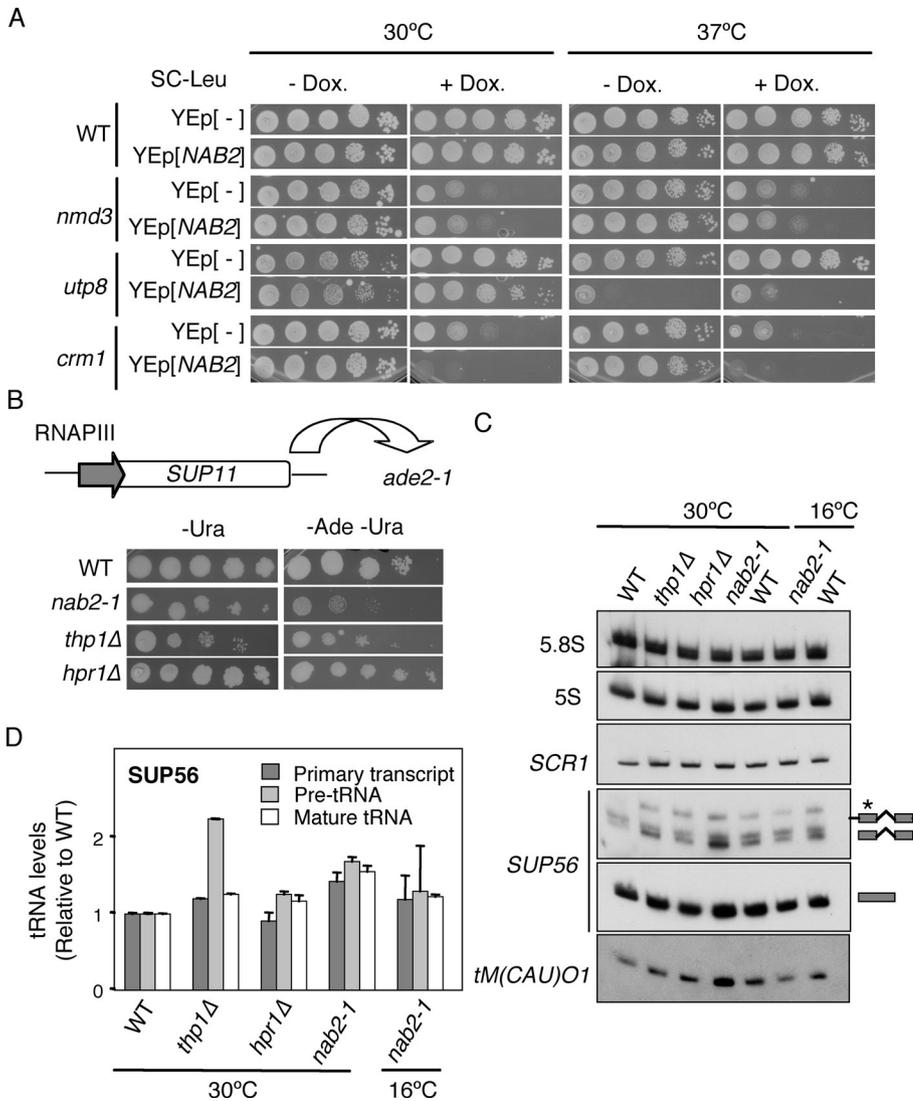


FIGURE 6: Effect of Nab2 on tRNA and rRNA biogenesis. (A) Overexpression of *NAB2* in conditional mutants of tRNA and rRNA biogenesis factors. Tenfold serial dilutions of WT (R1158), *tet::NMD3* (TH-7592), *tet::UTP8* (TH-6066), and *tet::CRM1* (TH-7764) conditional mutants were transformed with multicopy plasmids YEpNAB2 and YEp351 and plated on selective medium with 20 g/ml (*tet* repressed) or without (*tet* active) doxycycline. Photographs were taken after 3 d of growth at 30 or 37°C. (B) Effect of Nab2 mutation in tRNA metabolism using plasmid pUN60 containing the RNAPIII-transcribed *SUP11* allele that encodes a tRNA suppressor of *ochre* stop-codon of *ade2-1*. The efficiency of *SUP11* tRNA expression is measured by the ability to suppress the *ade2-1* mutation as determined by the capability of growth in medium without adenine. Tenfold serial dilutions of WT (WT-YLR454), *nab2-1* (NAYL-3D), *thp1Δ* (WFBEO46), and *hpr1Δ* (U768-4C) strains were transformed with the plasmid pUN60 and plated on selective medium with or without adenine. Photographs were taken after 4 d of growth at 30°C. (C) Northern analyses of *thp1Δ* (WFBEO46), *hpr1Δ* (U768-4C), their isogenic WT strain (W303-1A), *nab2-1* (*nab2-1*), and its isogenic WT strain (ACY429). Cells were cultured in YPD medium at 30°C. In the case of *nab2-1*, cells were grown at 30°C up to the exponential phase, and half of the culture was transferred to 16°C for 8 h. Scheme of the primary transcript, intermediates stages, and mature transcript of *SUP56* tRNA is shown. Asterisk marks the signal corresponding to 5S rRNA. The ³²P-labeled ssDNA probes used are described in Supplemental Table S3. (D) Quantification of *SUP56* tRNA levels in the different mutants described in C. RNA levels were normalized with respect to the wild-type value, taken as 1.

were increased (primary transcript, pre-tRNAs, and mature tRNAs) (Figure 6D). These results indicate that expression of tRNAs seems to be altered in *nab2-1* and *thp1Δ* mutants. Because Northern analysis shows that RNAPIII transcription is not

Genome-wide impairment of transcription caused by quick Nab2 depletion

To determine how general the impact of Nab2 in gene expression is, the expression profile of the total yeast RNAPII genes was

