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Molecular Evidence That the Eukaryotic THO/TREX Complex Is Required for Efficient Transcription Elongation*

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Ana G. Rondón‡, Sonia Jimeno§, María García-Rubio, and Andrés Aguilera¶

From the Departamento de Genética, Facultad de Biología, Universidad de Sevilla, Avenida Reina Mercedes 6, 41012 Sevilla, Spain

THO/TREX is a conserved eukaryotic complex formed by the core THO complex plus proteins involved in mRNA metabolism and export such as Sub2 and Yra1. Mutations in any of the THO/TREX structural genes cause pleiotropic phenotypes such as transcription impairment, increased transcription-associated recombination, and mRNA export defects. To assay the relevance of THO/TREX complex in transcription, we performed *in vitro* transcription elongation assays in mutant cell extracts using supercoiled DNA templates containing two G-less cassettes. With these assays, we demonstrate that *hpr1*Δ, *tho2*Δ, and *mft1*Δ mutants of the THO complex and *sub2* mutants show significant reductions in the efficiency of transcription elongation. The mRNA expression defect of *hpr1*Δ mutants was not due to an increase in mRNA decay, as determined by mRNA half-life measurements and mRNA time course accumulation experiments in the absence of Rrp6p exoribonuclease. This work demonstrates that THO and Sub2 are required for efficient transcription elongation, providing further evidence for the coupling between transcription and mRNA metabolism and export.

mRNA synthesis in eukaryotes is a multistep process mediated by RNA polymerase II (RNAPII)[1] and consists of three major stages, *i.e.* initiation, elongation, and termination. During elongation, RNAPII has to overcome situations derived from transient pausing caused by regulatory signals with the help of transcriptional elongation factors. These factors associate with RNAPII to facilitate elongation through either particular DNA sequences or chromatin (1, 2). Among these factors, there is functional evidence for roles in transcription elongation for TFIIS (3, 4), TFIIF (5, 6), human elongin (7), human 11-19 lysine-rich leukemia (ELL) (8), human FACT/yeast Spt16-Pob3 (9–11), human DSIF/yeast Spt4-Spt5 (12, 13), human NELF (14), and the 19 S proteasome subunit (15). In addition, there is a set of eukaryotic factors that might also have an effect in transcription elongation. One such factor is THO/TREX.

THO was identified in yeast as a four-protein complex containing Tho2, Hpr1, Mft1, and Thp2 (16). Null mutations in each of the genes encoding the subunits of THO confer increased recombination between direct repeats, high levels of plasmid and chromosome instability, and defects in gene expression. This is particularly evident for long and GC-rich DNA sequences such as *lacZ* (16–19). The observation that transcription of some DNA sequences is impaired in THO mutants and that hyper-recombination only occurs at actively transcribed sequences whose transcription is THO-dependent suggests that transcription elongation is impaired in these mutants (17, 19, 20). However, in contrast to mutants of *bona fide* transcription elongation factors, THO mutants show transcription-dependent genetic instability and are not sensitive to 6-azauracil (6-AU) (17), a hallmark phenotype associated with transcription elongation defects (21).

Recently, THO has been shown to be present in a larger complex, termed TREX, together with components of the mRNA export machinery such as Sub2 and Yra1 (22). In addition, *sub2* and *yra1* mutants show the same gene expression defects and transcription-associated hyper-recombination as those of the THO mutants (23, 24). This, together with the mRNA export defect of THO mutants, the THO-like gene expression and recombination phenotypes of mutants of the Mex67-Mtr2 mRNA export factor, and the ability of THO to bind RNA *in vitro*, suggests that THO might be functionally involved in mRNP biogenesis and export (23). This conclusion is strengthened by the observation that mutants of the Thp1·Sac3 complex, which interacts with RNA and the nuclear pore complex (NPC) and participates in mRNA export (25, 26), confer the same transcription and hyper-recombination phenotypes as do THO/TREX mutations (25, 27).

Sub2 is a conserved eukaryotic protein of the family of the DEAD box RNA helicases. First identified as a protein functioning at multiple steps during spliceosome assembly (28–30), Sub2 and its human (hUAP56) and *Drosophila* (HEL) orthologues have also been involved in mRNA transport (31–34). Indeed, Sub2 binds *in vivo* to Yra1, an RNA binding protein (35) with RNA-RNA annealing activity (36). Yra1 interacts with the Mex67-Mtr2 mRNA export factor (32, 37), a heterodimer that mediates the interaction of the mRNP with the nuclear pore complex (35, 38). A number of observation suggest that THO may form a core complex apart from Sub2. First, removal of Sub2 in cells does not affect the integrity of the purified THO complex (23). Second, in a highly purified THO complex, Sub2 is a minor component and Yra1 is absent (22). Finally, there is an important part of Sub2 in the cell that is not in association with the THO complex, as observed in purification experiments using tandem affinity purification (TAP)-tagged Sub2 (22). Despite the cumulative *in vivo* evidence

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¶ To whom correspondence should be addressed. Tel.: 34-954-557107; Fax: 34-954-557104; E-mail: aguilo@us.es.

[1] The abbreviations used are: RNAPII, RNA polymerase II; mRNP, mRNA and heterogeneous nuclear ribonucleoprotein complex; WCE, whole cell extract; WT, wild-type; nt, nucleotide.

