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

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\textbf{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 transcription defects that are detected by polysome profiling. Genome-wide analysis of expression of a conditional degron \textit{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.

\textbf{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 susceptible to be degraded by the nuclear exosome (Schmid and Jensen, 2008).

One example of this coupling is found in the essential Nab2 protein of \textit{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...
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 Thp6, 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 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 a rDNA, U6 snDNA, and 18S rDNA genes and their flanking regions in the wild-type strain (NAT).

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.

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 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
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, the 5S rDNA in the genome corresponding to the fragment amplified by PCR covers the 3′-end, 5′-end, and promoter regions of the PMA1 gene.

As performed with the RNAPIII-driven PMA1 gene, we analyzed whether the association of Nab2 with the SUP56, 5S rRNA, and U6 snRNA 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 THSC and THO complexes and the Tho1 hnRNP. Nab2 recruitment to the genes analyzed was also reduced in THO and THSC mutants. Normalization of the Nab2-TAP levels with respect to RNAPIII 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 RNAPII factors. For this, ChIP experiments were performed with the Nab2-TAP tagged protein in mutants of the THO complex (hpr1Δ mutant), the Tho1 hnRNP (thp1Δ), 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).

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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-cell 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 THF factors (González-Aguilera et al., 2008). To test this possibility, transcription elongation was analyzed by determining the in vivo distribution of RNAPII by ChIP analysis. RNAPII recruitment was assayed at the chromosomal GAL1pr::YLR454w fusion construct in the five aforementioned regions covering the entire ORF. Although the amount of total RNAPII in the nab2-1 mutant was similar to that in the wild type in 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 RNAPII that has a critical role in transcription elongation by virtue of its interaction with the transcription elongation factor TFIIIS (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

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.
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 TDI3 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 rrpΔ double mutants were viable. Thus the rrpΔ 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 rrpΔ) or 16°C (as nab2-1) (Supplemental Figure S4B). Of interest, the levels of lacZ and YLR454w mRNAs in nab2Δ rrpΔ and nab2-1 rrpΔ 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 RNAPII 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 several essential genes were under the doxycycline-repressible tet system. Unexpectedly, the genetic cross revealed that nab2Δ rrpΔ double mutants were viable. Thus the rrpΔ 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 rrpΔ) or 16°C (as nab2-1) (Supplemental Figure S4B). Of interest, the levels of lacZ and YLR454w mRNAs in nab2Δ rrpΔ and nab2-1 rrpΔ 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.
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 conferring adenine auxotrophy (Figure 6B). Considering that in nab2-1 background the expression of ADE2 was not affected (Supplemental Table S1), we transformed an ade2-1o 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 30ºC 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 (26ºC) and induced degron strains (37ºC) 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.
cause Northern analysis shows that RNAPIII transcription is not 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 presented a wild type, whereas thp1Δ 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.

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 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
wild-type strains are identical, so only one is shown. As can be seen in Figure 8A, the expression profiles at 30 and 75 min of degron 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 RNAP II 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 nab2-2 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,
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-degron mutant shows that 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 involved in mRNA biogenesis as well as tRNA and rRNA metabolism.

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.
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 rRNA (Figure 3). This is consistent with recent genome-wide studies showing that human RNAPII- and RNAPII-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 RNAPII genes are not reduced in nab2 mutants. Indeed, levels are slightly increased in nab2-1, suggesting the possibility that RNA 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 RNA export factors Crm1 and Utp8 (Krogan et al., 2006). Consequently, Nab2 would not be the only RNAPII functionally related protein with a role in RNAPIII RNA metabolism, as this is also the case of RNAPII 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, RNAPII, and RNAPII 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, tisAGA[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 degron-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 degron. 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 multiaverage method. The following statistical data analyses were performed using the limma package (affy/mGUI 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, >10; 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 RNAPII 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 A600 units) were resolved in 7%–50% sucrose gradients (OD600 0.5–0.8) as previously described (Kressler et al., 1997). Ten A600 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 degron-allele–containing cells strains harboring G-less cassette plasmids, pG-Leu-CYCds, or pCYC-LacZ were grown to an OD600 of 0.5 at 26°C, and the degron 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-S100) 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).

ACKNOWLEDGMENTS

We thank A. H. Corbett, J. A. Tercero, R. W. Davis, and E. Hurt for providing plasmids and strains; the Unidad de Genómica, Centro Andaluz de Biología Molecular y Medicina Regenerativa, for technical assistance with microarray analysis; P. Askjaer for critical reading of the manuscript; and D. Haun for aid with the language. This work was supported by grants from the Spanish Ministry of Science and Innovation (BFU2006-05260 and BFU2007-28647-E to A.A. and BFU2007-60151 to J.dlc.), the Junta de Andalucía (BIO-102 and CVI-2549 to A.A. and CVI-03508 to J.dlc.), and the European Union (FEDER). C.G.-A. was the recipient of a Formación del Profesorado Universitario predoctoral training grant from the Spanish Ministry of Science and Innovation. R.B. is a recipient of a fellowship from the Junta de Andalucía.

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