affected in these mutants, it is possible that tRNA edition, export, or stability is affected.

Because Nab2 binds the 5S rRNA gene and shows functional interactions with Crm1, we wondered whether Nab2 affected export of the ribosomal subunits (r-subunits). To address this question, the three mRNA-metabolism mutant strains *nab2-1*, *hpr1Δ*, and *thp1Δ* were transformed with plasmids expressing green fluorescent protein (GFP)-tagged forms of either the 60S r-protein L25 or the 40S r-protein S2. Cellular localization of these proteins was determined by fluorescence microscopy. As shown in Figure 7A, the distribution of L25-eGFP and S2-eGFP, as expected for r-proteins, was predominately cytoplasmic in wild type and *hpr1Δ* and *thp1Δ* mutants. However, L25-eGFP and to a lesser extent S2-eGFP exhibited a clear nuclear accumulation in the *nab2-1* mutant. These results suggest that transport of preribosomal particles is partially blocked in the *nab2-1* mutant.

Loss of Nab2 function leads to defective translation initiation

Considering that Nab2 seems to play a role in mRNA, tRNA, and rRNA metabolism, we also addressed whether *nab2-1* could affect negatively translation. To do this, the polysome profiles of *nab2-1* and wild-type strains were analyzed and compared. As shown in Figure 7B, the profile of *nab2-1* showed a marked increase in the 80S peak and a reduction in the polysome content compared with the wild-type strain. Salt treatment dissociated most of the 80S ribosomes into 40S and 60S r-subunits in *nab2-1* but not in the wild type, indicating that the large 80S peak found for the *nab2-1* mutant contained mostly nontranslating 80S couples not engaged in translation (unpublished data). These results open the possibility that the *nab2-1* mutation also leads to a reduction in the rate of translation initiation. To test whether the phenotype found for *nab2-1* cells is common among mRNA-export defective mutants, we also checked the polysome profiles of the *hpr1Δ* and *thp1Δ* mutants. As shown in Figure 7B, *hpr1Δ* cells lead to a different polysome profile (Figure 7B). Therefore the translation phenotype detected for the *nab2-1* mutant seems to be specific for the *nab2* mutation and not related to a general mRNA export defect.