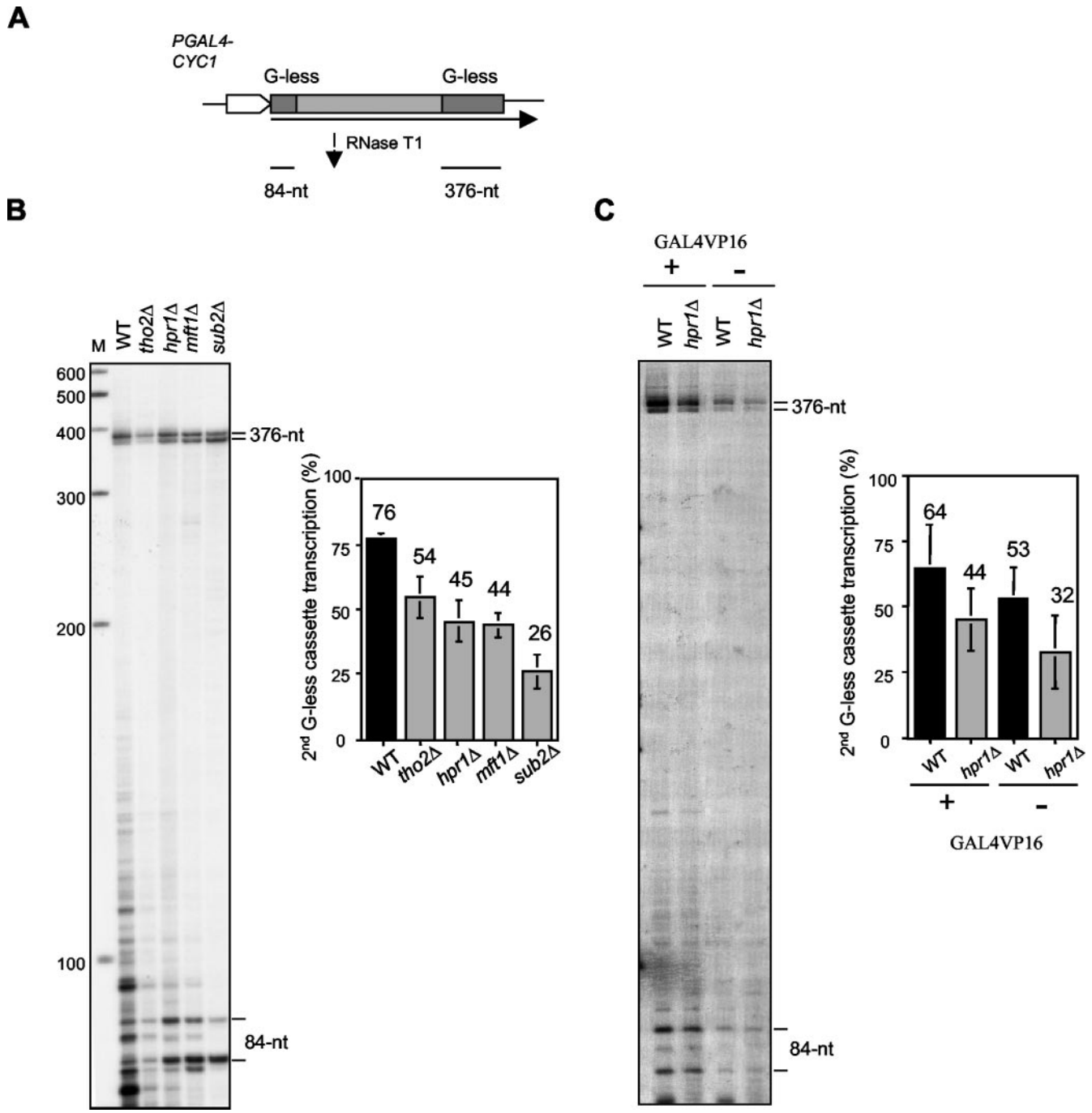

FIG. 1. ***In vitro* transcription elongation assays of WCEs of wild-type and mutants of the THO/TREX complex.** *A*, scheme of the double G-less cassette system used for the analysis of *in vitro* transcription elongation. RNase T1-treatment of the mRNA driven from the *GAL4-CYC1* promoter, which is activated by purified Gal4-VP16, render two fragments corresponding to the G-less cassettes. *B*, *in vitro* transcription assay of WCEs from W303-1A (*WT*), RK2-6C (*tho2*Δ), SChY58a (*hpr1*Δ), WMK-2A (*mft1*Δ), and DLY23sub2Δ (*sub2*Δ) strains. Each reaction was stopped after 30 min, treated with RNaseT1, and run in a 6% PAGE. Two bands from each G-less cassette were obtained, probably due to incomplete action of RNaseT1. Efficiency of transcription elongation was determined as the percentage of total transcripts that reach the 376-nt G-less cassette in respect to the transcripts that cover the 84-nt cassette. Radioactivity incorporated into the G-less cassettes was quantified in a Fuji FLA3000 and normalized with respect to the C content of each G-less cassette. The mean value and S.D. of three independent experiments are shown. *C, in vitro* transcription assay of WCEs from W303-1A (*WT*) and SChY58a (*hpr1*Δ) with or without addition of the Gal4-VP16 activator to the reaction. Other details are as described for *panel B*.

indicating that THO and Sub2 mutants confer pleiotropic phenotypes on transcription and mRNA metabolism (17, 18, 22, 23), no direct *in vitro* proof exists yet that transcription elongation is defective in these mutants.

