

# The Prp19 complex is a novel transcription elongation factor required for TREX occupancy at transcribed genes

Sittinan Chanarat, Martin Seizl, and Katja Strässer<sup>1</sup>

Gene Center Munich, Department of Biochemistry, Ludwig-Maximilians-University Munich, 81377 Munich, Germany, and Center for Integrated Protein Science Munich (CIPSM), Ludwig-Maximilians-University Munich, 81377 Munich, Germany

**Different steps in gene expression are intimately linked. In *Saccharomyces cerevisiae*, the conserved TREX complex couples transcription to nuclear messenger RNA (mRNA) export. However, it is unknown how TREX is recruited to actively transcribed genes. Here, we show that the Prp19 splicing complex functions in transcription elongation. The Prp19 complex is recruited to transcribed genes, interacts with RNA polymerase II (RNAPII) and TREX, and is absolutely required for TREX occupancy at transcribed genes. Importantly, the Prp19 complex is necessary for full transcriptional activity. Taken together, we identify the Prp19 splicing complex as a novel transcription elongation factor that is essential for TREX occupancy at transcribed genes and that thus provides a novel link between transcription and messenger ribonucleoprotein (mRNP) formation.**

[*Keywords:* transcription; mRNA export; TREX; Syf1; Prp19 complex]

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Transcription of protein-coding genes by RNA Polymerase II (RNAPII) is facilitated by a multitude of transcription factors. Transcription initiation factors regulate transcription and recruit RNAPII to the promoter. When RNAPII starts to synthesize the messenger RNA (mRNA), initiation factors are exchanged for elongation factors (Pokholok et al. 2002). Transcription elongation factors are thought to increase the processivity of RNAPII by facilitating chromatin passage as well as mRNA processing (Hirose and Manley 2000; Orphanides and Reinberg 2000, 2002; Ahn et al. 2004; Mason and Struhl 2005; Perales and Bentley 2009). Consistently, it has been shown in recent years that transcription and downstream processes such as capping, splicing, polyadenylation, cleavage, packaging of the mRNA into a mature messenger ribonucleoprotein (mRNP), mRNP quality control, and mRNP export are intimately linked (Maniatis and Reed 2002; Reed 2003; Stutz and Izaurralde 2003; Iglesias and Stutz 2008; Komili and Silver 2008; Carmody and Wenthe 2009).

In *Saccharomyces cerevisiae*, the conserved TREX complex couples transcription to mRNP export (for review, see Reed and Cheng 2005; Kohler and Hurt 2007; Carmody and Wenthe 2009; Katahira and Yoneda 2009;

Rondon et al. 2010). TREX consists of the heterotetrameric THO complex (comprised of the proteins Tho2, Hpr1, Mft1, and Thp2), the intranuclear mRNA export factors Sub2 and Yra1, the SR proteins Gbp2 and Hrb1, and Tex1 (Strasser et al. 2002). The THO subcomplex facilitates efficient transcription elongation (Rondon et al. 2003). In addition, it prevents hyperrecombination, most likely by preventing the formation of DNA–RNA hybrids, so-called R-loops, during transcription elongation, thus linking transcription to genome stability (Jimeno et al. 2002; Huertas and Aguilera 2003). Furthermore, TREX functions in 3' end processing and transcription-coupled DNA repair (TCR) (Gaillard et al. 2007; Rougemaille et al. 2008).

THO recruits the other components of the TREX complex to actively transcribed genes (Strasser et al. 2002; Zenklusen et al. 2002). In addition, the 3' end processing factor Pcf11 has been reported to recruit Yra1 to genes (Johnson et al. 2009). Sub2 and Yra1 are then thought to be transferred to the nascent mRNA and function as intranuclear mRNA export factors that recruit the mRNA export receptor Mex67–Mtr2 to the mRNP (Strasser and Hurt 2000, 2001; Libri et al. 2001; Luo et al. 2001; Zenklusen et al. 2001; Lei and Silver 2002; Abruzzi et al. 2004). TREX leaves the transcribing polymerase downstream from the polyadenylation site (i.e., before RNAPII terminates transcription), most likely together with the mRNP (Hurt et al. 2004; Kim et al. 2004).

<sup>1</sup>Corresponding author.

E-MAIL [strasser@imb.uni-muenchen.de](mailto:strasser@imb.uni-muenchen.de); FAX 49-89-218076945.

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In higher eukaryotes, TREX is recruited to the mRNA during splicing (Masuda et al. 2005; Wahl et al. 2009). This might reflect the fact that splicing is more prominent in higher cells. However, since splicing takes place cotranscriptionally, the recruitment of the human TREX complex to spliced mRNAs is indirectly coupled to transcription through splicing. Furthermore, the overall functions of human TREX and Tap-p15, the human homolog of the yeast mRNA export receptor Mex67-Mtr2, are conserved (for review, see Katahira and Yoneda 2009 and references therein). However, despite the well-known and conserved function of the TREX complex, it has been a long-standing question as to how THO/TREX is recruited to transcribed genes.

Here, we show that the Prp19 complex, a non-snRNP protein complex with an essential role in splicing (Will and Lührmann 2006 and references therein), is necessary for TREX occupancy at transcribed genes. Deletion of the C terminus of Syf1, a component of the Prp19 complex, leads to dissociation of the Prp19 complex from RNAPII and the loss of the Prp19 complex and, consequently, TREX from actively transcribed genes. Importantly, Syf1 function is needed for efficient transcription and full processivity of RNAPII. Taken together, the Prp19 complex has a second, independent function in gene expression; namely, transcription elongation by stabilizing the recruitment of TREX to transcribing RNAPII.

## Results

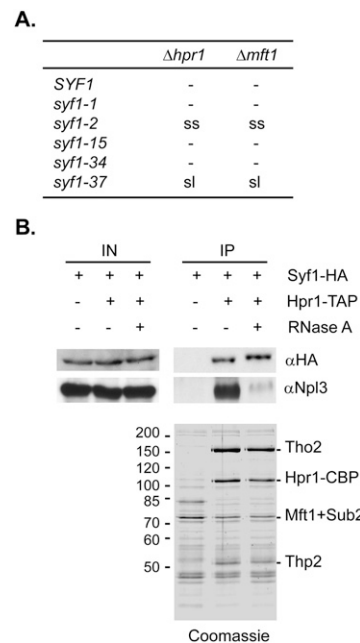
### *Syf1 interacts genetically and biochemically with the THO complex*

In *S. cerevisiae*, the conserved TREX complex couples transcription to mRNP export, is recruited to actively transcribed genes, and travels along the gene together with RNAPII (for review, see Reed and Cheng 2005; Kohler and Hurt 2007; Katahira and Yoneda 2009; Rondon et al. 2010). However, it is not known how TREX is recruited to the transcription machinery or which proteins or protein complexes mediate this interaction. Interestingly, Syf1, a component of the Prp19 splicing complex, is homologous to human XAB2 (XPA-binding protein 2), a protein involved in splicing, but also in transcription and TCR (Nakatsu et al. 2000; Kuraoka et al. 2008). Since XAB2 and TREX have similar functions, we hypothesized that Syf1 functions in transcription in conjunction with TREX.