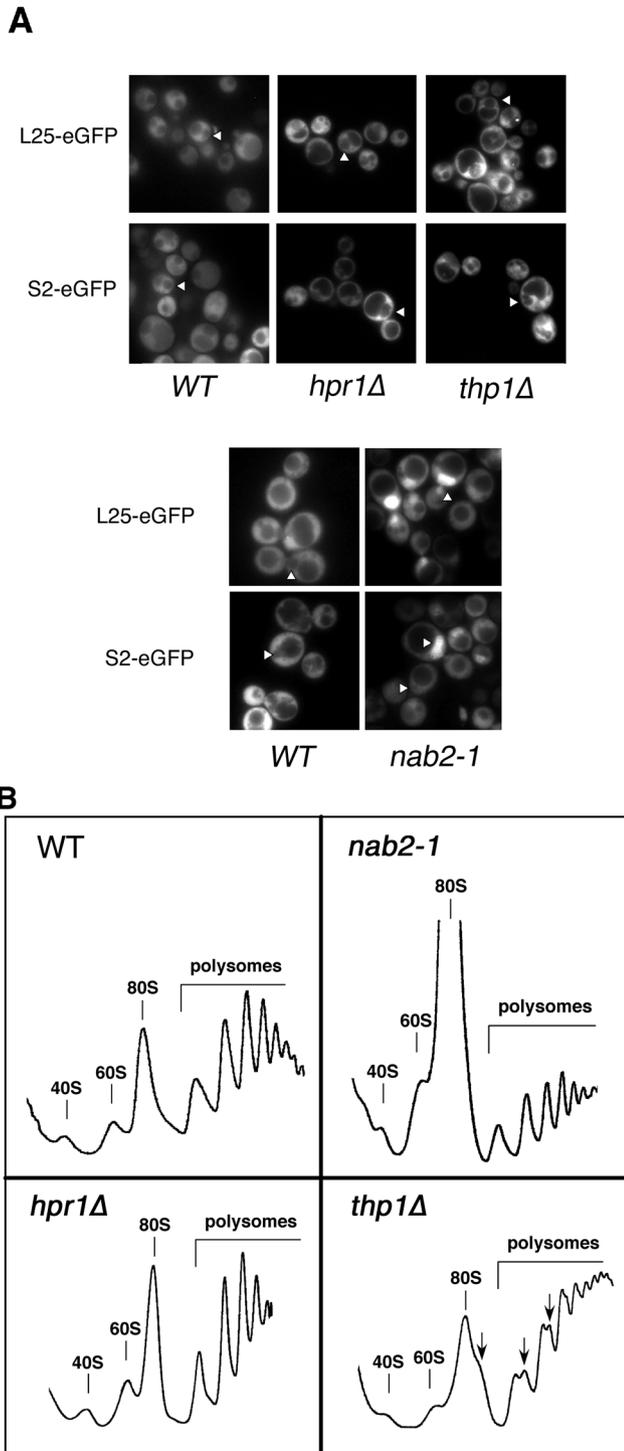


FIGURE 7: Effect of Nab2 in ribosomal subunit export and translation. (A) Steady-state subcellular localization of ribosomal-subunit GFP reporters. The WT (W303-1A), *hpr1Δ* (U768-4C), *thp1Δ* (WFBE046), *nab2-1* (NAB2-1) and its isogenic ACY429 WT strain expressing either the 60S r-subunit reporter L25-eGFP or the 40S r-reporter were grown at 30°C in selective SC medium to an OD₆₀₀ of 0.5 and then subjected to fluorescence microscopy. Triangles indicate the position of the nucleus of a representative cell in each case. (B) Polysome analyses of the same strains shown in A. For details see *Materials and Methods*. The sedimentation is from left to right. The peaks of free 40S and 60S ribosomal subunits, 80S couples/monosomes, and polysomes are indicated. Half-mer polysomes are indicated by arrows. Polysome profiles of both W303-1A and ACY429 wild-type strains are identical, so only one is shown.

