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Nab2p and the Thp1p-Sac3p Complex Functionally Interact at the Interface between Transcription and mRNA Metabolism*

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***THP1* is a conserved eukaryotic gene whose null mutations confer, in yeast, transcription and genetic instability phenotypes and RNA export defects similar to those of the THO/TREX complex null mutations. In a search for multicopy suppressors of the transcription defect of *thp1*Δ cells, we identified the poly(A)$^+$ RNA-binding heterogeneous nuclear ribonucleoprotein Nab2p. Multicopy *NAB2* also suppressed the RNA export defect of *thp1*Δ cells. This result suggests a functional relationship between Thp1p and Nab2p. Consistently, the leaky mutation *nab2-1* conferred a transcription defect and hyper-recombination phenotype similar to those of *thp1*Δ, although to a minor degree. Reciprocally, a purified His$_6$-tagged Thp1p fusion bound RNA *in vitro*. In a different approach, we show by Western analyses that a highly purified Thp1p-Sac3p complex does not contain components of THO/TREX and that *sac3*Δ confers a transcription defect and hyper-recombination phenotype identical to those of *thp1*Δ. mRNA degradation was not affected in *thp1*Δ mutants, implying that their expression defects are not due to mRNA decay. This indicates that Thp1p-Sac3p is a structural and functional unit. Altogether, our results suggest that Thp1p-Sac3p and Nab2p are functionally related heterogeneous nuclear ribonucleoproteins that define a further link between mRNA metabolism and transcription.**

In eukaryotes, the processing and export of mRNA from the nuclear transcription site to the cytoplasm require a large number of proteins, a fraction of which act cotranscriptionally. RNA polymerase II (pol II)[1]-driven transcription elongation is coupled with 5′-end capping, splicing, 3′-end cleavage, and poly(A)$^+$ addition (1, 2). Thus, capping enzymes access the 5′-end of nascent mRNAs via the pol II C-terminal domain; splicing occurs cotranscriptionally in association with the transcription elongation complex via the pol II C-terminal domain (3–6); and cleavage of the poly(A)$^+$ site is dependent on the pol II C-terminal domain (7). The connection between transcription and mRNA metabolism is supported by the accumulation of unspliced pre-mRNAs in mutants with a truncated pol II C-terminal domain (8) or the retention at the site of transcription of human β-globin pre-mRNAs defective in either splicing or 3′-end formation (9). This connection is also extended to mRNA export. The mRNA is not exported from the nucleus unless the transcript has been properly processed (10).

Nuclear mRNA export seems to be connected to transcription at both functional and physical levels. It has been shown that hyperadenylated transcripts accumulate at the site of transcription after different mRNA export blockages (11). Importantly, a conserved TREX complex that contains Sub2p/UAP56, Yra1p/ALY, Tex1p, and the THO complex has been identified in yeast and humans (12). Yra1p/ALY and Sub2p/UAP56 are both involved in mRNA nuclear export (13). THO was previously identified as a four protein-containing complex involved in transcription elongation and the maintenance of genome stability (14). Yeast mutations in any of the four genes encoding the THO complex (*THO2*, *HPR1*, *MFT1*, and *THP2*) confer transcription elongation defects and a strong hyper-recombination phenotype that is transcription-dependent (14–16). Functional analysis of *sub2* and *yra1* mutants as well as *mex67* and *mtr2* mutants, defective in the Mtr2p-Mex67p essential heterodimeric mRNA export factor (17, 18), revealed that they have defective transcription and high levels of transcription-dependent direct-repeat recombination (19). All these factors represent a subset of conserved eukaryotic proteins involved in mature mRNA biogenesis and processing steps that are important for efficient transcription. Regardless of the specific function of these factors along the different steps of mRNA metabolism from 5′-end capping to 3′-end cleavage, poly(A)$^+$ addition, and export, failure to perform appropriately any of these steps may affect the other steps, leading to nuclear mRNA accumulation and transcription impairment and causing transcription-associated hyper-recombination.

In a recent search for yeast mutants with high levels of genetic instability, we identified *THP1*, a gene conserved in eukaryotes whose null mutations confer the same transcription defect and hyper-recombination phenotype as those of THO mutants (20). To gain new insight into Thp1p function, we undertook two experimental approaches. First, in a search for multicopy suppressors of the gene expression defect of *thp1*Δ, we identified *NAB2*, a gene encoding an hnRNP that specifically associates with nuclear poly(A)$^+$ RNA *in vivo* (21) and is required for poly(A) tail length control and nuclear RNA export (22, 23). Second, we purified the Thp1p-Sac3p complex, which has also been purified in a parallel study (24); characterized it for the presence of components of the THO/TREX complex; and

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[1] The abbreviations used are: pol II, RNA polymerase II; hnRNP, heterogeneous nuclear ribonucleoprotein; mRNP, mRNA and heterogeneous nuclear ribonucleoprotein complex; HA, hemagglutinin; MALDI-reTOF, matrix-assisted laser desorption ionization reflectron time-of-flight; SC medium, synthetic complete medium; dsDNA, double-stranded DNA.

determined whether Thp1p-Sac3p acts as a functional unit in mRNP metabolism. Sac3p was first identified as an actin suppressor (25) and has been shown to be involved in nuclear protein export (26) and recently in RNA export (24, 27). We show by genetic and molecular analyses that *sac3*Δ and *nab2-1* strains have transcription and hyper-recombination phenotypes similar to those of *thp1*Δ. In addition, we show that purified His_6-Thp1p from *Escherichia coli* binds RNA *in vitro*. We conclude that Nab2p and Thp1p-Sac3p are functionally related hnRNP-like factors that act in the same process as THO/TREX and Mtr2p-Mex67p in mRNP metabolism.

EXPERIMENTAL PROCEDURES

Strains—We used the wild-type (W303-1A), *hpr1*Δ::*HIS3* (U678-1C), *tho2*Δ::*KAN* (RK2-6C) (16), *thp1*Δ::*KAN* (WFBE046) (20), and *mex67-5* (WMC1-1A) (19) strains that have been reported previously. Strain LSY267 (*pep4*Δ) (28) isogenic to W303-1A was used for protein complex purification. Mutant *nab2-1* (22) is a *nab2*Δ strain carrying the *nab2-1* mutant allele in a centromeric plasmid. The *sac3*Δ::*KAN* (Y03517) and *thp1*Δ::*KAN* (Y01764) strains were obtained from EUROSCARF (Frankfurt, Germany). Isogenic derivatives were obtained by genetic crosses. These include wild-type strains SAY3-2B, NAL-3A, and NAL-3B and mutant strains SAY3-2A (*sac3*Δ) and NAL-11B and NAL-5B (*nab2-1*). All comparative analyses were performed with mutants and their isogenic wild-type strains on either the W303 (R. Rothstein) or BY4741 (EUROSCARF) genetic background, with the exception of *nab2-1*, for which we used its own genetic background (22).