To determine whether mRNA elongation was defective in mutants of the THO/TREX complex, we performed *in vitro* transcription elongation assays with two G-less cassette DNA templates (39). In this study, we show that cell extracts of THO complex mutants (*hpr1*Δ, *tho2*Δ, and *mft1*Δ) and *sub2* have significantly reduced transcription elongation efficiencies *in vitro*. In addition, we demonstrate that the decrease in mRNA accumulation of *hpr1*Δ mutants is not due to an increase in mRNA decay. It is likely that THO is not a *bona fide* transcription elongation factor but rather a protein complex involved in mRNP biogenesis during transcription elongation. Yet, our work provides unequivocal molecular evidence that transcrip-

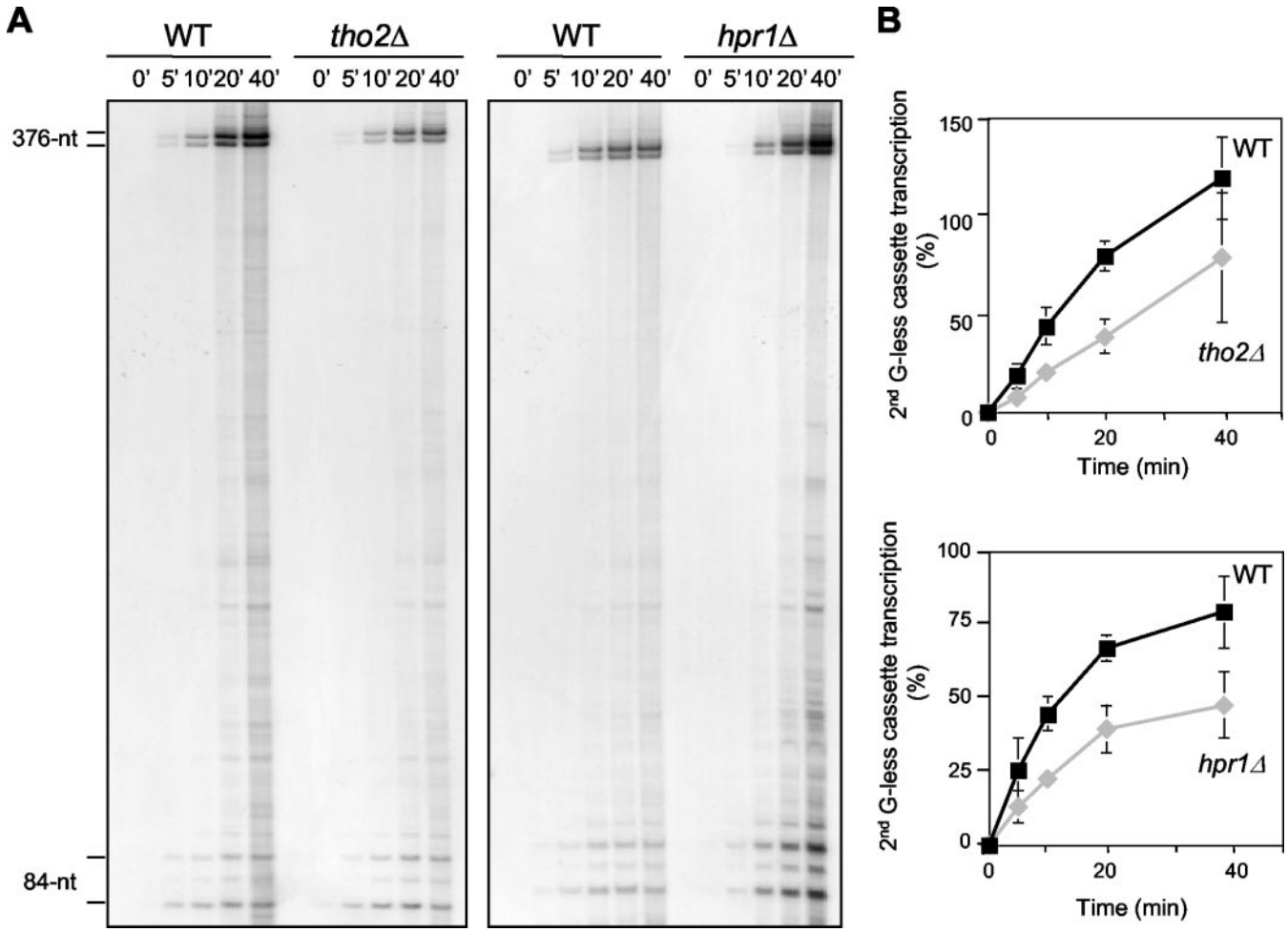

FIG. 2. ***In vitro* transcription elongation analysis of wild-type, *tho2*Δ, and *hpr1*Δ WCEs.** *A*, time course analysis of transcription elongation of WCEs from W303-1A (*WT*), RK2-6C (*tho2*Δ), and SChY58a (*hpr1*Δ) strains. Each reactions was stopped at the specified time, treated with RNaseT1, and run in a 6% PAGE. *B*, efficiency of transcription elongation was determined as the percentage of total transcripts that reach the downstream 376-nt G-less cassette in respect to the transcripts that cover the upstream 84-nt cassette. The mean value and S.D. of 3–4 independent experiments are shown. Other details are as described in the Fig. 1 legend.

tion elongation efficiency is reduced in THO/TREX mutants, which is in agreement with the idea that transcription and mRNA metabolism and export are coupled in the cell.