analyzed by microarray analysis with *nab2-td* cells and their isogenic wild-type strain. This analysis was performed at permissive conditions in which Nab2 is not depleted (time 0) and after 30 and 75 min of Nab2 degradation induced by a shift to 37°C and galactose. As can be seen in Figure 8A, the expression profiles at 30 and 75 min of degenron induction were alike each other but different from the time 0 control (Figure 8A). As an early response to Nab2 depletion (time 30 min), 100 genes covering many functions and locations of the cell (Supplemental Figure S6) were deregulated at least 1.5-fold with respect to the wild-type strain. One of these genes was *NAB2*, which increases its expression level threefold compared with wild type (Supplemental Table S1). This observation demonstrates that under this condition, in *nab2-td*, the Nab2 protein was depleted but transcription was not completely abolished, probably due to the presence of traces of copper in the medium. However, this result allows us to validate the assay, since it was previously known that Nab2 negatively regulates its own expression (Roth et al., 2009). In such a short period of time, an Nab2-dependent gene should be down-regulated. Indeed, most of the deregulated genes were down-regulated (72 of 100), but some were up-regulated (28 of 100). Under these conditions, up-regulation is likely the result of a cellular response to Nab2 depletion, and thus the genes up-regulated may not represent primary targets of Nab2. This could also be the case for some down-regulated genes. Consistent with the role of Nab2 in the poly(A)⁺-tail length control, the genes up-regulated were significantly enriched in those involved in mRNA polyadenylation or mRNA 3'-end processing. Of interest, genes down-regulated were significantly enriched in nuclear and RNA metabolism genes (Table 1). Between them, the most representative ones were components of the U6 snRNP involved in splicing and mRNA degradation (Table 1), but also found were components of the RNAPII transcription machinery, mRNA export factors, and proteins related to tRNA and rRNA metabolism (Figure 8C). As a later response (75 min after the induction of Nab2 depletion), 1059 genes were deregulated in the *nab2-td* strain (379 up-regulated, 680 down-regulated), which represent 17% of the genome. These genes cover many functions and locations of the cell (Supplemental Figure S6) and also included most of the genes already deregulated at 30 min (Figure 8B). Up-regulated genes were enriched in those involved in amino acid, organic acid, or cellular ketone metabolism, as well as in cell wall and membrane functions, and down-regulated genes were enriched in those involved in protein modification by small-protein conjugation processes and in genes encoding cytoplasmic and mitochondrial proteins (Table 1).

To complete the analysis, we also analyzed the expression profile in *nab2-1* mutant. In this case, 1561 genes were deregulated (862 up-regulated and 699 down-regulated). The down-regulated genes were enriched in genes related with membranes and general transporter activity, whereas in the up-regulated genes no category was significant represented (Supplemental Table S4). The number of affected genes in *nab2-1* mutant was similar to that observed in *nab2-td* after 75 min of induction, especially in the down-regulated genes. However, when we analyzed the nature of these genes we observed that only 202 of them were present in both *nab2-td* and *nab2-1* alleles (Figure 8B). This suggests that probably the expression pattern in *nab2-1* is due to an adaptation of the cell to the mutation, and it does not reflect the direct target of Nab2. However, when we analyzed the common genes deregulated in both *nab2-1* and *nab2-td* mutants we found an enrichment in genes related to amino acid and glucose metabolism in the down-regulated genes, whereas the up-regulated genes were enriched in genes related to morphogenesis and the cell wall (Supplemental Table S5). Of interest,