Plasmids—Plasmids p416-GAL1-lacZ and pSCh202 (15), used for the studies of recombination and transcription, and plasmids YEp351-SUB2 (19) and pSUP38 (16) have been published previously. pRS316LΔNS and pRS316L are identical to pRS314LΔNS and pRS314L (29), but in vector pRS316. For the isolation of multicopy suppressors of *thp1*Δ, we used the pCM184-LAUR plasmid (19). We used MW90 constructed in YEp351 as a yeast multicopy library (30).

For the purification of Thp1p from *E. coli*, we constructed plasmid pT7-HA-THP1 containing an N-terminal His_6-HA-Thp1p translational fusion under the control of the T7 RNA polymerase promoter. For this purpose, the HA-*THP1* fragment was obtained by PCR using primers GATATCATCGATCTCGAGTCACCAAAGAACGTGAG and GGATCCTCTAGAGCTAGCTATCCCTATGACGTTCCCTCTCACGTTTTGTGGTCCATGGAAAAGAGAAG and subcloned into the *Nhe*I-*Cla*I site of plasmid pT7-7-His_6 (31).

Purification of His_6-HA-Thp1p from E. coli—E. coli strain BL21(DE3) transformed with plasmid pT7-HA-THP1 was grown in 1 liter of LB medium supplemented with 100 μg/ml ampicillin up to A_{600} = 0.8. Expression was induced for 4 h by adding isopropyl-β-D-thiogalactopyranoside to a final concentration of 0.5 mM. Cells were lysed in buffer A (50 mM NaH_2PO_4 and 300 mM NaCl, pH 8) containing 10 mM imidazole. His_6-HA-Thp1 was partially purified by affinity chromatography on a nickel-nitrilotriacetic acid column (QIAGEN Inc.) in buffer A containing increasing concentrations of imidazole (20 and 250 mM).

Thp1p-Sac3p Complex Purification and Western Analyses—The Thp1p-Sac3p complex was purified using a C-terminally TAP-tagged Thp1 protein constructed for this study. For this, we replaced the wild-type copy of *THP1* with the *THP1-TAP* fusion obtained by PCR with primers GTTAATGAACGAATCACCAAGATGTTTCCTGCCCATTCTCACGTTTTGTGGTCCATGGAAAAGAGAAG and CAGCATAATGTCGCTCCTTCTCTTCTATTTCCTATATATATCTACGACTCACTATAGGG in wild-type strain LSY267. Purified TAP-tagged proteins were purified essentially as described (32) with modifications as published (19). Purified complexes were run on a 10% SDS-polyacrylamide gel and transferred to nylon membranes. After blocking with phosphate-buffered saline containing 0.1% Tween 20 and 5% milk, proteins were detected using antibodies against Mft1p and Sub2p and peroxidase-conjugated goat anti-rabbit IgG. Blots were washed with phosphate-buffered saline and 0.1% Tween 20 and developed by enhanced chemiluminescence reactions (ECL, Amersham Biosciences).

Protein Identification—Gel-resolved proteins were digested with trypsin and partially fractionated, and the resulting peptide mixtures were analyzed by matrix-assisted laser desorption ionization reflectron time-of-flight (MALDI-reTOF) mass spectrometry (Reflex III, Bruker Daltonics, Bremen, Germany) as described (33) and also using an electrospray ionization triple quadrupole tandem mass spectrometry instrument (API300, ABI/MDS Sciex, Thornhill, Canada) modified with an ultrafine ionization source (34). Selected masses from the MALDI-reTOF spectra were taken to search the yeast segment of a protein non-redundant data base. Tandem mass spectra were inspected for y'' ion series to compare with the computer-generated fragment ion series of the predicted tryptic peptides.

mRNA Export Assays—In situ mRNA localization assays were performed as described (35) using digoxigenin-labeled oligo$(dT)_{16}$.

In Vitro RNA and DNA Binding Assays—In vitro RNA and DNA band shifting assays were performed following the procedures described previously (19) using identical RNA and DNA probes.

Miscellaneous—Recombination frequencies were obtained from two to three different transformants for each genotype. They were determined as the median frequency of 6–12 independent values for each transformant as reported previously (29). β-Galactosidase assays and Northern analyses were performed according to previously published procedures (15).

RESULTS

*Multicopy NAB2 Suppresses the Gene Expression Defect of thp1*Δ—To obtain new insights into the functional role of Thp1p, we searched for genes that could potentially have an overlapping function with Thp1p. For this, we used our newly developed genetic assays for the analysis of gene expression based on plasmid pCM184-LAUR carrying a 4.15-kb *lacZ-URA3* translational fusion under the control of the P_{tet} promoter (Fig. 1*A*) (19). We have previously reported that *thp1*Δ mutants are defective in transcription of the *lacZ* sequence (20). Here, we first showed that, as expected, *thp1*Δ mutants containing the LAUR expression system were unable to form colonies on synthetic complete medium without uracil (SC−Ura) and showed reduced levels of β-galactosidase activity (Fig. 1*B*) (data not shown).

From ~80,000 *thp1*Δ transformants with the multicopy library MW90 (30) plated on SC−Ura, we selected one that recovered the capacity to grow in the absence of uracil. Isolation and restriction and sequencing analysis of the plasmid (YEp351-1.9) carried by this transformant revealed a 6.5-kb insert of the *NAB2* region covering three complete open reading frames. Complementation analysis of deletion derivative versions of the original plasmid indicated that the ability of this plasmid to suppress the gene expression defect of *thp1*Δ was abolished only if *NAB2*, but not the other two open reading frames, was deleted (data not shown). Subcloning of the *NAB2* open reading frame into YEp351 confirmed that multicopy *NAB2*, and not the adjacent genes of the original insert, suppressed the inability of *thp1*Δ cells to express the *lacZ-URA3* fusion as determined by growth on SC−Ura and by Northern analysis (Fig. 1, *B* and *C*). This suppression was specific to *thp1*Δ, and not to other mutants with the *thp1*-like phenotype such as *hpr1*Δ and *tho2*Δ (Fig. 1*B*). Quantification of mRNA levels showed that suppression of the transcription defect of *thp1*Δ cells was partial. Multicopy *NAB2* made *thp1*Δ cells reach 11.6% of the wild-type levels of *lacZ-URA3* expression.

Finally, recombination analyses of the chromosomal direct-repeat system *leu2-k*::*ADE2-URA3*::*leu2-k* showed that multicopy *NAB2* reduced recombination in a *thp1*Δ background to levels (1.1×10^{-1}) 1.5-fold below *thp1* levels (7.1×10^{-2}). These results indicate that suppression of *thp1*Δ by *NAB2* overexpression is partial. It is detectable at the transcriptional level, but it is not enough to yield a significant effect on direct-repeat recombination.