EXPERIMENTAL PROCEDURES

Yeast Strains and Plasmids—Strains used were wild-type (W303-1A) and the isogenic mutants *tho2*Δ::*KAN* (RK2-6D) (19), *hpr1*Δ::*KAN* (SChY58-a), *hpr1*Δ::*HIS3* (U678-1C) (17), *mft1*Δ::*KAN* (WMK-2A) (16), *sub2*Δ::*HIS3* (Ura$^-$ segregant of DLY23), and *sub2-201* (DLY33/sub2-201) (28). We have also used wild-type BY4741 and its isogenic deletion *rrp6*Δ::*KAN* (YOR001w) (Euroscarf, Frankfurt, Germany). Strains HRK-1A (WT), HRK-2B (*rrp6*Δ::*KAN*), HRK-3C (*hpr1*Δ::*HIS3*), and HRK-44C (*hpr1*Δ::*HIS3 rrp6*Δ::*KAN*) come from genetic crosses between U678-1C and YOR001w. His_6-tagged Gal4-VP16 recombinant protein was expressed in *Escherichia coli* from plasmid pRJRGAL4-VP16 (M. Ptashne, Sloan-Kettering Institute, New York, NY). We used plasmid pGCYC1-402 (39) for *in vitro* transcription elongation assays and p416*GAL1lacZ* (40) for expression analysis.

Preparation of Yeast Whole Cell Extracts (WCEs)—Yeast cells were grown in rich YEPD (1% yeast extract, 2% peptone, and 2% dextrose) medium at 30 °C to an A_{600} of 1. WCEs were prepared as described (41), with the exception that the extraction buffer was 0.2 M Tris, pH 7.5, 0.39 M ammonium sulfate, 10 mM $MgSO_4$, 20% glycerol, 1 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 2.7 mM benzamidine, 0.3 μg/ml leupeptin, and 1.4 μg/ml pepstatin A. Precipitation and dialysis were performed as described (42). WCEs were distributed in aliquots and frozen in liquid nitrogen. They were stable after repeated cycles of freezing and thawing.

In Vitro Transcription Assay with Whole Cell Extracts—Each reaction was performed in a final volume of 40 μl of buffer A 0.5 (20 mM HEPES, pH 7.5, 20% glycerol, 1 mM EDTA, 1 mM dithiothreitol, and 500 mM potassium acetate) with 100 μg of whole cell extracts and 100 ng of Gal4-VP16 purified as described (43) and dialyzed in buffer A 0.05 (same as A 0.5 but with 50 mM potassium acetate). Final potassium acetate concentration should be <150 mM. The reaction was set up adding 20 μl of transcription buffer (2×) (final concentration was 40 mM HEPES-KOH, pH 7.5, 5 mM $MgCl_2$, 1 mM ATP, 1 mM GTP, 0.5 mM UTP, 0.03 mM CTP, 40 mM phosphocreatine, 32 μg of creatine kinase, 5 mM dithiothreitol, and 7.5 units of the RNase inhibitor RNAguard (Amersham Biosciences)). After 20 min of preincubation at room temperature, 400 ng of pGCYC402 and 1 μl of [α^{32}P]CTP (3000 Ci/mmol) were added. The reaction was stopped at indicated times by the addition of 200 μl of stop buffer (10 mM Tris-HCl, pH 7.5, 0.3 M NaCl, and 5 mM EDTA) and 200 units of RNaseT1 for 15 min at room temperature. Samples were treated with proteinase K, which was phenol-extracted and run in a sequencing gel as described (44). The amount of radioactivity incorporated was quantified with a Fuji FLA3000.

In Vivo Analysis of Gene Expression and mRNA Decay—Ten micrograms of total RNA was prepared from induced cultures and used for Northern analysis following standard procedures (17). DNA filters were hybridized first with ^{32}P-labeled DNA probes as specified. For determination of the total amount of RNA used, filters were stripped and re-hybridized with ^{32}P-labeled 25 S rDNA obtained by PCR as described (17).

Miscellaneous—DNA isolation, ^{32}P-radiolabeling, genetic crosses, and yeast transformations were performed according to standard procedures.