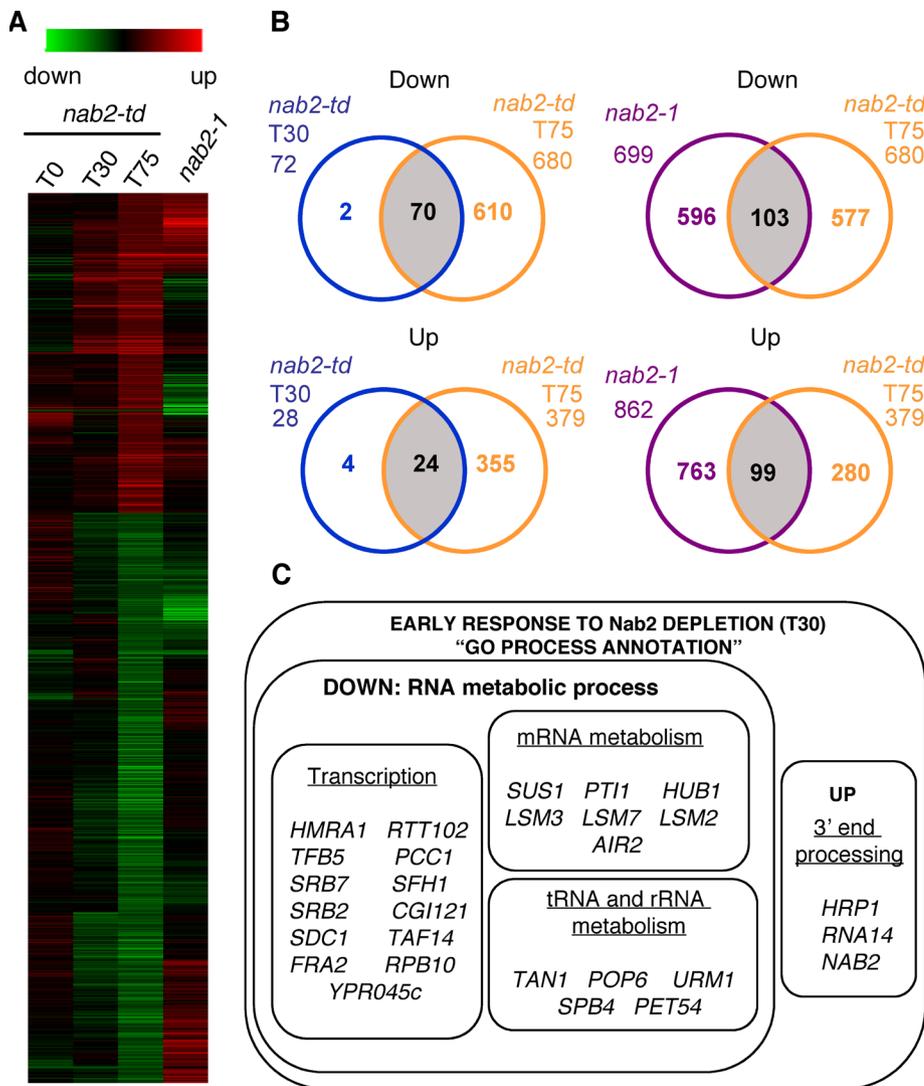


FIGURE 8: Gene expression profiles of *nab2-td* and *nab2-1* mutants. (A) Hierarchical cluster of the 1060 genes deregulated ≥ 1.5 -fold with respect to the wild-type strain in *nab2-td* at time 75 min after degenon activation and comparison with the levels of the same genes in *nab2-td* at times 0 and 30 min and *nab2-1*. (B) Venn diagrams of the genes up- and down-regulated ≥ 1.5 -fold with respect to the wild-type strain in *nab2-1* and *nab2-td* in the different conditions tested. (C) Diagram of the genes altered in *nab2-td* after 30 min of induction included in the Gene Ontology (GO) "process" categories shown in Table 1 and arranged according to their function.

RNA metabolism genes were also significantly up-regulated in the common genes present in both *nab2-1* and *nab2-td*, including genes with function in mRNA, tRNA, and rRNA metabolism (Supplemental Table S5).

Taken together, the changes in the genome-wide pattern of expression caused by mutation or depletion of Nab2 are consistent with Nab2 having a general role in the control of gene expression and being involved in mRNA, tRNA, and rRNA metabolism.

DISCUSSION

Here we showed that the poly(A)⁺-binding protein Nab2 works in RNAPII transcription and RNAPIII metabolism. Our results uncovered a general function of Nab2 that is not restricted to mRNA 3'-end processing but covers the biogenesis and activity of RNAs in general, playing a key role in the control of gene expression from transcription to translation.

Role of Nab2 in mRNA transcription

Nab2 is an hnRNP involved in mRNA 3'-end formation and export (Green *et al.*, 2002; Hector *et al.*, 2002). Here we show that Nab2 has a function in RNAPII transcription. This is supported by the synthetic lethality of *nab2-1* with mutations in Rpb9 and Spt4 and a decrease in the amount of RNAPII toward the 3 end of the genes *in vivo*, as well as a defect in *YLR454w* and *lacZ* mRNAs synthesis (Figure 4; Gallardo *et al.*, 2003). A connection between transcription elongation and mRNA processing has been reported for the THO and THSC complexes (Strasser *et al.*, 2002; González-Aguilera *et al.*, 2008). In contrast to THO, THSC mutations also confer an *in vivo* defect in transcription elongation that is not observed *in vitro* (González-Aguilera *et al.*, 2008). Because the THSC complex is associated with NPCs, its function during transcription elongation might be coupled to mRNA export at the NPC. Of interest, a functional relationship between Nab2 and the THSC complex has been shown by the suppression of THSC mutations by Nab2 overexpression (Gallardo *et al.*, 2003). This suggests that all of these factors are involved in the same process, consistent with the genetic and physical interaction observed between Nab2 and the rest of them (Jimeno *et al.*, 2006; Fasken *et al.*, 2008; Iglesias *et al.*, 2010). However, in contrast to THO and THSC, the role of Nab2 in transcription elongation seems to be minor, since we did not detect transcription elongation defects either *in vitro* or *in vivo* in the different mutants and conditions assayed (Figures 4B and 5F; Tous *et al.*, 2011). Considering the reduction of RNAPII recruited to the ORFs of transcribed genes in *nab2* mutants (Figure 4C), the low mRNA levels observed in *nab2* cells are consistent with a defect in transcription, likely initiation or termination. Although we cannot rule out a reduction in mRNA stability, our results indicate that it

would not be related to the nuclear exosome but to other mRNA surveillance mechanisms (Supplemental Figure S4).