As a first indication for an overlapping function, we assessed whether *SYF1* interacts genetically with THO components. To do this, we generated temperature-sensitive (ts) alleles of *SYF1* and tested them for synthetic lethality with deletions of *HPR1* and *MFT1*, two of the four components of the THO complex. Five different ts mutants of *SYF1* were obtained by random mutagenesis: *syf1-1*, *syf1-2*, *syf1-15*, *syf1-34*, and *syf1-37*. All five *SYF1* ts mutants grow approximately like wild-type cells at the permissive temperature (30°C), but do not grow at the nonpermissive temperature (37°C) (Supplemental Fig. 1). Interestingly, only one of the five *SYF1* ts alleles, *syf1-37*

(for further information on *syf1-37* see the legend for Fig. 1A), is synthetic lethal with  $\Delta hpr1$  and  $\Delta mft1$  (Fig. 1A). Thus, *SYF1* interacts genetically and in an allele-specific manner with *HPR1* and *MFT1*.

Second, we assessed whether Syf1 interacts biochemically with the TREX complex. TREX was purified using a strain expressing Hpr1-TAP, and copurification of HA-tagged Syf1 was assessed by Western blotting. Syf1 copurified with the TREX complex in the absence and presence of RNaseA, indicating that Syf1 interacts with TREX in an RNA-independent manner (Fig. 1B,  $\alpha$ HA). Nevertheless, the interaction between TREX and Syf1 might be mediated by other proteins. Taken together, Syf1 interacts with TREX genetically and biochemically.



**Figure 1.** *SYF1* interacts genetically and biochemically with the THO complex. (A) *SYF1* interacts genetically with *HPR1* and *MFT1*, two components of the THO complex. *SYF1 HPR1* and *SYF1 MFT1* shuffle strains were transformed with an empty plasmid or plasmids encoding wild-type *HPR1* or *MFT1*, respectively, and a plasmid encoding wild-type *SYF1* or one of the *SYF1* ts alleles (*syf1-1* [W40G, R319G, K367N, E419G, I544V], *syf1-2* [V69A, Y123C, Q596P, E752G], *syf1-15* [E27V, V425A, T570P, F615L], *syf1-34* [Y729F], and *syf1-37* [I5E, R129G, Y453C, N658M, V659G, I660stop; caused by a frameshift mutation]). A synthetic lethal interaction was assessed by restreaking the respective transformants on 5-fluoroorotic acid (5-FOA)-containing plates. (sl) Synthetic lethal; (ss) synthetic sick; (–) no genetic interaction. (B) Syf1 interacts with TREX independently of RNA. The TREX complex was purified using TAP-tagged Hpr1 and copurification of HA-tagged Syf1 assessed by Western blotting with  $\alpha$ HA antibodies ( $\alpha$ HA). A nontagged strain served as negative control. To assess whether the interaction between TREX and Syf1 is mediated by RNA, extracts were treated with RNaseA prior to purification. Npl3, an SR protein involved in mRNA export that interacts with TREX in an RNA-dependent manner, served as a positive control for the RNase treatment ( $\alpha$ Npl3). (IN) Input; (IP) immunoprecipitation; (Coomassie) TEV eluate of the TAP purification.

*Deletion of the C-terminal domain of Syf1 causes sensitivity to 6-azauracil (6-AU) and synthetic lethality with  $\Delta hpr1$  and  $\Delta mft1$*

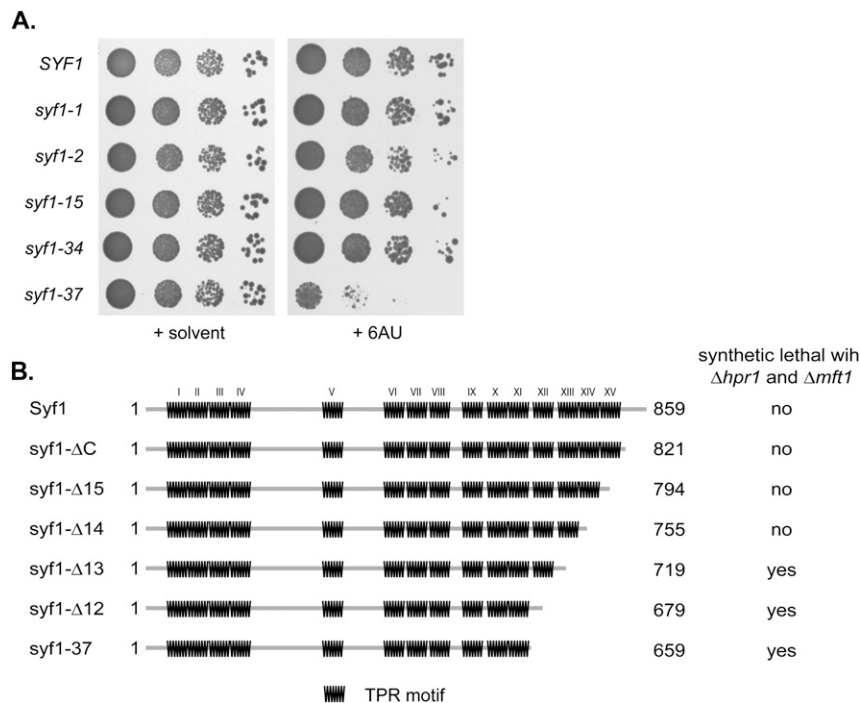
Syf1 and the Prp19 complex are known to function in splicing (Tarn et al. 1994; Ben-Yehuda et al. 2000; Will and Lührmann 2006). However, the function of XAB2, the human homolog of Syf1, in transcription and TCR (Nakatsu et al. 2000; Kuraoka et al. 2008) as well as the genetic interaction between *SYF1* and the THO complex components shown here suggest that Syf1 may also function in transcription and/or DNA repair. To obtain further evidence for a function of Syf1 in transcription, we tested *syf1-ts* cells for sensitivity to 6-AU, a drug that impairs transcription elongation by decreasing the intracellular pools of the nucleotides GTP and UTP. Interestingly, of the five *ts* alleles, only *syf1-37*, the allele that is synthetic lethal with  $\Delta hpr1$  and  $\Delta mft1$  (Fig. 1A), confers 6-AU sensitivity (Fig. 2A). This further indicates that Syf1 might be involved in transcription elongation, and that the genetic interaction between *SYF1* and THO is caused by overlapping functions in transcription elongation.

Strikingly, of the five *syf1 ts* alleles, only the *syf1-37* allele carries a C-terminal truncation (Fig. 2B, *syf1-37*, caused by a frameshift mutation, see legend for Fig. 1A). Thus, we assessed whether this C-terminal truncation causes the genetic interaction with *HPR1* and *MFT1*. Syf1 consists mainly of 15 tetratricopeptide repeat (TPR) motifs (Fig. 2B, Syf1). TPR motifs consist of 34 amino acids with the highly degenerate consensus of W-LG-Y-A-F-A-P (Lamb et al. 1995). Despite their sequence diversity, TPR motifs have a conserved three-dimensional structure of a helix–turn–helix motif with adjacent TPR motifs