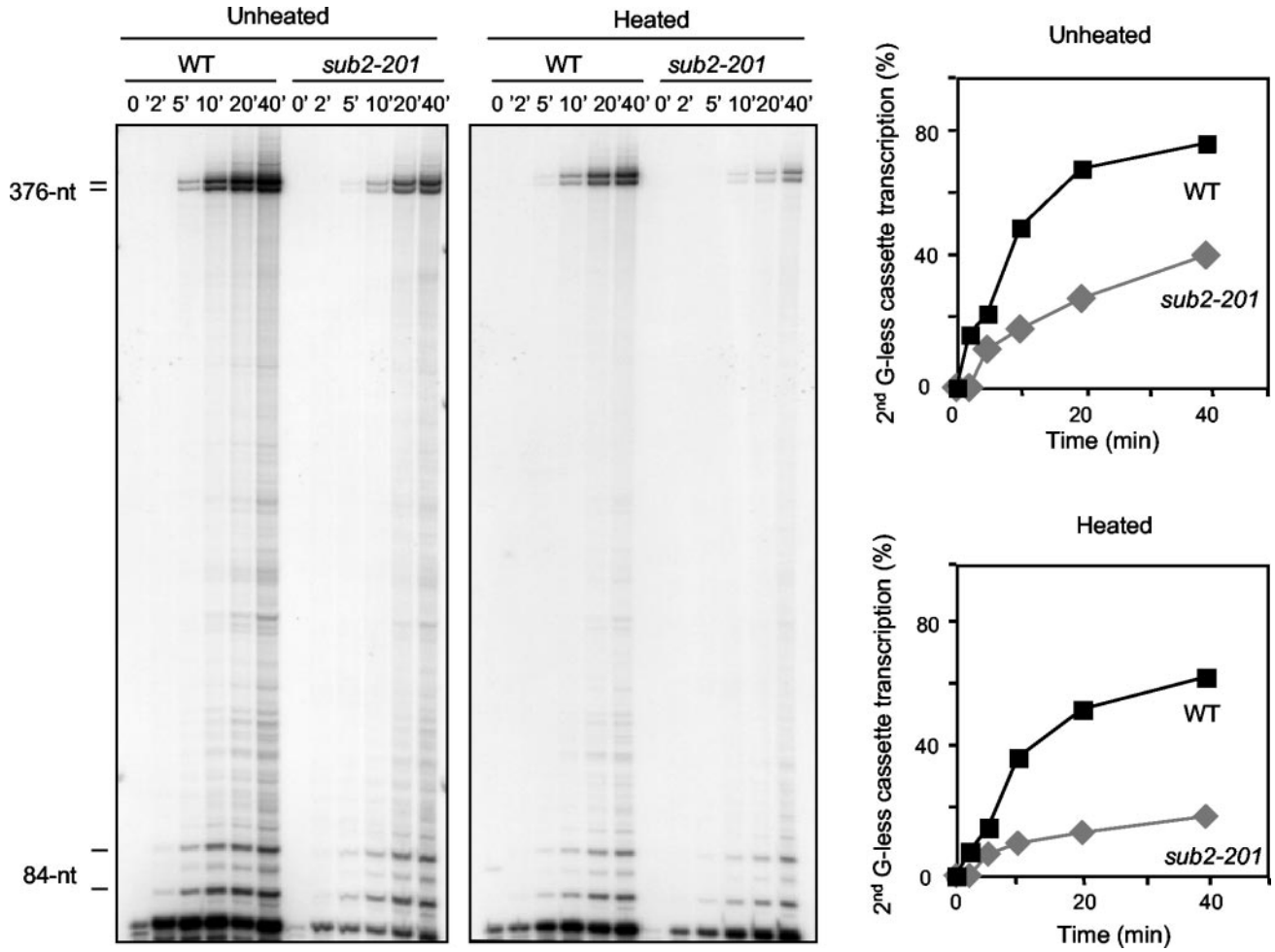

FIG. 3. ***In vitro* transcription analysis of wild-type and *sub2-201* WCEs.** Time course analysis of transcription elongation of WCEs from W303-1A (*WT*) and DLY33sub2-201 (*sub2-201*) strains unheated or heated for 15 min at 37 °C before being added to the reaction. Reactions were stopped at the indicated times, incubated with RNaseT1, and run in a 6% PAGE. Other details are as described in the Figs. 1 and 2 legends.

RESULTS

Whole Cell Extracts of Mutants of the THO Complex Are Impaired in Transcription Elongation—To determine whether mutants of the THO complex were impaired in transcription elongation, we used our newly reported *in vitro* assay (39). This assay is based on a plasmid (pGCYC1-402) in which a hybrid *GAL4-CYC1* promoter containing a Gal4 binding site is fused to a 1.88-kb DNA fragment coding two G-less cassettes separated by 1.4 kb. The first G-less cassette is right downstream of the promoter and is 84-nt-long. The second is located at 1.48 kb from the promoter and is 376-nt-long. In this assay, transcription activated by Gal4-VP16 leads to an mRNA that, after digestion with RNase T1, which degrades all G-containing mRNA sequences, leaves the two G-less cassettes intact (Fig. 1*A*). The efficiency of transcription elongation is determined in WCEs by the values of the ratio of accumulation of the 376-*versus* the 84-nt-long G-less RNA fragments. We have recently proved with this assay that transcription elongation is defective in *spt4*Δ WCE (39).

As can be seen in Fig. 1*B*, after 30 min of transcription *tho2*Δ, *hpr1*Δ, and *mft1*Δ cell extracts carrying deletions of structural genes of the THO complex fully transcribed the 376-nt G-less cassette with efficiencies ranging from 58 to 71% of the wild-type levels. These results indicate that removal of any subunit of the THO complex leads to a similar reduction in the efficiency of transcription elongation *in vitro*. To determine whether or not the addition of Gal4-VP16 could affect transcription elongation in wild-type and THO mutants differently, we repeated the experiments in the absence and the presence of externally added Gal4-VP16. We first showed that, as expected, transcription initiation efficiency in the absence of Gal4-VP16, as determined by the accumulation of the 84-nt G-less cassette, was lower (22%) than in the presence of Gal4-VP16. Furthermore, as can be seen in Fig. 1*C,* the addition of Gal4-VP16 diminished the efficiency of transcription elongation similarly in *hpr1*Δ and wild-type cells. Therefore, the lower efficiency of transcription elongation observed in the THO mutants is independent of the transcription activator.

To further demonstrate that elongation was less efficient in mutants of the THO complex, we determined the kinetics of transcription elongation in time course experiments in mutants of the two largest subunits of the complex, *tho2*Δ and *hpr1*Δ. As can be seen in Fig. 2, transcription elongation efficiency in both mutant extracts was 46–66% of the wild-type, depending on the time the reaction was run. We conclude that THO complex depletion causes a deficiency of transcription elongation in whole cell extracts. It is worth noting that this deficiency is independent of the growth rate of the strains tested, because whereas *mft1*Δ cells grow like wild-type cells and *hpr1*Δ cells grow poorly, both mutations have similar effects on transcription elongation.