The genome-wide analysis of the expression pattern in *nab2-td* and *nab2-1* alleles reveals significant differences from the wild type. The profile of gene expression of the conditional-degenon mutant shows that the 17% of the genome, covering genes with all types of functions, was deregulated after shifting to nonpermissive conditions. In addition, there is a high degree of coincidence between these deregulated genes and the mRNAs physically bound to Nab2 (Batisse *et al.*, 2009). This, together with the quick response of these genes to Nab2 depletion, suggests that most of them may be primary target of Nab2. In addition, it indicates that the effect of Nab2 on transcription is all over the genome rather than on a specific subset of genes.

It is significant that after 30 min of Nab2 depletion, nuclear protein-encoding genes were significantly deregulated, most of them related to RNA metabolism (Figure 8). These include genes

		CF	BF	p value
	Process			
<i>nab2-td</i> T30 up	mRNA polyadenylation	10.7% (3/28)	0.4% (31/7164)	2.9e-03
	mRNA 3'-end processing	10.7% (3/28)	0.3% (18/7164)	1.5e-02
<i>nab2-td</i> T30 down	RNA metabolic process	34.7% (25/72)	15.9% (1136/7164)	1.5e-02
<i>nab2-td</i> T75 up	Amino acid biosynthetic process	6.4% (24/379)	1.5% (104/7164)	2.8e-07
	Organic acid biosynthesis	10.1% (38/379)	4.8% (344/7164)	5.4e-03
	Cellular ketone metabolic process	10.1% (38/379)	5% (357/7164)	
<i>nab2-td</i> T75 down	Protein modification by small protein conjugation or removal	4.1% (28/680)	1.8% (126/7164)	1.1e-02
	Component			
<i>nab2-td</i> T30 down	snRNP U6	4.2% (3/72)	0.1% (9/7164)	6.8e-03
	Nucleus	47.2% (34/72)	28.5% (2039/7164)	4.6e-02
<i>nab2-td</i> T75 up	Cell wall	7.2% (27/379)	1.4% (100/7164)	1.1e-11
	Plasma membrane	8.8% (33/379)	3.9% (280/7164)	1.6e-03
<i>nab2-td</i> T75 down	Mitochondria	22.5% (153/680)	15.4% (1103/7164)	5.4e-05
	Cytoplasm	63.1% (430/680)	52.5% (3764/7164)	8.5e-07

Function and localization of the genes deregulated ≥ 1.5 -fold with respect to the wild-type strain in the different conditions tested. Go-Term Finder (<http://yeastgenome.org>) was used, and the hits with $p < 0.05$ were selected. BF, background frequency; CF, cluster frequency.

TABLE 1: GO annotations of the genes altered in *nab2-td*.

encoding transcription, polyadenylation, and mRNA export factors, as well as factors involved in pre-mRNA splicing and tRNA and rRNA metabolism. This opens the possibility that Nab2 may be functionally related to such factors. This is the case of the mRNA polyadenylation or mRNA 3'-end processing genes that respond by increasing their mRNA levels (Figure 8 and Supplemental Table S1), likely compensating for the lack of Nab2 activity. Moreover, the rapid response to Nab2 depletion indicates that the effect is direct and not mediated by the altered expression of other genes. Altogether we conclude that beyond its role in mRNA 3'-end processing and export, Nab2 plays an important role in transcription.

A novel role of Nab2 in tRNA and rRNA metabolism

An unexpected observation of this study is the involvement of Nab2 in tRNA and rRNA metabolism. In addition to protein-coding genes, Nab2 is associated with RNAPIII-transcribed genes in a THSC/TREX-2 and, to a lesser extent, THO-dependent manner. Indeed, both Thp1 and Hpr1 bind to RNAPIII genes, but they do it preferentially to the 5S rDNA (Figure 3). This is consistent with recent genome-wide studies showing that human RNAPII- and RNAPIII-associated factors associate with RNAPIII genes (Barski et al., 2010; Raha et al., 2010). In addition, previous microarray gene expression analyses in *thp1Δ* and THO mutants revealed a clear decrease in the expression level of some tRNAs, rRNAs, and snRNAs (Marin et al., 2003). However, recruitment to RNAPIII genes is not a general feature of all mRNA-processing factors, since Tho1, for example, is not present in these genes (Figure 3).