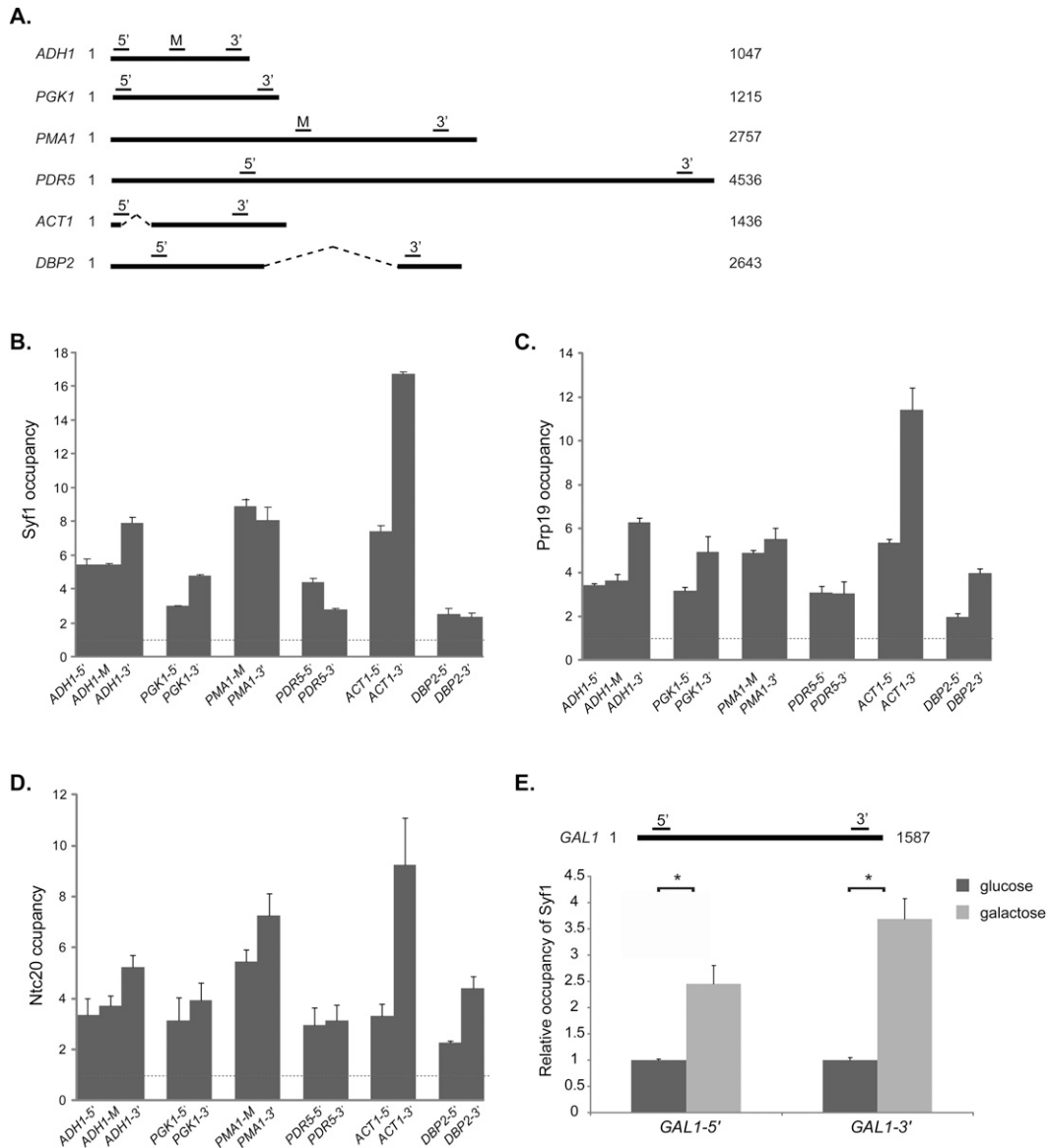
packed into anti-parallel  $\alpha$  helices (Das et al. 1998). Proteins containing TPR motifs typically act as scaffolds for the assembly of multiprotein complexes (Blatch and Lassle 1999). In order to test whether and how large a deletion of the C terminus of Syf1 causes the genetic interaction with THO, we progressively truncated Syf1 from the C terminus and tested for synthetic lethality of the resulting *syf1* alleles with  $\Delta hpr1$  and  $\Delta mft1$ . Deletion of the very C-terminal part of Syf1 (*syf1- $\Delta$ C*) and the last (15th) and last two (14th and 15th) TPR motifs (*syf1- $\Delta$ 15* and *syf1- $\Delta$ 14*, respectively) did not lead to synthetic lethality with  $\Delta hpr1$  and  $\Delta mft1$  (Fig. 2B). However, additional deletion of the 13th TPR motif (*syf1- $\Delta$ 13*) caused synthetic lethality in combination with  $\Delta hpr1$  or  $\Delta mft1$  (Fig. 2B). Thus, the deletion of the C-terminal part of Syf1 is responsible for the genetic interaction with THO components.

*Syf1 functions in transcription*

The function of XAB2 in transcription, the genetic interaction of *SYF1* with THO, and the 6-AU sensitivity of the *syf1-37* mutant indicate a function of Syf1 in transcription. If this is the case, Syf1 should be present at actively transcribed genes. Thus, we assessed recruitment of Syf1 to four intronless genes (*ADH1*, *PGK1*, *PMA1*, and *PDR5*) and two intron-containing genes (*ACT1* and *DBP2*) by chromatin immunoprecipitation (ChIP) experiments (Fig. 3A). Occupancy of these six genes by Syf1 was calculated as the enrichment of Syf1 at the respective gene relative to a nontranscribed region (NTR), which served as a negative control (NTR1, 174131–174200 on chr. V) (Rother et al. 2010). Syf1 is specifically recruited to all six of these genes (Fig. 3B), but not to two other NTRs



**Figure 2.** Truncation of the Syf1 C terminus leads to 6-AU sensitivity and synthetic lethality with  $\Delta hpr1$  and  $\Delta mft1$ . (A) The *ts* mutant *syf1-37* is sensitive to 6-AU. Ten-fold serial dilutions of yeast cells expressing wild-type *SYF1* or one of five different *ts* alleles of *SYF1* and containing pRS316 were spotted onto SDC(-ura) plates containing solvent or 50  $\mu$ g/mL 6-AU and incubated for 2 d at 30°C. (B) The C terminus of Syf1 is important for the genetic interaction of *SYF1* with *HPR1* and *MFT1*. Schematic diagram showing the domain structure of Syf1, with each TPR motif indicated by a zigzag. Deletion of the three most C-terminal TPR motifs (*syf1- $\Delta$ 13*) leads to synthetic lethality with  $\Delta hpr1$  and  $\Delta mft1$ .



**Figure 3.** The Prp19 complex is recruited to transcribed genes. (A) Schematic diagram of the *ADH1*, *PGK1*, *PMA1*, *PDR5*, *ACT1*, and *DBP2* genes. The ORFs are represented by a solid line and the introns are indicated by a hatched line. Bars above the genes show the positions of the primer pairs used for the ChIP analysis. (B) Syf1 is recruited to transcribed genes. The occupancy of Syf1 at the intronless *ADH1*, *PGK1*, *PDR5*, and *PMA1* genes and the intron-containing *ACT1* and *DBP2* genes was calculated as the enrichment of Syf1 at the respective gene relative to its presence at an NTR that served as a negative control (NTR1, 174131–174200 on chr. V). Correspondingly, the occupancy at an NTR is “1” as indicated by a dashed line. (C,D) Experiment as in B for Prp19 and Ntc20, two other components of the Prp19 complex. (E) Recruitment of Syf1 to a gene is transcription-dependent. Occupancy of Syf1 at the *GAL1* gene under repressive condition (cells grown in glucose-containing medium; dark-gray bars), which was set to 1, and induced conditions (cells grown in galactose-containing medium; light-gray bars). Primer pairs amplify the 5' and 3' region of *GAL1*, as indicated in the top panel.

(NTR2, NTR3) (Supplemental Fig. 2). Thus, even though Syf1 is a splicing factor, it is recruited to intronless genes, indicating that it might have a general function in transcription. In order to test whether Syf1 alone or the whole Prp19 complex is recruited to genes during transcription, we assessed the occupancy of two further Prp19 complex components, Prp19 and Ntc20, at these six genes. Prp19 and Ntc20 were also present at these genes (Fig. 3C,D),

indicating that the whole Prp19 complex is most likely recruited to transcribed genes.