Whole Cell Extracts of Mutants of Sub2 Show Transcription Elongation Impairment—The THO complex is associated with proteins involved in mRNA export, such as Sub2 and Yra1, in a larger complex termed TREX. However, THO may form a core complex apart from Sub2 (22). This, together with the fact that

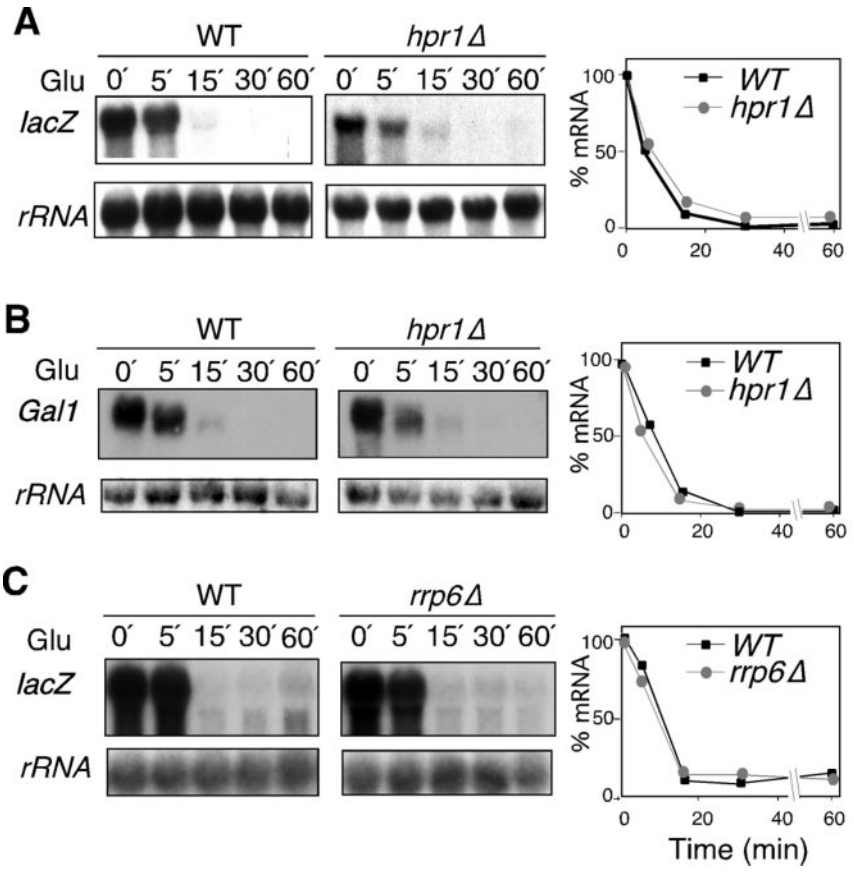

FIG. 4. **Kinetics of degradation of *lacZ* and *GAL1* mRNAs in *hpr1*Δ and wild-type cells.** *A*, Northern analysis of *lacZ* mRNA decay in wild-type (W303-1A) and *hpr1*Δ (U678-1C) cells. Mid-log phase cultures of wild-type and *hpr1*Δ cells harboring p416*GAL1lacZ* were grown in 2% galactose synthetic medium for 3 h and transferred to 2% glucose-synthetic medium before samples were taken. *B*, Northern analysis of mRNA decay from endogenous *GAL1* transcripts. Experiments were performed as described for *panel A*. *C*, Northern analysis of the *lacZ* mRNA decay in isogenic BY4741 (*WT*) and YOR001w (*rrp6*Δ) strains transformed with p416*GAL1lacZ*. Experiments were performed as described for *panel A*. The 0.75-kb *Pvu*II-*Ava*I internal *GAL1* fragment, the 3-kb *Bam*HI-*Bgl*II *lacZ* fragment, and an internal 589-bp 25 S rRNA fragment obtained by PCR were used as ^{32}P-labeled DNA probes. Northern blots were quantified in a Fuji FLA 3000. RNA levels are given in arbitrary units normalized with respect to the rRNA levels of each sample.

Sub2 and Yra1 are proteins with functional roles in mRNA splicing and/or mRNA export, makes it important to know whether Sub2 is also required for efficient transcription elongation.

We applied *in vitro* transcription analysis to *sub2* mutants. As can be seen in Fig. 1*B*, after 30 min of transcription, *sub2*Δ WCEs fully transcribed the 376-nt G-less cassette with 36% of the efficiency of the wild-type. To get further evidence of the role of Sub2 in transcription elongation, we determined the kinetics of transcription elongation of whole cell extracts with time course experiments using WCEs of the *sub2-201* mutant. It has been reported that the *sub2-201* mutation causes a splicing phenotype at 37 °C in WCEs *in vitro* that is not observed at the permissive temperature (28). Elongation assays were performed by maintaining the WCEs at 23 °C (unheated) or shifting them to 37 °C for 15 min (heated) immediately before starting the experiment. As can be seen in Fig. 3, transcription elongation was reduced in unheated WCEs (54% of the wild-type levels after 40 min), indicating that *sub2–101* was leaky at the permissive temperature for transcription elongation. However, the reduction in transcription elongation efficiency was stronger in heated *sub2-201* WCEs in which the elongation levels were similar to those of *sub2*Δ cells (30% of the wild-type levels). This result indicates that Sub2 is required for efficient elongation.

*The Gene Expression Defect of hpr1*Δ *Is Not Due to an Increase in mRNA Decay*—Recently, the question has been raised of whether the low mRNA levels observed *in vivo* in mutants of the THO/TREX complex could be due to a higher rate of mRNA degradation and not to a transcription defect (37, 45). It was previously observed that steady-state levels of *lacZ* mRNA driven from a *GAL10* promoter in *yra1–8 rrp6*Δ mutants were ~2–3-fold higher than those in *yra1–8* mutants (37). Also, the 5′ end of *HSP104* mRNA accumulates ~2 times above the 3′-end in *hpr1*Δ and *sub2-201* single mutants but not in *hpr1*Δ *rrp6*Δ and *sub2-201 rrp6*Δ double mutants (45). However, these differences are very low as compared with the 20-fold decrease in expression of genes such as *lacZ* observed in time course experiments (16) and do not explain the gene expression and recombination phenotypes of THO mutants.