*Multicopy NAB2 Partially Suppresses the mRNA Export Defect of thp1*Δ *Mutants*—Given the phenotypic similarity of *thp1*Δ mutants to mRNA export mutants and THO/TREX mutants (12, 19), we first showed, by *in situ* hybridization with digoxigenin-labeled oligo(dT) and fluorescein-conjugated anti-digoxigenin antibody, that Thp1p was also required for proper mRNA nuclear export at both 30 and 37 °C (Fig. 1*D*) (data not shown). This is agreement with recently reported data from a parallel study (24). Consistent with this result, we observed

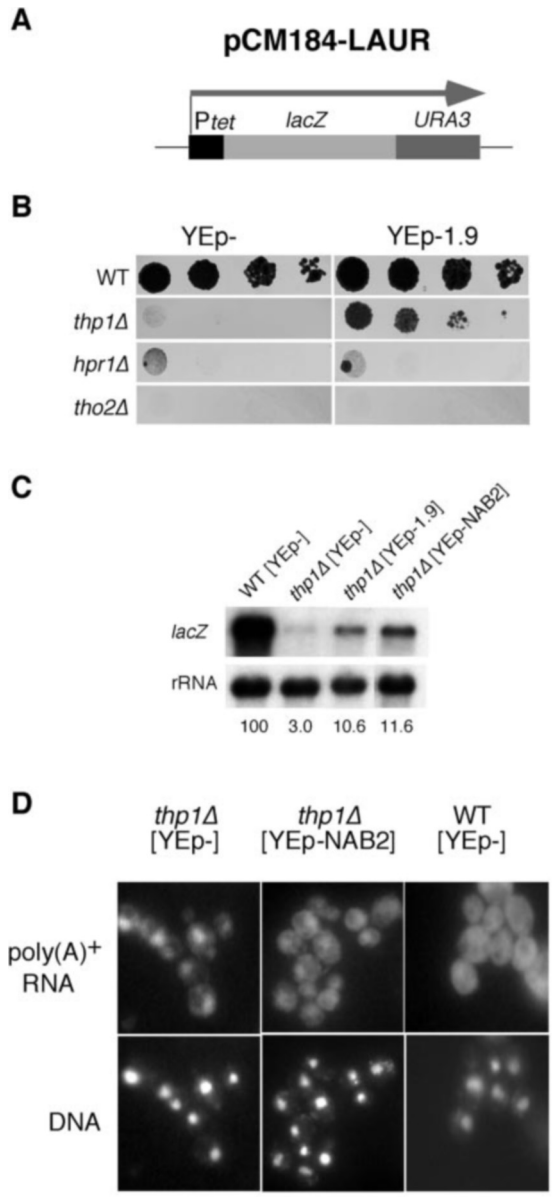

FIG. 1. **Suppression of the transcription and mRNA export defects of *thp1*Δ by multicopy *NAB2*.** *A*, scheme of the P_{tet}::*lacZ-URA3* fusion construct carried by plasmid pCM184-LAUR. *B*, phenotypic analysis of the wild-type (*WT*; W303-1A), *thp1*Δ (WFBE046), *hpr1*Δ (U678-1C), and *tho2*Δ (RK2-6C) strains carrying plasmid pCM184-LAUR and transformed with either vector YEp351 (*YEp-*) or plasmid YEp351-1.9 (*YEp-1.9*) containing *NAB2*. The capacity of each strain to form colonies on SC−Ura after 6 days at 30 °C is shown. *C*, Northern analysis of the expression of the P_{tet}::*lacZ-URA3* fusion construct. RNA was isolated from mid-log phase cells carrying pCM184-LAUR grown in SC medium. The 0.5-kb 5′-end *lacZ Bam*HI-*Hpa*I fragment and a 589-bp 25 S rDNA internal fragment obtained by PCR were used as DNA probes. *Numbers* below each lane indicate the percentage of the complete *lacZ* RNA with respect to the wild-type value, which was taken as 100%. Northern blots were quantified in a Fuji FLA 3000. *D*, subcellular localization of poly(A)$^+$ RNA analyzed by *in situ* hybridization with a digoxigenin-labeled oligo(dT) probe. Samples were taken from exponentially growing wild-type (W303-1A) and *thp1*Δ (WFBE046) cells transformed with multicopy plasmid YEp351 or YEp351-NAB2 that had been shifted to 37 °C for 4 h in SC−Leu. Nuclear DNA was stained with 10 μg/ml 4,6-diamidino-2-phenylindole. All analyses were performed with as Olympus AHBT3 microscope.

that *thp1*Δ *mex67-5* and *sac3*Δ *mex67-5* double mutants were also inviable (data not shown), a phenotype previously observed for mutants that accumulate mRNA in the nucleus (18, 19, 36).

Nab2p is a major nuclear protein associated with nuclear poly(A)$^+$ RNA (21) required for nuclear mRNA export (22, 23). As the transcription defect of *thp1*Δ was accompanied by mRNA accumulation in the nucleus, we assayed whether this phenotype was also suppressed by multicopy *NAB2*. As shown in Fig. 1*D*, *thp1*Δ cells carrying multicopy *NAB2* showed significantly lower nuclear accumulation of poly(A)$^+$ RNA compared with *thp1*Δ cells, even though some nuclear mRNA accumulation could still be observed after 4 h of shifting cells to 37 °C. These results confirm that *thp1*Δ is suppressed by multicopy *NAB2*, but only at partial levels, and that the gene expression and mRNA export phenotypes are linked.

nab2-1 Strains Are Defective in lacZ Transcription and Stimulate Direct-repeat Recombination—As our results showing that multicopy *NAB2* suppresses *thp1*Δ suggest that the function of Nab2p might be related to that of Thp1p, we investigated whether *nab2* mutants also show expression and recombination phenotypes similar to the those of *thp1*Δ mutants. Because *NAB2* is an essential gene, we used *nab2-1* for our studies. This mutation is leaky at 30 °C, and it was previously shown that *nab2-1* strains accumulate mRNA in the nucleus at this temperature (22). As shown in Fig. 2*A*, *nab2-1* reduced β-galactosidase activity to 20% of the wild-type levels. Kinetic analyses of transcription of the *GAL1pr*::*lacZ* and *GAL1pr*::*PHO5* fusion constructs revealed that *lacZ* mRNA accumulated at a much slower rate than the wild-type strains, whereas only a small difference was found for *PHO5*. As shown for *thp1*Δ, this implies that the main transcription step impaired in *nab2-1* mutants is transcription through *lacZ*, and not activation of the *GAL1* promoter. Next, we determined the effect of *nab2-1* on recombination between direct repeats. For this, we constructed *nab2-1* strains carrying the chromosomal direct-repeat system *leu2-k*::*ADE2-URA3*::*leu2-k* containing two 2.16-kb repeat units. Recombination between the *leu2-k* repeats was scored in this system as deletions that lose the *ADE2-URA3* intervening sequence plus one *leu2-k* copy. The frequency of deletions of *nab2-1* strains was 25-fold above wild-type levels (Fig. 2*B*). The intensity of the phenotypes of *nab2-1* was weaker than that of the phenotypes of *thp1*Δ, but this may be due to the leaky activity of the *nab2-1* allele (22). To conclude, *nab2-1* shows a transcription defect and hyper-recombination phenotype similar to those of *thp1*Δ, although to a lesser degree.