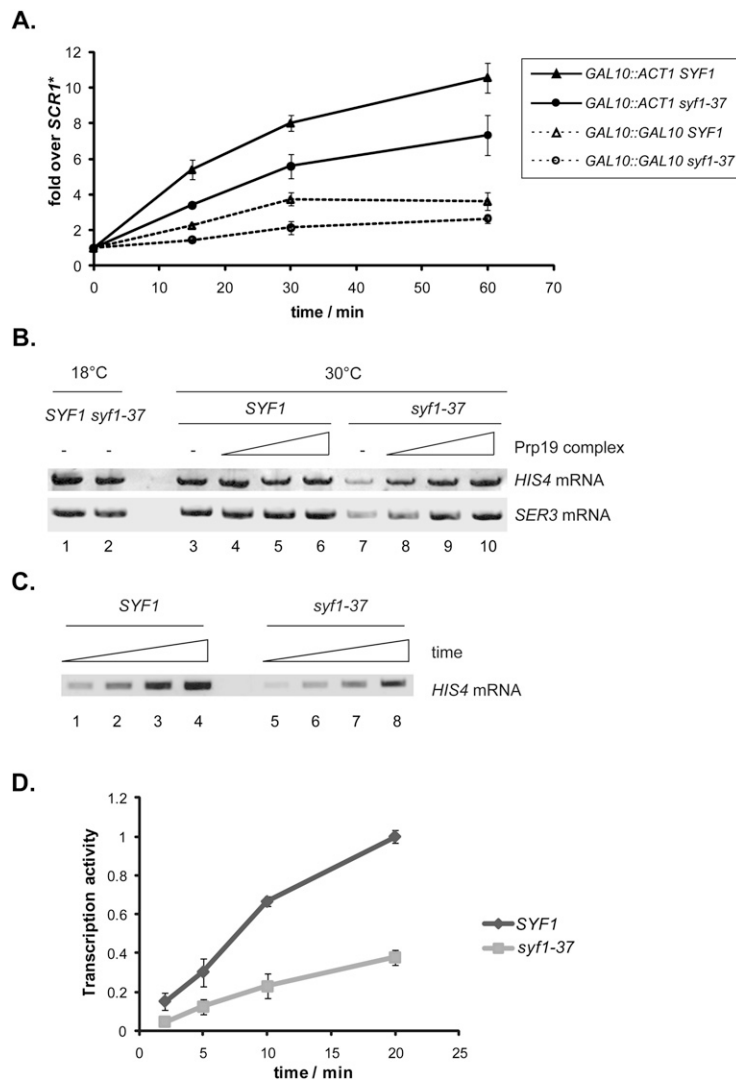
To assess whether recruitment of Syf1 to genes depends on active transcription, we assessed the occupancy of Syf1 at the regulatable *GAL1* gene (Fig. 3E). The *GAL1* gene is repressed when cells are grown in glucose-containing medium and induced when cells are grown in galactose-containing medium. Syf1 is not recruited to the

*GAL1* gene under repressive conditions (Fig. 3E, glucose, dark-gray bars) relative to a nontranscribed control region (NTR1, 174131–174200 on chr. V). In contrast, Syf1 is present at the *GAL1* gene when transcription of *GAL1* is induced (Fig. 3E, galactose, light-gray bars), showing that recruitment of Syf1 to an intronless gene is transcription-dependent.

To obtain more direct evidence that the Prp19 complex indeed functions in transcription, we tested whether mutation of *SYF1* affects transcription in vivo. Among the *SYF1* truncation mutants, *syf1-Δ13* is the longest one that is synthetic lethal with *Δhpr1* and *Δmft1* (Fig. 2B). However, since *syf1-Δ13* grows more slowly than the *syf1-37* ts mutant (Supplemental Fig. 3A), we chose the *syf1-37* allele for further experiments in order to avoid unspecific effects due to slow growth. To ensure that the activity of the *syf1-37* allele does not reflect residual activity of wild-type Syf1 produced by frameshifting during translation of the *syf1-37* allele, we tested the levels of truncated and full-length Syf1 in *syf1-37* and *SYF1* cells. Syf1-37 and Syf1 are produced at approxi-

mately the same levels, and no expression of full-length Syf1 in the *syf1-37* cells could be detected (Supplemental Fig. 3B). Thus, the phenotypes observed for the *syf1-37* allele (see below) are most likely due to truncation of the Syf1 C terminus.

Two reporter constructs were used to assess mRNA synthesis in vivo—*ACT1* as an intron-containing gene and *GAL10* as an intronless gene—both of which were driven by the galactose-inducible *GAL10* promoter. *SYF1* and *syf1-37* cells were grown in raffinose-containing medium at 30°C (permissive temperature) and expression of either reporter gene induced with galactose. RNA was isolated 0, 15, 30, and 60 min after induction, and the amount of synthesized *ACT1* and *GAL10* mRNA was quantified. The levels of *ACT1* and *GAL10* mRNA were decreased in the *syf1-37* mutant (Fig. 4A). Since this defect is observed at the permissive temperature, it is not due to a complete functional loss of the *syf1-37* ts allele. Thus, Syf1 function is needed to produce normal levels of intron-containing as well as intronless mRNAs in vivo.



**Figure 4.** Syf1 function is needed for efficient transcription. (A) Mutation of *SYF1* leads to lower mRNA levels in vivo. *GAL10::ACT1* and *GAL10::GAL10* were used as reporters for an intron-containing and an intronless gene, respectively. The level of *ACT1* (solid line) and *GAL10* (dotted line) mRNA of cells grown at the permissive temperature (30°C) was assessed by primer extension 0, 15, 30, and 60 min after induction with galactose and normalized to the amount of *SCR1* RNA, an RNAPIII transcript. (B) Mutation of *SYF1* leads to impaired transcription in vitro. The transcription activity of nuclear extracts from *SYF1* and *syf1-37* cells was assessed at 18°C (permissive temperature; lanes 1,2) and 30°C (nonpermissive temperature; lanes 3,7) by an in vitro transcription assay using one of two templates containing a *HIS4* or *SER3* promoter, respectively. (Lanes 7–10) Add-back of 50, 125, or 250 ng of Prp19 complex purified from *S. cerevisiae* rescues the impaired transcription activity of the *syf1-37* extract at the nonpermissive temperature. (C) The transcription activity of a *syf1-37* nuclear extract is lower after 2, 5, 10, and 20 min (lanes 5–8) compared with the activity of a *SYF1* extract (lanes 1–4) at the nonpermissive temperature. (D) Quantification of the experiment shown in C.

Since Syf1 functions in splicing (Tarn et al. 1994; Ben-Yehuda et al. 2000; Will and Lührmann 2006), we tested whether splicing is affected in the *syf1-37* strain under the same conditions as used for the in vivo transcription assay. Expression of the *GAL10::ACT1* reporter was induced for 1 h, and the amount of spliced and unspliced *ACT1* mRNA was assessed. As expected for a ts mutant of a protein involved in splicing, nonspliced transcripts accumulated in the *syf1-37* strain at the nonpermissive temperature (Supplemental Fig. 3C). Importantly, though, the *syf1-37* mutant did not display a defect in splicing of the *ACT1* mRNA at the permissive temperature (Supplemental Fig. 3C). Thus, the *syf1-37* mutation does not cause a severe splicing defect at the permissive temperature, making it unlikely that the decrease in *ACT1* mRNA levels in the *syf1-37* strain (Fig. 4A) is a secondary effect of defective splicing.