We decided to assay whether the gene expression defects of THO/TREX mutants reflected transcription defects rather than higher mRNA degradation rates. We measured the half-life of two differently affected mRNAs in THO mutants, *i.e.* the *lacZ* and *GAL1* mRNAs, both driven from the *GAL1* promoter. It was shown previously that *lacZ* was poorly expressed *in vivo* in THO mutants, whereas *GAL1* levels of expression were proximal to wild-type levels (16, 17). As can be seen in Fig. 4, *A* and *B*, *hpr1*Δ and wild-type cells have similar kinetics of degradation of both *lacZ* and *GAL1* mRNAs. Indeed, we do not detect higher levels of mRNA stability in *rrp6*Δ mutants (Fig. 4*C*). That is, mRNA stability is the same in both wild-type and mutant strains, regardless of whether or not the expression of the mRNA analyzed was affected by *hpr1*Δ. Therefore, failure of the *hpr1*Δ mutant and, by extension, THO/TREX mutants to accumulate mRNAs such as *lacZ in vivo* (17) was not due to an increase in mRNA decay but to low levels of transcript formation. This is consistent with the transcription elongation defect detected *in vitro*. The prediction of this result is that *rrp6*Δ should not significantly suppress the defect in the kinetics of accumulation of *lacZ* mRNA caused by *hpr1*Δ. As expected, Fig. 5 shows similar kinetics of transcription in *hpr1*Δ and *hpr1*Δ *rrp6*Δ strains that, in both strains, occur at 42 and 22% of the wild-type levels after 1 h of induction, respectively.

DISCUSSION

Using an *in vitro* transcription elongation assay based on supercoiled DNA templates containing two G-less cassettes, we provide molecular evidence that the THO/TREX complex, with a role at the interface between transcription and mRNA export,

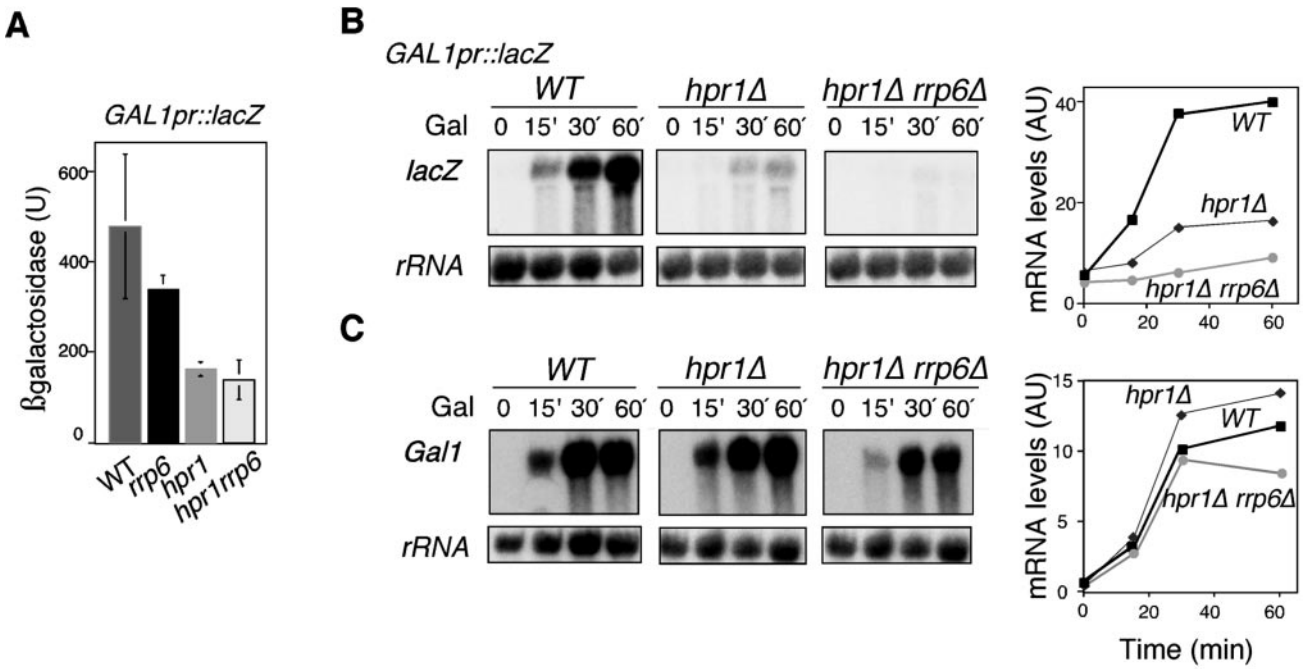

FIG. 5. **Expression analysis of *GAL1pr::lacZ* construct in HRK-1A (*WT*), HRK-2B (*hpr1*Δ), and HRK-44C (*hpr1*Δ *rrp6*Δ) congenic strains.** *A*, β-galactosidase activity of strains transformed with plasmid p416*GAL1lacZ*. Each value represents the average of two different transformants. Only the data of induced expression (2% galactose) are given. Under repression conditions (2% glucose), values were below detection levels in all cases. *U*, units. *B*, Northern analysis of *lacZ* mRNA driven from the *GAL1* promoter and *GAL1* endogenous locus. Mid-log phase cultures of cells transformed with plasmid p416*GAL1lacZ* were diluted in synthetic complete (SC) 3% glycerol-2% lactate media lacking uracil to an A_{600} of 0.5 and incubated for 16 h at 30 °C. 2% galactose was then added, and samples were taken at different times, as specified. Other details are as described in the Fig. 4 legend. *AU*, arbitrary units.

is required for transcription elongation. The different mutants of the THO core complex (*hpr1*Δ, *tho2*Δ, and *mft1*Δ) and *sub2*Δ and *sub2-201* showed inefficient transcription elongation *in vitro*, consistent with previously reported *in vivo* mRNA accumulation defects. We excluded experimentally the possibility that the mRNA expression defect of *hpr1*Δ mutants and, by extension, THO/TREX mutants, was due to high mRNA degradation rates.