The role of Nab2 in tRNA and rRNA metabolism seems to be posttranscriptional, since transcription levels of RNAPIII and RNAPI genes are not reduced in *nab2* mutants. Indeed, levels are slightly increased in *nab2-1*, suggesting the possibility that tRNA stability could be affected (Figure 6). In addition, the detection of genetic interactions between Nab2 and proteins of tRNA and rRNA metabolism such as Utp8 and Crm1 (Figure 6) strongly suggests a direct role of Nab2 in RNAPIII RNA metabolism. Consistently, *nab2* mutants exhibit r-subunit export defects (Figure 7). In *nab2-1* mutants, export of

the 60S and 40S r-subunits is clearly affected (Figure 7). The observation that the defect in the export of 60S r-subunits seems to be stronger than that of the 40S r-subunit could likely be due to the fact that the 5S rRNA, the only rRNA transcribed by RNAPIII, is in the 60S r-subunit. The effect of Nab2 in 40S r-subunit export could be due to the interaction between Nab2 and the rRNA export factors Crm1 and Utp8 (Krogan et al., 2006). Consequently, Nab2 would not be the only RNAPIII functionally related protein with a role in RNAPIII RNA metabolism, as this is also the case of RNAPIII elongation factors such as Dst1 (TFIIS) and Elongator (Svejstrup, 2007; Ghavi-Helm et al., 2008) or the transcriptional coactivator Sub1 (Rosonina et al., 2009).

The biological relevance of the functional role of Nab2 in both tRNA and rRNA metabolism, however, is supported by a reduction in translation initiation of *nab2-1*, as indicated by the high accumulation of 80S ribosomes and the decrease in polysomes (Figure 7). This translation defect is likely not linked exclusively to the defect in mRNA metabolism of *nab2* mutants, since other mRNA-processing and export factor mutants, such as *hpr1Δ* or *thp1Δ*, show a wild-type or a different profile, respectively. In the case of *thp1Δ*, the polysome profile suggests a defect in 40S/60S r-subunit joining (Figure 7), similar to the phenotype previously observed in *mex67* mutants that impair 60S r-subunit export and prevent binding of Mex67 to 5S rRNA (Yao et al., 2007), whereas the translation initiation defect of *nab2-1* mutants seems to occur at an earlier stage. Of interest, Gle1, a conserved mRNA export factor that physically interacts with Nab2 and Mex67, also shows the same polysome profile as Nab2 (Bolger et al., 2008), so it is possible that Nab2 and Gle1 could be acting together during translation initiation.

The role of RNA-binding factors involved in transcription and in the metabolism of RNAs produced by the three types of eukaryotic RNA polymerases, such as Nab2, opens the possibility that some of these factors may play a role in multiple control of gene expression. The transcription and translation defects of *nab2* cells, together with the previously known role of Nab2 in 3'-end mRNA processing and export, support the conclusion that Nab2 is a key regulator of gene expression. In sum, our study has served to identify novel functions

for Nab2 in transcription and RNA metabolism from processing to translation, indicating that Nab2 is a central player in the general control of gene expression.

MATERIALS AND METHODS

Strains and plasmids

See Supplemental Data for a detailed description of yeast strains and plasmids. Yeast strains used are listed in Supplemental Table S2.

Chromatin immunoprecipitation

For ChIP experiments in *PMA1*, *RNAPIII*, and *RNAPI* genes, yeast strains were grown in yeast extract–peptone–dextrose (YPD) at 30°C up to an OD₆₀₀ of 0.5. For ChIP in the *YLR454w* gene, strains were grown in synthetic complete medium (SC), 3% glycerol–2% lactate, up to an OD₆₀₀ of 0.5, after which the culture was split in two. Then one half was supplemented with 2% glucose (repressed transcription) and the other with 2% galactose (activated transcription) for 4 h. ChIP assays were performed as described (Hecht and Grunstein, 1999). Immunoprecipitations were performed with immunoglobulin–Sepharose for TAP-tagged proteins and with monoclonal anti–Rpb1-CTD antibody 8WG16 (Berkeley Antibody Company, Richmond, CA) and protein A–Sepharose for RNAPII immunoprecipitation. We used the PCR of the intergenic region at positions 9716–9863 of chromosome V as a negative control. The sequences of primers used for the amplification of *SUP56*, *ts(AGA)A*, *U6snDNA*, *5S rDNA*, and *18S rDNA* regions are detailed in Supplemental Table S3. The relative abundance of each DNA fragment was calculated by normalizing IP/input ratios as described previously (González-Aguilera et al., 2008). In all cases ChIPs were performed from three independent cultures, and quantitative PCRs were repeated three times for each culture.