In order to assess whether the lower *ACT1* and *GAL10* mRNA levels in *syf1-37* cells (Fig. 4A) are caused by impaired transcription in this mutant, we performed in vitro transcription assays. We used a plasmid-based in vitro transcription assay with a *HIS4* or *SER3* promoter (Koschubs et al. 2009) and prepared nuclear extracts from *SYF1* and *syf1-37* cells. At the permissive temperature (18°C), the *syf1-37* nuclear extract had a slightly lower transcriptional activity than the wild-type extract, which could be caused by the *SYF1* mutation or simply by variations in the extract preparation procedure (Fig. 4B, lanes 1,2). Importantly, though, when the temperature during the transcription reaction was raised (nonpermissive temperature, 30°C) the activity of the nuclear extract prepared from the *syf1-37* ts cells decreased significantly relative to the wild-type extract (Fig. 4B, lanes 3,7). This decreased transcriptional activity of the *syf1-37* extract at the nonpermissive temperature was observed after different incubation times (Fig. 4 C,D). In order to assure that this decrease in transcriptional activity of the *syf1-37* extract is due to impaired Syf1 function, we performed add-back experiments with Prp19 complex purified from *S. cerevisiae*. Addition of the Prp19 complex rescued transcriptional activity of the *syf1-37* extract (Fig. 4B, lanes 7–10) showing that the decreased transcriptional activity of the *syf1-37* extract is caused by impaired Syf1 function. Importantly, RNAPII was not associated with the Prp19 complex purified under the conditions used for this experiment (Supplemental Fig. 4), and thus did not cause the stimulatory effect. In addition, since addition of the Prp19 complex did not increase the activity of the wild-type extract (Fig. 4B, lanes 3–6), rescue of the *syf1-37* extract is most likely also not caused by addition of other transcription factors copurifying with the Prp19 complex. Thus, Syf1 is required for activated transcription in vitro. Taken together, Syf1 functions in transcription independently of its function in splicing.

#### *Syf1 is necessary for RNAPII processivity*

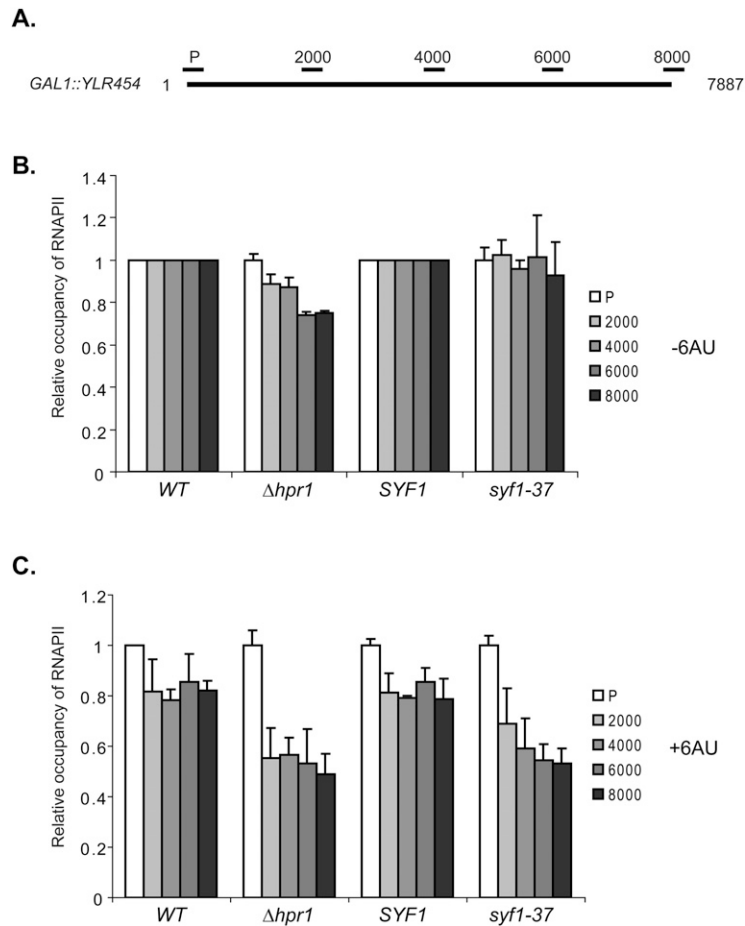
In principle, a decrease in transcription elongation can be caused by a decrease in either the rate of transcription (i.e., the speed of transcribing RNAPII) or the processivity

of RNAPII (i.e., the ability of elongating RNAPII to travel the entire length of the gene). Mason and Struhl (2005) developed an assay to measure the rate and processivity of RNAPII in vivo. None of an array of transcription elongation mutants affected the rate of RNAPII in vivo (Mason and Struhl 2005). For RNAPII processivity, the well-known transcription elongation factors fall into three categories: factors the deletion of which (1) causes a decrease in processivity of RNAPII, (2) causes a decrease in processivity only in the presence of 6-AU, and (3) does not affect processivity at all. Interestingly, deletion of THO complex components reduces RNAPII processivity (Mason and Struhl 2005). Since Syf1 interacts genetically with THO, we tested whether mutation of *SYF1* also affects RNAPII processivity. Processivity was assessed by measuring the level of RNAPII at five positions along the 8-kb-long *YLR454* gene under control of the *GAL1* promoter in cells grown in galactose-containing medium at the permissive temperature (30°C) by ChIP (Fig. 5A; Mason and Struhl 2005), and the values for the wild-type strain were normalized to 1.0 at each position.

As shown previously, deletion of *HPR1* caused a decrease in RNAPII processivity (Fig. 5B,  $\Delta hpr1$ ; Mason and Struhl 2005), and addition of 6-AU exacerbated the processivity defect of  $\Delta hpr1$  cells (Fig. 5C,  $\Delta hpr1$ ). RNAPII processivity was not affected by the *syf1-37* mutation in the absence of 6-AU (Fig. 5B, *syf1-37*). Importantly, though, in the presence of 6-AU, processivity of RNAPII was reduced in *syf1-37* cells at the permissive temperature (30°C) (Fig. 5C, *syf1-37*). Thus, as observed for other transcription elongation factors (Mason and Struhl 2005), the *syf1-37* allele affects RNAPII processivity in the presence of 6-AU (see also the Discussion).