*mRNA Degradation Rates Are Unaffected in thp1*Δ *Mutants*—The observation that transcription driven by the same promoter is defective in *thp1*Δ cells for some DNA sequences (*i.e. lacZ*), but not for others (*i.e. PHO5*), indicates that the transcription defect occurs at a post-initiation step (20). The fact that hyper-recombination of *thp1*Δ cells is linked to transcription elongation is good evidence that these mutants have a transcription defect responsible for hyper-recombination. Nevertheless, to confirm that the lower rate of mRNA formation observed in time course kinetic experiments is not due to high rates of mRNA decay caused by *thp1*Δ, we determined the degradation rate of two mRNAs, *GAL1* and *lacZ*, driven by the same regulated *GAL1* promoter. For this, we first allowed cells to accumulate mRNA in 2% galactose. After repressing transcription by shifting cells to 2% glucose, the kinetics of degradation of the accumulated mRNA was determined in time course experiments. Whereas *thp1*Δ strains formed the *GAL1* mRNA at half the wild-type rate (20), Fig. 3 shows that the rate of degradation of this mRNA was not higher in *thp1*Δ *versus* wild-type cells (half-lives of 17 and 12 min, respectively). Also, whereas *thp1*Δ cells, in contrast to wild-type cells, did not form detectable *lacZ* mRNA after 3 h of transcription activation (20), Fig. 3 shows that the rate of *lacZ* mRNA degradation was not higher in *thp1*Δ *versus* wild-type cells (half-lives of 7.5 and 4.5