Microarray analysis of gene expression

The Nab2 degenon-allele-containing cells were cultured in YPD-rich medium supplied with 0.1 mM CuSO₄ at 26°C up to an OD₆₀₀ of 0.6 and then transferred to YPGal without CuSO₄ at 26°C for 30 min to allow the expression of Ubr1 (*t* = 0). Next the temperature was shifted to 37°C to induce the degenon. The *nab2-1* and its isogenic wild-type strain were cultured in YPD medium at 30°C up to an OD₆₀₀ of 0.6. Microarray data analysis was performed in triplicate using GeneChip Yeast Genome 2.0 array (Affymetrix, Santa Clara, CA) and processed using the robust multiarray average method. The following statistical data analyses were performed using the limma package (affyGUI interface) of the R Bioconductor project (<http://www.bioconductor.org/>). For each condition, the expression profile was compared with the same condition of its isogenic wild-type strain, and genes showing at least 1.5-fold expression change were considered as altered (parameters: absolute difference between signal in mutant vs. wild-type strain, >100; difference *p* value, <0.05). The hierarchical cluster was made with the MultiExperiment Viewer 4.3 program (Saeed et al., 2003), using complete linkage clustering. The data discussed here have been deposited at the National Center for Biotechnology Information's Gene Expression Omnibus (Edgar et al., 2002) and are accessible through GEO series accession numbers GSE19302 and GS26303.

Northern analysis

RNA was prepared and analyzed by Northern blot following standard procedures. RNAPII mRNAs were electrophoresed in formaldehyde–agarose gels and hybridized with dsDNA ³²P–dCTP probes. RNAPI and RNAPIII small RNAs were electrophoresed in urea–acrylamide gels and hybridized with ssDNA probes as described in Supplemental Table S3.

RNA export assays

In situ poly(A)⁺ mRNA localization assays were performed in the *nab2-td* strain grown in the same conditions used for microarray analysis and using digoxigenin-labeled oligo(dT)₁₈ as described (Amberg et al., 1992). For preribosomal particle export, cells carrying plasmids harboring L25-eGFP or S2-eGFP fusion protein were grown to midlog phase in selective liquid medium and the experiment performed as described (Babiano and de la Cruz, 2010).

Sucrose gradient analyses of polysomes

Polysome preparations were obtained from exponential cultures grown in liquid YPD medium at 30°C. Similar amounts of cell extracts (10 A₂₆₀ units) were resolved in 7%–50% sucrose gradients (OD₆₀₀ 0.5–0.8) as previously described (Kressler et al., 1997). Ten A₂₆₀ units of extracts was loaded in each gradient. An Isco UV-6 gradient collector (Teledyne Isco, Lincoln, NE) with continuous monitoring at A₂₅₄ was used to record the profiles.

In vivo G-less–based run-on (GLRO) assays of transcription elongation

The Nab2 degenon-allele-containing cells strains harboring G-less cassette plasmids, pG–Leu–CYCs, or pCYC–LacZ were grown to an OD₆₀₀ of 0.5 at 26°C, and the degenon was induced as described earlier at 37°C. Run-on products were digested with RNaseT1, which cannot degrade G-less RNA, and resolved by 6% PAGE. Transcription run-on assays were carried out as described (Steinmetz and Brow, 2003). Dried gels were analyzed with a Phosphorimager (Fuji FLA-5100) using ImageQuant software (Molecular Dynamics, Sunnyvale, CA). For each sample, the ratio of total counts in the 132-nt band divided by total counts in the 272-nt band was determined.

Miscellaneous

Western blot experiments were performed following standard procedures using antibodies against the hemagglutinin (HA) epitope (Roche, Mannheim, Germany). In vitro transcription elongation assays were performed as described (Rondon et al., 2003).

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