#### *The C terminus of Syf1 is required for recruitment of the Prp19 complex to transcribed genes and the interaction of the Prp19 complex with RNAPII*

The *syf1-37* allele lacks the four most C-terminal TPR motifs of Syf1, and TPR motifs are known to function as scaffold domains for the interaction with other proteins (Blatch and Lassle 1999). Thus, an explanation for the reduced transcription in *syf1-37* cells could be that Syf1 and the Prp19 complex are not recruited to the transcription machinery in this mutant. In order to test this, we assessed recruitment of *syf1-37* to genes (Fig. 3A, *ADH1*, *PMA1*, and *DPB2*). Whereas the occupancy of RNAPII did not change in the *syf1-37* strain compared with wild type (Supplemental Fig. 5), the occupancy of Syf1 (relative to RNAPII) decreased in the *syf1-37* strain to ~50% compared with wild-type cells (Fig. 6A). Importantly, this effect is observed at the permissive temperature (30°C); i.e., under conditions where function of the *syf1-37* ts mutant is not impaired due to elevated temperature. Thus, the C terminus of Syf1 is required to recruit Syf1 efficiently to the transcribed gene. Since Syf1 is a component of the Prp19 complex, we wondered whether recruitment of not only Syf1 but the whole complex is impaired in the *syf1-37* strain. Thus, we assessed the occupancy of two



**Figure 5.** RNAPII processivity is decreased in  $\Delta hpr1$  and *syf1-37* cells. (A) Schematic diagram of the *GAL1::YLR454* reporter gene used to analyze the processivity of RNAPII in wild-type,  $\Delta hpr1$ , *SYF1*, and *syf1-37* cells grown at 30°C (permissive temperature). Positions of primer pairs used for the ChIP analysis are indicated above the gene. (B) Relative RNAPII occupancy within the *YLR454* gene in wild-type,  $\Delta hpr1$ , *SYF1*, and *syf1-37* cells grown in galactose-containing medium. The occupancy in wild-type cells at each position was set to 1. (C) Relative RNAPII occupancy as in B in the presence of 50  $\mu\text{g}/\text{mL}$  6-AU.

further Prp19 complex components (Prp19 and Ntc20) in *SYF1* and *syf1-37* cells. Indeed, the occupancy of Prp19 and Ntc20 also decreased to  $\sim 50\%$  in *syf1-37* compared with wild-type cells (Fig. 6B,C). This indicates that Syf1 functions in transcription elongation most likely as part of the Prp19 complex.

The *syf1-37* mutation results in lower levels of the Prp19 complex at the site of transcription. In order to find out whether this is due to a compromised interaction of *syf1-37* with TREX or RNAPII we assessed their respective interaction by purifying TAP-tagged versions of Syf1 and *syf1-37*. Both Syf1 and *syf1-37* copurified equal levels of Prp19 complex components and Hpr1 (Fig. 6D, Coomassie and Hpr1-HA), suggesting that *syf1-37* interacts with the Prp19 complex and TREX. However, copurification of Rpb1, the largest subunit of RNAPII, with *syf1-37* was greatly reduced (Fig. 6D, Rpb1), indicating that the direct or indirect interaction of the Prp19 complex with RNAPII is impaired by truncation of the C terminus of Syf1. Taken together, the C terminus of Syf1 is necessary for the interaction between the Prp19 complex and RNAPII and thus for recruitment of the Prp19 complex to transcribed genes. In addition, these findings indicate that the Prp19 complex functions in transcription elongation, since its occupancy on genes is reduced in the *syf1-37* mutant that displays the transcription defects.

#### The Prp19 complex is required for TREX occupancy at genes

The function of Syf1 in transcription and its genetic interaction with THO suggest that the Prp19 and TREX complexes might have a related function in transcription. Thus, we tested whether the occupancy of Hpr1, one of the THO components, depends on Syf1 and vice versa. The occupancy of Syf1 relative to RNAPII was not affected by deletion of *HPR1* (Supplemental Fig. 6). In contrast, Hpr1 occupancy relative to RNAPII decreased in *syf1-37* cells to  $\sim 50\%$  compared with wild-type cells (Fig. 7A), reflecting the reduced recruitment of the Prp19 complex in these cells (Fig. 6A–C). Again, this effect is observed at the permissive temperature (30°C) and thus is not caused by a general loss of function of the *syf1-37* allele, but by deletion of its C terminus. Loss of THO is especially observed at the 3' end of genes (also see the Discussion). Importantly, this decrease of THO recruitment in *syf1-37* cells is not caused by a general, and thus nonspecific, loss of transcription factors from the transcription machinery, since recruitment of two other transcription elongation factors (Paf1 [a component of the PAF complex] and Spt5) was not affected by mutation of *SYF1* (Supplemental Fig. 7). Therefore, the Prp19 complex is required for THO occupancy at transcribed genes.

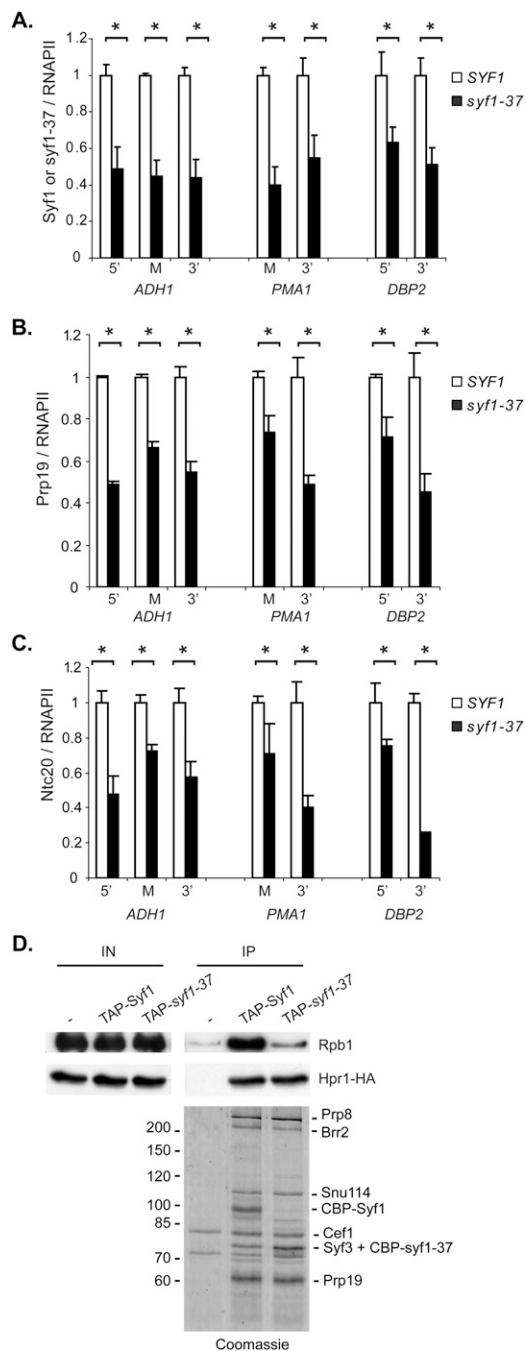
The THO subcomplex is needed to recruit the other TREX components—such as the intranuclear mRNA export factors Sub2 and Yra1—to the gene. In order to test whether recruitment of the entire TREX complex is affected by the *syf1-37* mutation and the resulting loss of THO from genes, we assessed recruitment of Sub2 and Yra1 in *syf1-37* cells. Indeed, like Hpr1, the occupancy of Sub2 and Yra1 was decreased in *syf1-37* cells (Fig. 7B,C). However, the observed decrease in occupancy of Sub2 and Yra1 at genes is lower than the decrease for Hpr1. This is expected, since Sub2 and Yra1 are still partially recruited to genes even in the complete absence of Hpr1

(Zenklusen et al. 2002). In summary, the Prp19 complex functions in transcription by recruiting TREX to transcribing RNAPII.