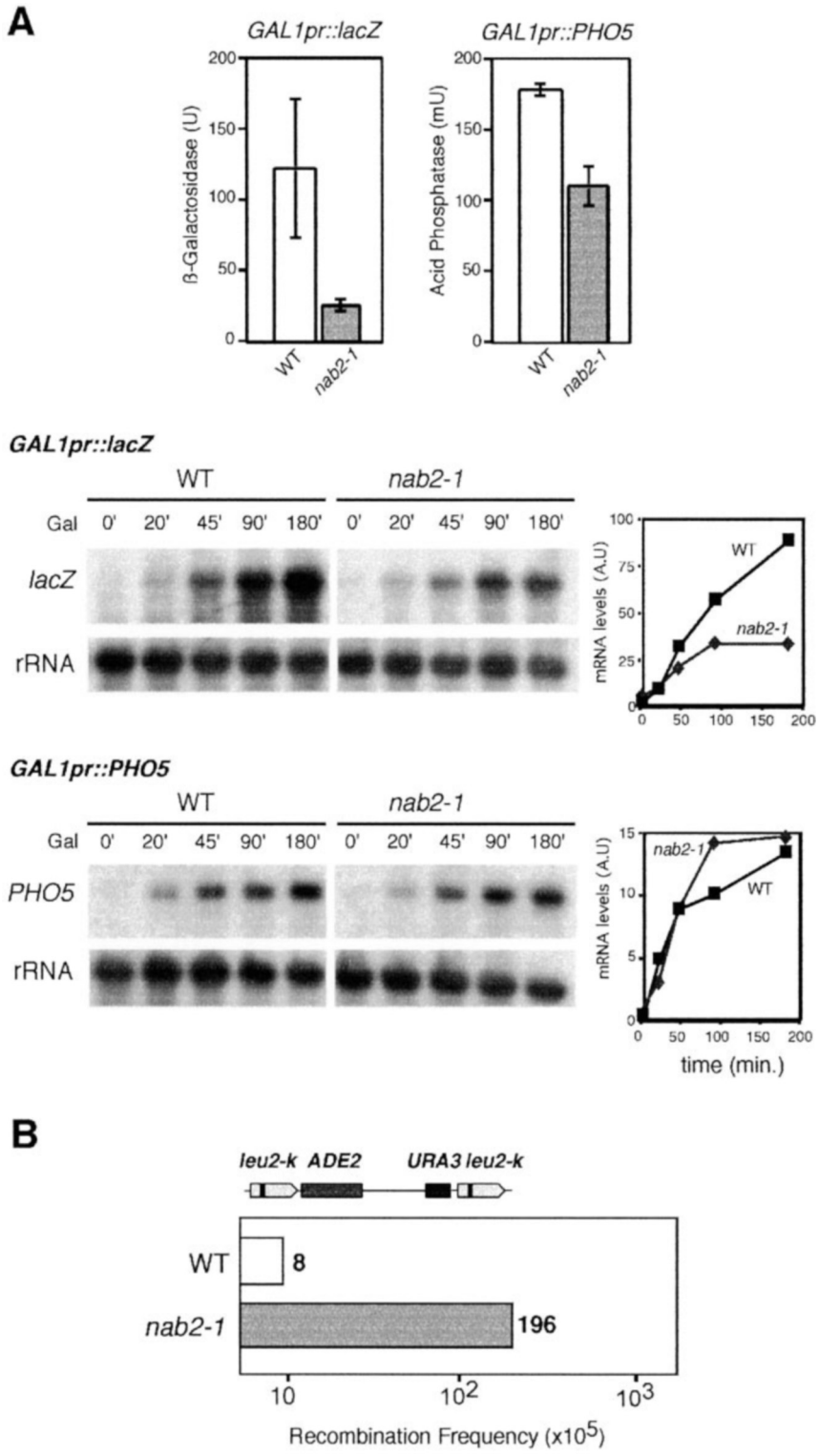

FIG. 2. **Expression analysis of *GAL1pr::lacZ* and *GAL1pr::PHO5* constructs in *nab2-1* mutants.** *A*: *upper panels*, β-galactosidase and acid phosphatase activities of the wild-type (*WT*; NAL-3A) and *nab2-1* (NAL-11B) strains transformed with plasmids p416-GAL1-lacZ and pSCh202. Each value represents the average of two to three different transformants. Only the data of induced expression (2% galactose) are given. Under repression conditions (2% glucose), values were below detection levels in all cases. *Lower panels*, Northern analysis of *lacZ* and *PHO5* mRNAs driven by the *GAL1* promoter. Mid-log phase cells transformed with plasmid p416-GAL1-lacZ or pSCh202 were diluted in 3% glycerol- and 2% lactate-containing SC−Ura to A_{600} = 0.5 and incubated for 16 h at 30 °C. Galactose was then added, and samples were taken at different times as indicated. The 0.9-kb *PHO5 Eco*RV internal fragment was used as the *PHO5* probe. For other details, see the legend to Fig. 1. *B*: recombination frequencies of the chromosomal direct-repeat system *leu2-k*::*URA3-ADE2*::*leu2-k* in the wild-type (NAL-3B) and *nab2-1* (NAL-5B) strains. All experiments were performed at 30 °C. Recombination values are the median of a total of 12 independent colonies obtained from SC medium. Ura^- recombinants were scored on SC medium containing 5-fluoroorotic acid. *A.U.*, arbitrary units.

min, respectively). We can therefore conclude that the strong defect in the kinetics of mRNA formation of *thp1*Δ cells is caused by defective transcription, and not by increased mRNA degradation.

Thp1p Binds RNA in Vitro—Because null mutants of *THP1* show phenotypes similar to those of mutants of the THO/TREX complex, Nab2p, and mRNA export factors Mtr2p and Mex67p, all of which bind RNA (17, 19, 21, 37), we investigated whether Thp1p associates with either RNA or DNA *in vitro*. We first purified His_6-Thp1p from *E. coli*. RNA band shifting assays using a 90-nucleotide long RNA molecule showed that Thp1p bound RNA (Fig. 4*A*). Binding was directly proportional to the amounts of Thp1p used. No binding activity was observed with the negative control, *i.e.* purified material obtained from *E. coli* transformed with the empty vector pT7-7-His_6. The ability to bind RNA is not conferred by the His_6 tail because we have shown that other His_6-tagged proteins did not bind RNA in our assays.[2]

RNA-binding activity competed strongly with unlabeled

[2] A. G. Rondón and A. Aguilera, unpublished data.

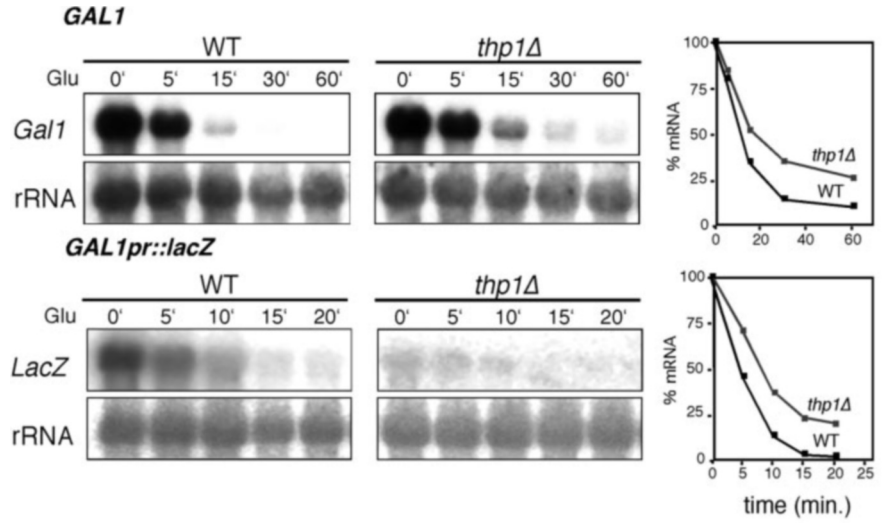

FIG. 3. **Kinetics of degradation of *GAL1* and *lacZ* mRNAs in *thp1*Δ mutants.** Shown are the results from Northern analysis of the mRNA decay of the endogenous *GAL1* mRNA and the *lacZ* mRNA driven by the *GAL1* promoter in cells transformed with p416-GAL1-lacZ. Mid-log phase wild-type (*WT*; W303-1A) and *thp1*Δ (WFBE046) cells grown in 2% galactose-containing SC medium either for 3 h (*GAL1*) or overnight (*lacZ*) were transferred to 2% glucose-containing SC medium before samples were taken at the indicated times. The 0.75-kb *GAL1 Pvu*II-*Ava*I internal fragment, the 3-kb *lacZ Bam*HI-*Bgl*II fragment, and the 589-bp 25 S rRNA internal fragment obtained by PCR were used as ^{32}P-labeled DNA probes.