Several results suggest that THO has a role in mRNP biogenesis and export. First, the tandem affinity purification-tagged THO complex copurified with mRNA export proteins Sub2 and Yra1 (22). Second, mutants of the mRNA export factors Sub2, Yra1, Mex67, Mtr2, Thp1, and Sac3 showed transcription-dependent hyper-recombination and gene expression phenotypes like those of THO mutants (23–25,27). Third, THO mutants are affected in mRNA export (22, 46). It was therefore important to prove that the gene expression defect observed in mutants of the THO complex was the result of an impairment of transcription elongation. Our *in vitro* transcription elongation assay unequivocally shows that, regardless of its particular function, THO is required for efficient transcription elongation. In an assay designed to specifically quantify transcription elongation efficiency, the *hpr1*Δ, *tho2*Δ, and *mft1*Δ mutants of the THO complex reduced the efficiency of transcription elongation to 58–71% of the wild-type levels (Figs. 1 and 2).

THO does not have an effect on the rate of mRNA decay that could explain the differences of gene expression of THO mutants (Figs. 4 and 5). Degradation rates of mRNAs are the same in *hpr1*Δ and wild-type mutants, regardless of whether or not expression of the gene studied (*lacZ* or *GAL1*) was negatively affected by *hpr1*Δ (Fig. 4). In addition, *rrp6*Δ, a null mutation of the Rrp6 exoribonuclease component of the nuclear exosome, does not significantly suppress the *lacZ* expression defect caused by *hpr1*Δ. As expected from this result, *rrp6*Δ does not suppress the hyper-recombination phenotype of *hpr1*Δ mutants.[2] Our mRNA stability results are consistent, therefore, with the *in vitro* analysis showing a defect in transcription elongation and with the hallmark phenotype of THO mutants, a transcription elongation-dependent hyper-recombination.

Sub2 is a putative RNA helicase of the DEAD box family that has been shown to have a role in mRNA splicing and export (28, 29, 31–33). In our transcription elongation assays, *sub2* confers a reduction in transcription elongation efficiency of 30–36% of the wild-type levels (Figs. 1 and 3). This proves clearly that Sub2 is also required for efficient transcription elongation *in vitro*. Examples of the stimulatory effects of proteins involved in mRNA metabolism, such as splicing proteins, on transcription elongation has been reported previously. Thus, in HeLa cell extracts, specific U1 and U2 small nuclear RNPs and Sm splicing proteins are loaded onto the transcription machinery via positive transcription elongation factor b (P-TEFb) interaction and are necessary for efficient transcription elongation (47). As shown previously for mRNA export activity, the Sub2 requirement for transcription elongation is splicing-independent. This conclusion is based on two pieces of evidence. First, the elongation defect is observed in an intron-free system (double G-less cassette). Second, at permissive temperatures *sub2-201* extracts are splicing-proficient (28), but transcription is elongation-deficient (Fig. 3).

The observation that transcription elongation in heat-inactivated *sub2-201* extracts is more severely impeded than in THO mutant extracts indicates that Sub2 might be a key element in facilitating transcription elongation. This supports the idea that Sub2-Yra1 and THO represent two distinct subcomplexes of TREX. It has been hypothesized that THO may be loaded onto RNAPII-mRNP structures prior to Sub2, helping to recruit it (22, 37). This is in accordance with our *in vitro* results and the observations that multicopy *sub2* suppresses both the

[2] P. Huertas and A. Aguilera, unpublished results.

transcription and recombination phenotypes of *hpr1*Δ cells (23, 24).

The negative effect on transcription elongation of the THO/TREX mutations does not necessarily indicate that THO or Sub2-Yra1 are *bona fide* transcription elongation factors (48). In this sense, *spt4*Δ mutants show similar *in vitro* and *in vivo* transcription elongation defects as do THO mutants. However, in contrast to THO mutants, in *spt4*Δ cells the *in vitro* transcription elongation defect is stronger; impairment of transcription does not lead to hyper-recombination, and there are no mRNA export defects (39). The interconnection between the different steps of mRNA metabolism from transcription to mRNA processing and export (49) suggests the possibility that a set of proteins without a primary role in transcription may also affect transcription elongation. Consistent with this view, mutations in the Mex67·Mtr2 and Thp1·Sac3 complexes confer similar transcription and recombination phenotypes as the THO mutants do (23, 25, 27). The effect of THO and Sub2 on transcription elongation and mRNA metabolism and export suggests the possibility, already raised, that the nascent mRNA might have a role in transcription elongation efficiency and transcription-associated hyper-recombination (48).

In summary, this study shows that there is a set of conserved eukaryotic factors, like THO/TREX, that function at the interface between transcription and mRNA metabolism and that, regardless of their specific functional roles, are required for efficient transcription elongation. Deciphering the function of these factors will contribute to understanding the mechanisms of transcription elongation, how it is coupled with mRNA processing and export, and the mechanisms of transcription-associated genetic instability.

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Ana G. Rondón, Sonia Jimeno, María García-Rubio and Andrés Aguilera

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