## Discussion

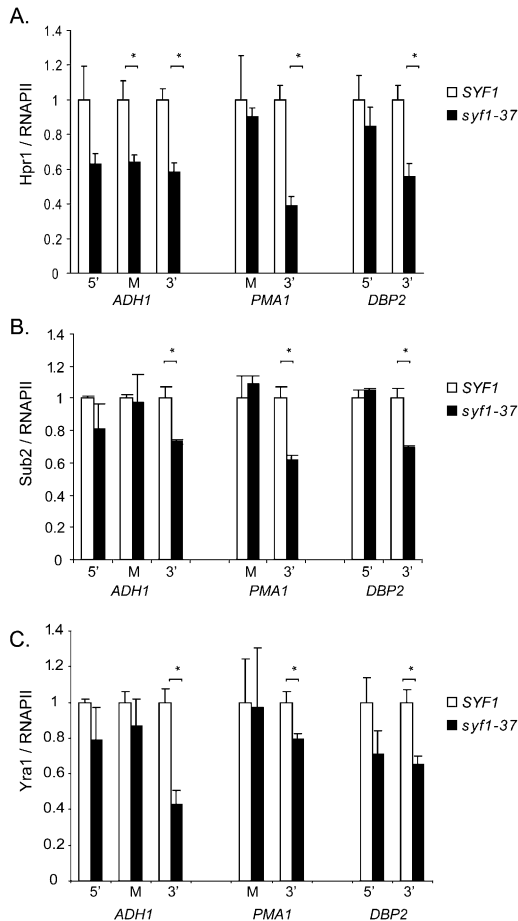
The Prp19 complex has a well-established and conserved function in splicing (Will and Lührmann 2006 and references therein). It forms a heteromeric complex that is also known as the NTC (nineteen complex) in *S. cerevisiae* (Tarn et al. 1994) and the hPrp19/CDC5L complex in humans (Ajuh et al. 2000; Makarova et al. 2004). The Prp19 complex is one of numerous non-snRNP proteins that play an essential role during splicing (Will and Lührmann 2006), and is required for the activation of the spliceosome (Ohi and Gould 2002; Chan et al. 2003).

Here, we show that, in yeast, the Prp19 complex has a second function in gene expression; namely, in transcription elongation. The Prp19 complex is recruited to intron-containing as well as intronless genes in a transcription-dependent manner (Fig. 3). In addition, mutation of *SYF1*, one of the conserved components of the Prp19 complex, causes a reduced presence of the Prp19 complex at transcribed genes and a concomitant decrease in transcriptional activity (Figs. 4A,B, 6A–D). The *syf1-37* mutation decreases RNAPII processivity, suggesting that the Prp19 complex most likely functions in transcription elongation (Fig. 5). This novel function of the Prp19 complex is most likely independent of its function in splicing, since splicing is not affected in *syf1-37* cells at the permissive temperature (Supplemental Fig. 3C),



**Figure 6.** The C terminus of Syf1 is needed for the interaction of the Prp19 complex with the transcription machinery. (A) Deletion of the C terminus of Syf1 leads to loss of Syf1 from the transcribed gene. ChIP of TAP-Syf1 or TAP-Syf1-37 and Rpb3-TAP (RNAPII) in *SYF1* and *syf1-37* cells grown at permissive temperature (30°C) were quantified by real-time PCR using the primer pairs shown in Figure 3A. The ratio of Syf1 over RNAPII occupancy over an NTR (NTR1) was calculated, and the values for *SYF1* were set to 1. (B,C) The C terminus of Syf1 is required to recruit the Prp19 complex to the transcribed gene. Experiment as in A, but the occupancy of Prp19 (B) and Ntc20 (C) relative to RNAPII on the indicated genes was assessed in *SYF1* and *syf1-37* cells grown at permissive temperature (30°C). (D) The C terminus of Syf1 mediates the interaction between the Prp19 complex and RNAPII. The interaction of *syf1-37* with RNAPII is strongly reduced, while its interaction with the Prp19 and TREX complex is not affected. TAP-Syf1 and TAP-syf1-37 were purified from cells expressing Hpr1-HA and copurification of RNAPII and Hpr1 assessed by Western blotting using the 8WG16 antibody, which recognizes Rpb1, the largest subunit of RNAPII, or an  $\alpha$ HA antibody (Hpr1-HA), respectively. Copurifying proteins were identified by mass spectrometry and are indicated to the right: Prp8, Brr2, and Snu114 are components of the U5 snRNP, and Cef1, Syf3, and Prp19 are components of the Prp19 complex. The other components of the Prp19 complex (Isy1, Cwc2, Syf2, Snt309, and Ntc20) have a molecular weight between 16 and 38 kD, and thus are not present on this gel. A nontagged strain served as negative control. (IN) Input; (IP) immunoprecipitation; (Coomassie) EGTA eluates of the TAP purifications.





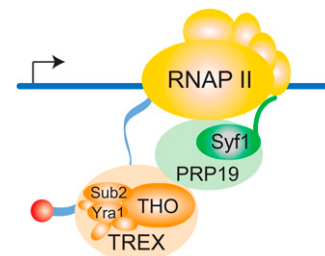
**Figure 7.** Syf1 ensures TREX occupancy at genes. (A) Mutation of *SYF1* leads to loss of the THO complex from the transcribed gene. ChIP of Hpr1-TAP (THO) in *SYF1* and *syf1-37* cells grown at permissive temperature (30°C) were quantified by real-time PCR using the primer pairs shown in Figure 3A. The ratio of Hpr1 relative to RNAPII occupancy over an NTR (NTR1) was calculated, and the values for *SYF1* were set to 1. (B,C) Syf1 is required to recruit the TREX complex to the gene. Experiment as in A to determine the occupancy of the TREX components Sub2 (B) and Yra1 (C) relative to RNAPII in *SYF1* and *syf1-37* cells.

where all of the transcription defects are evident (Figs. 2A, 4–7). Furthermore, the occupancy of the TREX complex, which functions in transcription elongation and mRNA export, is specifically reduced in *syf1-37* cells (Fig. 7). Thus, we identify for the first time a factor required for full occupancy of TREX on transcribed genes. Interestingly, for some genes such as *PMA1* and *DBP2*, the Prp19 complex is not required for TREX occupancy at the 5' end (Fig. 7). Thus, there might be another factor responsible to recruit TREX to the transcription machinery at the 5' end of genes. Importantly, though, corresponding to the decrease of the Prp19 complex, the occupancy of the TREX components Hpr1, Sub2, and Yra1 is reduced to ~50% in the *syf1-37* mutant. Thus, Prp19 complex function is essential for the presence of TREX on the transcribed gene. Based on this data, we propose the

model that the Prp19 complex functions in transcription elongation by ensuring occupancy of the TREX complex at the transcribed gene (Fig. 8).

The transcription defect observed in the *syf1* mutant is most likely due to the reduced presence of the THO complex at genes in this mutant. In contrast to THO, the occupancy of two other transcription elongation factors (Paf1 and Spt5) is not affected by mutation of *SYF1* (Supplemental Fig. 7). It is interesting to note that the PAF complex leaves the transcribing polymerase downstream from the polyadenylation site, as does TREX (Kim et al. 2004), but obviously depends on factors other than TREX for recruitment. THO is important for efficient transcription elongation by ensuring the stability of the RNAPII elongation machinery on the chromatin template (Mason and Struhl 2005). Consistent with a requirement of the THO complex for RNAPII processivity, *syf1-37* cells show impaired processivity of RNAPII in the presence of 6-AU (Fig. 5). Thus, the reduced amount of THO/TREX in the *SYF1* mutant seems to be sufficient for full RNAPII processivity under optimal conditions, but causes reduced RNAPII processivity when transcription is impaired in the presence of 6-AU. Likewise, even though deletion mutants of THO components show a mild mRNA export defect, we did not observe an mRNA export defect in the *syf1-37* mutant (data not shown). Thus, the 50% of TREX present at genes in *syf1-37* cells are sufficient for mRNA export.