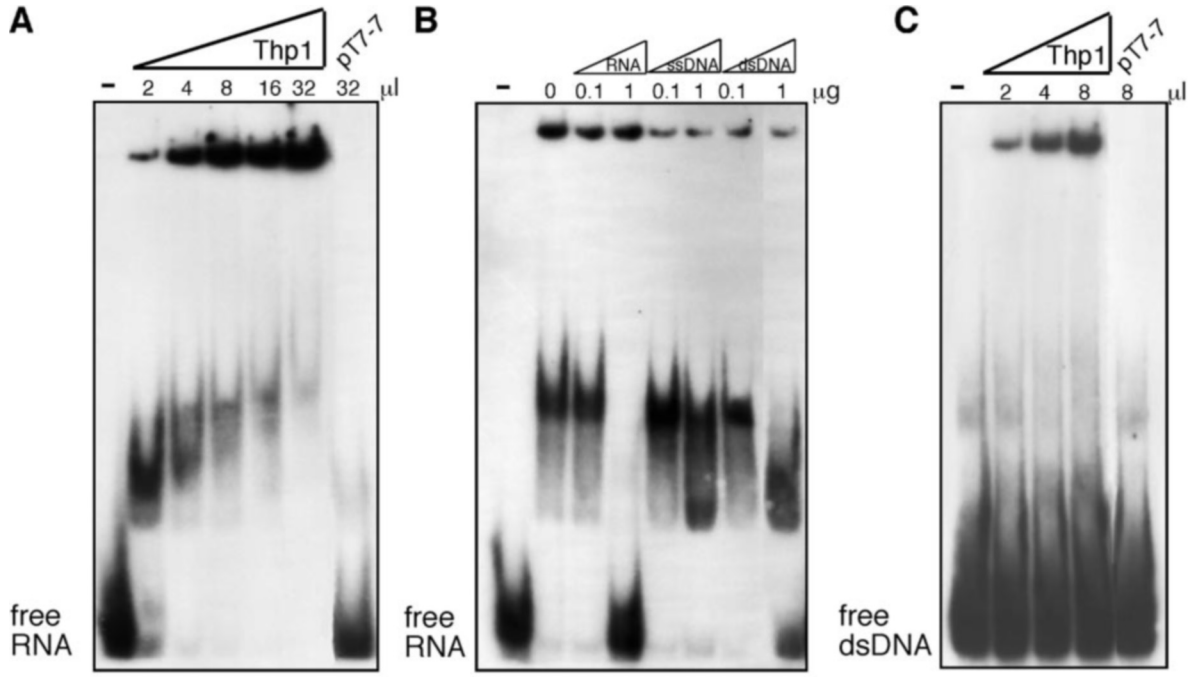

FIG. 4. ***In vitro* RNA and DNA binding assays of purified His-Thp1p.** *A*, RNA binding assay using the 90-bp ^{32}P-labeled RNA obtained from the pBluescript SK polylinker and increasing amounts of purified Thp1p from *E. coli*. Purified His-tagged extracts from *E. coli* transformed with the empty pT7-7-His$_6$ vector were used as negative controls. *B*, competition assays of the RNA-binding activity of Thp1p using as unlabeled competitors increasing amounts of the same 90-mer RNA used as probe, 60-mer single-stranded DNA (*ssDNA*), and 200-bp dsDNA. *C*, DNA binding assay using a 200-bp PCR-amplified ^{32}P-5′-end-labeled dsDNA and increasing amounts of purified Thp1p.

RNA and weakly with unlabeled double-stranded DNA (dsDNA), but not with unlabeled single-stranded DNA (Fig. 4*B*). Direct DNA band shifting assays using a labeled 200-bp dsDNA showed that Thp1p bound dsDNA, but to a lesser degree compared with RNA (Fig. 4*C*). These results are in line with the functional analyses of *thp1*Δ mutants, suggesting that Thp1p is involved in RNA metabolism. The binding to dsDNA suggests the possibility that Thp1p may also act at sites of transcription, as has been shown for the Yra1p, Hpr1p and Tho2p components of the THO/TREX complex (12, 38).

A Highly Purified Thp1p-Sac3p Complex Does Not Contain Components of the THO/TREX Complex—We constructed a yeast strain carrying the *THP1-TAP* gene fusion in which the TAP tag was fused to the C terminus of Thp1p at the chromosomal *THP1* locus. We used the stringent tandem affinity purification method involving two sequential affinity purification steps (32) to purify yeast proteins that bound to Thp1p. Fractionation of the eluted fraction by SDS-PAGE and subsequent silver staining revealed three polypeptides with apparent molecular sizes of ~140, ~100, and ~50 kDa plus other minor bands (Fig. 5*A*). Peptide mass fingerprinting using MALDI-reTOF mass spectrometry and Sequence-Tag/PepFrag Database searching using limited amino acid sequence data obtained by electrospray ionization tandem mass spectrometry (33) revealed that p140 was Sac3p, that p100 was an N-terminal fragment of Sac3p, and that p50 was Thp1p. Therefore, we conclude that Thp1p and Sac3p tightly associate in yeast cells. These results are in agreement with a parallel study showing that Thp1p and Sac3p are associated in a complex (24).

Sac3p is a nuclear protein export factor (26), trace amounts of which have recently been shown to copurify with TAP-tagged Sub2p (12). Given the possibility that the Thp1p-Sac3p complex could interact with THO/TREX, we determined whether small amounts of THO and Sub2p, not detectable on silver-

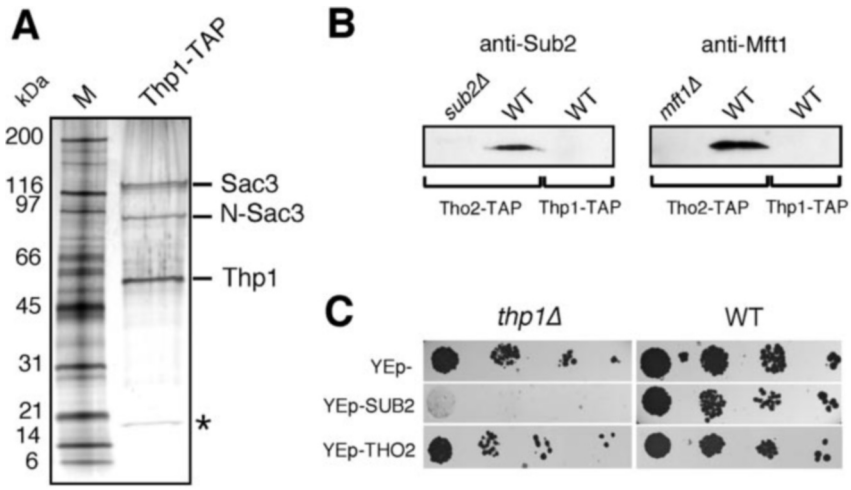

FIG. 5. **Purification and analysis of the Thp1p-Sac3p complex.** *A*, silver-stained SDS-polyacrylamide gel of the TAP-tagged Thp1p complex purified from yeast *pep4*Δ cells (LSY267). The lower abundance protein, indicated by an *asterisk*, corresponds to Cdc31p, which belongs to the superfamily of calmodulin proteins with a putative ability to bind the calmodulin-binding site of TAP. *Lane M*, molecular mass markers. *B*, Western blots of Thp1p-TAP- and Tho2p-TAP-purified complexes from the wild-type (*WT*), *sub2*Δ, and *mft1*Δ strains using anti-Sub2p and anti-Mft1p antibodies. Neither Sub2p nor Mft1p was detected in the Thp1p-Sac3p complex. *C*, effect of multicopy *SUB2* and *THO2* on cell viability of a *thp1*Δ strain. The wild-type (W303-1A) and *thp1*Δ (WFBE046) strains were transformed with the *URA3*-based pRS416-THP1 plasmid, containing a wild-copy of *THP1*, plus the *LEU2*-based YEp351 multicopy plasmid, containing a wild-copy of either *SUB2* (*YEp-SUB2*) or *THO2* (*YEp-THO2*). Transformants diluted by factors of 10 were plated on SC−Leu containing 500 μg/ml 5-fluoroorotic acid.

stained gels, were detectable by Western analysis with TAP-tagged Thp1p. Fig. 5*B* shows that TAP-tagged Thp1p did not bring together either the Mft1p component of THO or Sub2p, indicating that most Thp1p-Sac3p complexes may be found in the cell independent of either THO or Sub2p. Interestingly, whereas multicopy *SUB2* suppressed the transcription and recombination phenotypes of *hpr1*Δ, multicopy *SUB2* caused lethality in *thp1*Δ strains. Multicopy *THO2*, which encodes the most prominent component of the THO complex, did not suppress *thp1*Δ (Fig. 5*C*). This reflects a putative functional relationship between Sub2p and Thp1p, which is different from the THO complex.

*sac3*Δ *Confers Transcription and Recombination Phenotypes Similar to Those of thp1*Δ *Mutants*—To show that Thp1p-Sac3p acts as a functional unit, we performed a transcription and recombination analysis in *sac3*Δ mutants. In *sac3*Δ strains, expression of the bacterial *lacZ* sequence under the control of the *GAL1* promoter was almost abolished. Kinetic analysis of transcription activation of *sac3*Δ mutants showed an undetectable accumulation of *lacZ* mRNA for 170 min after activation (Fig. 6*A*). When an identical experiment was performed with a *GAL1pr*::*PHO5* fusion, accumulation of *PHO5* mRNA occurred with the same kinetics as the wild-type strain, reaching similar levels of transcription after 170 min of activation (Fig. 6*A*). These results are similar to those previously reported for *thp1*Δ (20) and suggest that transcription elongation through *lacZ*, and not activation of the *GAL1* promoter, is impaired in *sac3*Δ strains.

Analysis of recombination in the direct-repeat system *leu2-k*::*ADE2-URA3*::*leu2-k* revealed that *sac3*Δ increased the frequency of recombination 2145-fold above wild-type levels (Fig. 6*B*), a result similar to that previously obtained for *thp1*Δ (20). To determine whether the hyper-recombination phenotype of *sac3*Δ strains was dependent on transcription of the intervening sequence located between the repeats, we determined the effect of *sac3*Δ on recombination in the plasmid repeat systems L and LYΔNS. These systems are based on a 0.6-kb *leu2* repeat that contains either no intervening sequence or a 5.16-kb long one. In both cases, transcription was initiated from the external *LEU2* promoter and proceeded through the intervening region (29). As shown in Fig. 6*B*, whereas recombination frequencies in the L systems were the same in the *sac3*Δ and wild-type strains, recombination was increased 10-fold in the LYΔNS system. As previously shown for *thp1*Δ (20), these results are consistent with the idea that transcription of an intervening DNA sequence such as that in LYΔNS is required for hyper-recombination in *sac3*Δ mutants.

DISCUSSION

In this study, we have shown that the Thp1p-Sac3p complex is functionally related to the major mRNA-binding protein Nab2p and functions in the interface between transcription and mRNP metabolism. We link for the first time the functions of Sac3p and Nab2p, two proteins previously known to be involved in either protein or mRNA nuclear export, with transcription efficiency. Thp1p-Sac3p and Nab2p are functionally related factors that act in the same biological process as THO/TREX and Mtr2p-Mex67p, linking transcription and mRNP metabolism and affecting genome instability.

Thp1p is a conserved eukaryotic protein identified by the ability of null mutants to stimulate transcription-dependent hyper-recombination (20). Sac3p is a nuclear protein first identified as an actin suppressor (25) and is required for export of nuclear export signal-containing proteins (26). Thp1p-Sac3p behaves as a functional unit, as suggested by the *thp1*Δ and *sac3*Δ identical phenotypes of transcription impairment of *lacZ* sequences, transcription-dependent hyper-recombination, and defective mRNA nuclear export (Fig. 6) (20, 24). There are many phenotypic similarities between the mutants of Thp1p-Sac3p and the THO/TREX complex. However, THO/TREX and Thp1p-Sac3p are different complexes because, upon Western analysis, we found neither Sub2p nor Mft1p in the purified Thp1p-Sac3p complex (Fig. 5, *A* and *B*). Our observation that overexpression of Sub2p confers lethality on *thp1*Δ mutants indicates that Sub2p and Thp1p-Sac3p may functionally interact. Indeed, Sac3p copurifies with a Sub2p-TAP fusion (12). This interaction occurs apart from the THO complex because Sac3p is not copurified with TAP fusions of the THO components (12, 19). The specific functions of the THO and Thp1p-Sac3p complexes may be different. In favor of this conclusion are the observations that whereas multicopy *SUB2* suppresses the transcription and recombination phenotypes of *hpr1*Δ (19,

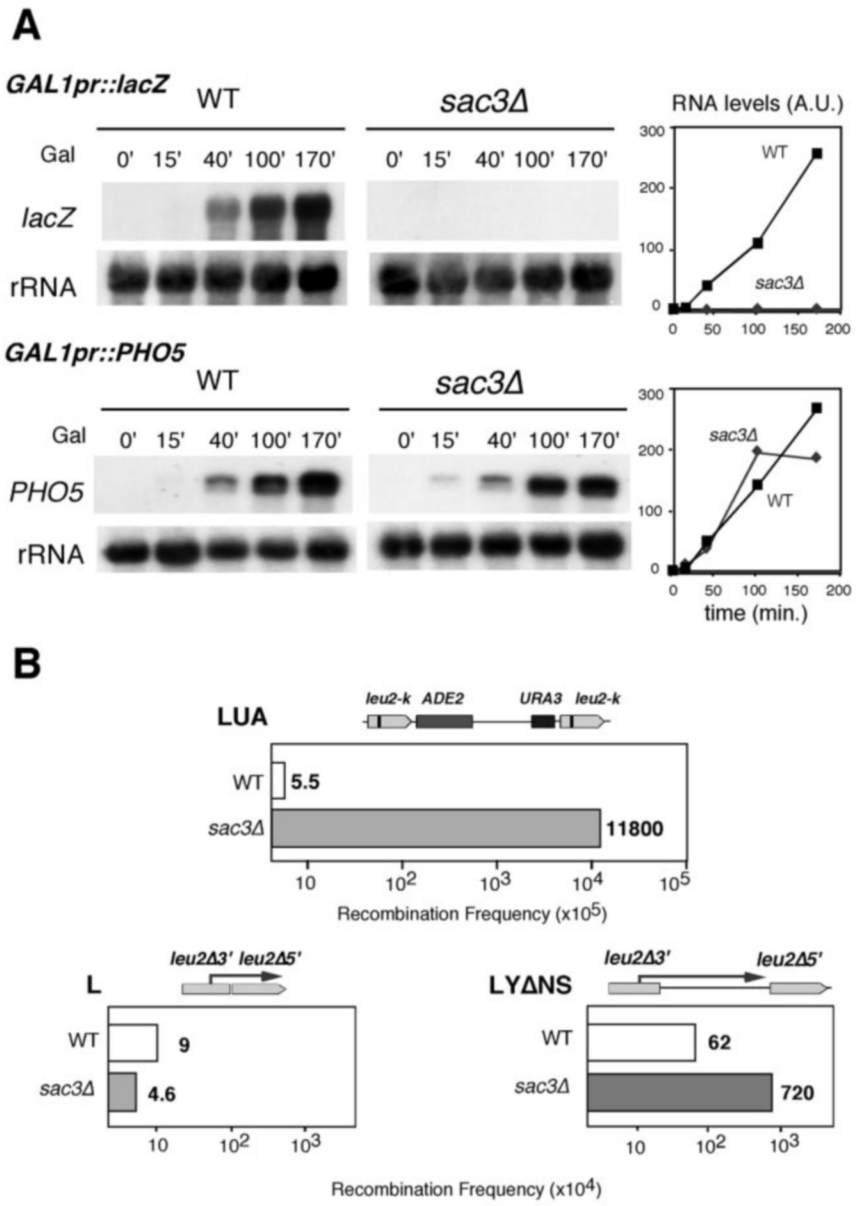

FIG. 6. **Transcription and recombination analyses of *sac3Δ* mutants.** *A*, Northern analysis of *lacZ* and *PHO5* mRNAs driven by the *GAL1* promoter in the wild-type (*WT*; BY4741) and *sac3*Δ (Y03517) strains transformed with plasmid p416-GAL1-lacZ or pSCh202. Mid-log phase cells were diluted in 3% glycerol- and 2% lactate-containing SC−Ura to A_{600} = 0.5 and incubated for 16 h at 30 °C. Galactose was then added, and samples were taken at different times as indicated. For details, see the legend to Fig. 2. *B*, recombination frequency of the chromosomal direct-repeat system LUA (*leu2-k*::*URA3-ADE2*::*leu2-k*) in the wild-type (SAY3-2B) and *sac3*Δ (SAY3-2A) strains and of plasmid direct-repeat recombination systems L and LYΔNS in the wild-type (BY4741) and *sac3*Δ (Y03517) strains transformed with pRS316L and pRS316LΔNS, respectively. A scheme of each direct-repeat recombination system is shown. Recombination values are the median of a total of 12 independent colonies obtained from SC medium (LUA) or SC−Ura (L and LYΔNS). Ura^- (LUA) and Leu^+ (L and LYΔNS) recombinants were scored on SC medium containing 5-fluoroorotic acid and on SC−Leu−Ura, respectively. *Arrows* indicate transcripts. Recombination values are the median of 12 independent colonies obtained from either SC complete medium. *A.U.*, arbitrary units.

39), multicopy *SUB2* causes lethality in the *thp1*Δ genetic background (Fig. 5*C*).

Nab2p is an RNA-binding hnRNP that strongly and specifically associates with nuclear poly(A)$^+$ RNA *in vivo* (21) and is required for poly(A)$^+$ tail length control and nuclear export of mRNA (22, 23). Our observations that multicopy *NAB2* suppresses the transcription and mRNA export defects of *thp1*Δ (Fig. 1*D*) and that *nab2-1* confers a similar inability to transcribe *lacZ* sequences and hyper-recombination phenotype compared with *thp1*Δ (Fig. 2) suggest that Nab2p and Thp1p-Sac3p function in the same biological pathway as THO/TREX, Mex67p, and Mtr2p. Consistently, it has recently been shown that Sac3p physically interacts with Mex67p, Mtr2p, and Mlp1p (24, 27) and that Mlp1p interacts with Nab2p (40).

In this study, we have shown that the defect in the kinetics of transcript formation observed in the mutants of the Thp1p-Sac3p complex (Fig. 6*A*) (20) is not due to increased mRNA decay (Fig. 3), in contrast to what had been suggested for other mutants of mRNP metabolism (41, 42). The degradation rates of transcripts such as *lacZ* and *GAL1*, which are formed at different rates, are lower in *thp1 versus* wild-type cells (Fig. 3). Therefore, the lower accumulation rate of particular mRNAs such as *lacZ* is mainly the consequence of defective transcription and consistent with the increase in recombination mediated by transcription impairment.

Most (if not all) mRNAs are associated with hnRNPs and are exported through the nuclear pore complex as mRNPs. During mRNA export, it is likely that proteins bound to the mRNA act as adapters for export factors to be translocated through the nuclear pore complex. In this sense, Nab2p is a classic shuttling hnRNP that requires ongoing transcription of poly(A)$^+$ RNA for its export from the nucleus. Preliminary results suggest that Mex67p is required for such an export (22). Thp1p-Sac3p interacts with Mex67p, Mtr2p, and other factors involved in mRNA export (24, 27). *Sac*III physically interacts with nucleoporins (24, 26), and it has been proposed that Sac3p mediates recruitment of the mRNA export machinery to the nuclear pore complex through this interaction (24). Thp1p and Sac3p have nuclear pore locations, which are dependent on the Nup1p nucleoporin (24). It has been shown by immunoelectron microscopy that Sac3p localizes to the cytoplasmic fibrils of the nuclear pore complex (27). In addition, Thp1p contains at least one leucine-rich nuclear export signal (**L**NYM**I**PTG**LIL**) starting at amino acid 268. Nuclear export signals were found in

several proteins that shuttle between the nucleus and the cytoplasm (43). In most cases, nuclear export signal-containing proteins are RNA-binding proteins that act as adapters and thus could be involved in the export of their cognate cargo RNA. Thp1p indeed has the ability to bind RNA *in vitro* (Fig. 4). Therefore, a possibility yet to be tested is whether the mRNA export defect observed in *thp1*Δ, *sac3*Δ, and even *nab2-1* mutants could be caused by the inability of the export machinery to contact hnRNPs absent in improperly assembled or processed mRNPs.

Given the increasing evidence of the connection between transcription, mRNA maturation, termination, and export (1, 2, 44, 45), it is likely that failure to perform a particular step during mRNP biogenesis has direct consequences on both upstream (*i.e.* transcription) and downstream (*i.e.* RNA export) steps. Thus, an mRNP is not exported from the nucleus unless the transcript has been properly processed (10). This could be the case for mutants of the THO/TREX complex (19). Reciprocally, there are indications that mRNA export blockage leads to accumulation of transcripts at the site of transcription (11). Regardless of the step of mRNP metabolism in which Thp1p-Sac3p and Nab2p function, it would be interesting to know whether mutations in their genes could lead to an accumulation of improperly formed mRNP intermediates at the site of transcription, promoting transcription impairment responsible for hyper-recombination.

The transcription-associated hyper-recombination phenotype of *thp1*Δ, *sac3*Δ, and *nab2-1* mutants is common to other mutants affected in both mRNP biogenesis and transcription (19, 46), but it is not observed in mutants of *bona fide* transcription elongation factors such as *spt4*Δ (47). We believe that failure to properly process or form the nascent mRNP molecules could lead to reactive nascent mRNA molecules that could interact with the DNA template located behind the advancing RNA polymerase, creating intermediates (*i.e.* RNA/DNA hybrids) responsible for both the elongation defect of the next elongating pol II and the increase in recombination, as we have previously proposed (46). Further biochemical and cell biology analyses are required to solve the molecular basis of this connection.

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