Interestingly, Prp19 and hPrp19 have E3 ubiquitin ligase activity in vitro (Hatakeyama et al. 2001; Ohi et al. 2003). Recently, it has been shown that this catalytic activity of Prp19 is important for structural rearrangements during the splicing reaction (Song et al. 2010). Prp19 ubiquitylates Prp3, a component of the U4 snRNP (Nottrott et al. 2002), forming nonproteolytic K63-linked chains. This modification of Prp3 increases the affinity of Prp3 for the U5 component Prp8 necessary to stabilize the U4/U6.U5.snRNP. The interaction of the Prp19 complex with the U5 snRNP does not seem to be affected in the *syf1-37* mutant (Fig. 6D); i.e., when transcription is impaired. Thus, the E3 ligase activity of



**Figure 8.** The Prp19 complex functions in transcription. The Prp19 complex functions in transcription elongation by stabilizing the recruitment of TREX to transcribed genes. TREX components then bind—together with other proteins—to the nascent mRNA to form an mRNP. Thus, the Prp19 complex links transcription to mRNP formation. The Prp19 complex is recruited to the transcription machinery by the C terminus of its component, Syf1. Illustration by Susanne Röther.

the Prp19 complex is most likely not affected in this *SYF1* mutant. Nevertheless, it is tempting to speculate that the E3 ligase activity of the Prp19 complex might be important for its function in transcription, in addition to its structural role to recruit the TREX complex. Notably, Hpr1 is polyubiquitylated and degraded, but ubiquitylated Hpr1 interacts with the UBA domain of the mRNA export receptor Mex67, an interaction that recruits Mex67 to transcribed genes and transiently protects Hpr1 from degradation (Gwizdek et al. 2006). However, when we purified Hpr1 from wild-type and *syf1-37* cells, Hpr1 ubiquitylation was not affected (data not shown), consistent with the notion that the Prp19 E3 ligase activity is not affected in *syf1-37* cells (see above). Thus, it remains an open question as to whether the E3 ligase activity of Prp19 is important for efficient transcription and what the target protein(s) might be.

Deletion of the last three TPR motifs (XIII–XV) of Syf1 significantly weakens the direct or indirect interaction between the Prp19 complex and RNAPII, and leads to a reduced Prp19 occupancy at transcribed genes in vivo (Fig. 6). Thus, the C-terminal domain of Syf1 is an important interface for the interaction between the Prp19 complex and RNAPII. However, since ~50% of the Prp19 complex is still recruited to the active transcription machinery in this mutant, another domain of the Prp19 complex for interaction with RNAPII must exist. Within the C terminus of Syf1, the 13th of the 15 TPRs of Syf1 seems to be specifically important for the interaction with RNAPII. Whereas deletion of the two most C-terminal TPRs (XV + XIV, *syf1-Δ14*) does not cause synthetic lethality with  $\Delta hpr1$ , additional deletion of the 13th TPR (*syf1-Δ13*) leads to synthetic lethality with  $\Delta hpr1$  (Fig. 2B). Fusion of six different combinations of two “other” TPR motifs (I + II, III + IV, VI + VII, VII + VIII, IX + X, or XI + XII) of Syf1 to the *syf1-Δ13* deletion mutant—with the same spacing between TPR 12 and the TRP motif C-terminal of it—are still synthetic lethal with  $\Delta hpr1$  (data not shown). Thus, the mere addition of TPR motifs to the 3' end of the truncated Syf1 does not rescue functionality of Syf1 in transcription, indicating that different TPR motifs might have specific functions. Similarly, deletion of the N-terminal five TPRs of Syf1 (I–V, *syf1-aa408–859*) does not lead to a synthetic lethal relationship with  $\Delta hpr1$  or  $\Delta mft1$  (data not shown), indicating that these TPRs are not important for the interaction of the Prp19 complex with the transcription machinery and the function of the Prp19 complex in transcription. Taken together, the 13th TPR of Syf1 might be important for the interaction of the Prp19 complex with RNAPII.

Interestingly, in higher cells, TREX is recruited to the mRNA during the splicing reaction (Masuda et al. 2005; Wahl et al. 2009), and, in yeast (where TREX is recruited in a transcription-dependent manner), a splicing complex recruits TREX to the gene. The spliceosome is a highly dynamic molecular machine that is assembled in a step-wise manner onto the mRNA to be spliced, leading to the formation of assembly intermediates called complex E, A, B, B\*, and C (Wahl et al. 2009 and references therein).

Briefly, binding of the U1 snRNP to the 5' splice site defines the E complex. Recognition of the pre-mRNA's branch-point sequence by the U2 snRNP leads to the A complex, and subsequent binding of U4, U5, and U6 as a preassembled tri-snRNP results in the B complex. The recruitment of additional factors as well as extensive structural rearrangements that destabilize the association of U1 and U4 snRNPs with the catalytic core lead to the catalytically activated B\* complex. The first transesterification reaction generates the C complex, which catalyzes the second step of splicing. Interestingly, the hPrp19 and hPrp19-related complexes containing XAB2/hSyf1 are present within the B complex, whereas TREX components are only present later, when the C complex has been formed (Wahl et al. 2009 and references therein). Since the Prp19 complex is part of the spliceosome before TREX components join, it might recruit TREX to the mRNA during the splicing reaction. Thus, the role of the Prp19 complex in recruiting TREX to the mRNA may be conserved in higher eukaryotes.

In summary, we identified the Prp19 splicing complex as a novel transcription elongation factor. In addition, we establish the Prp19 complex as the first factor essential for TREX complex occupancy at transcribed genes. In this respect, the Prp19 complex links transcription to mRNP formation.

## Materials and methods

### Yeast strains and plasmids

Yeast strains and plasmids are listed in Supplemental Tables S1 and S2, respectively.

### ChIP experiments

ChIP experiments were performed essentially as described previously (Rother et al. 2010) with some modifications. Briefly, cross-linked cells were lysed with an equal volume of glass beads by vortexing six times for 3 min with 3-min breaks, on ice. Chromatin lysate was used for immunoprecipitation with 15  $\mu$ L of IgG-coupled or anti-Yra1-coupled (Yra1 ChIP) Dynabeads for 3 h at 20°C. After washing and elution, the immunoprecipitation eluates as well as input samples were treated with proteinase K overnight at 65°C. DNA was purified by phenol/chloroform extraction. Quantitative PCR with input and immunoprecipitation samples was done on an Applied Biosystems StepOnePlus cycler, using Applied Biosystems' Power SYBR Green PCR Master Mix. As negative control, primers for three NTRs amplifying 174131–174200 on chr. V (NTR1) (Rother et al. 2010), 116689–116758 on chr. X (NTR2), and 131523–131582 on chr. V (NTR3) were used. PCR efficiencies (E) were determined with standard curves. The occupancy of each factor at the respective gene was calculated as its enrichment at the respective gene relative to NTR1 according to  $[E^{(C_{TIP}-C_{TInput})}]_{NTR} / [E^{(C_{TIP}-C_{TInput})}]_{gene}$ .

### Protein purification

Affinity purification of TAP-tagged proteins was performed as described previously (Puig et al. 2001; Strasser et al. 2002). Briefly, cells were lysed with glass beads in 2 vol of TAP buffer (50 mM Tris-HCl at pH 7.5, 100 mM NaCl, 1.5 mM MgCl<sub>2</sub>, 0.15% NP40, 1 mM DTT, 1.3  $\mu$ g/mL pepstatin A, 0.28  $\mu$ g/mL

leupeptin, 170  $\mu\text{g}/\text{mL}$  PMSF, 330  $\mu\text{g}/\text{mL}$  benzamidine). Crude extracts were centrifuged and supernatants were incubated with IgG-coupled sepharose beads. Subsequently, the IgG beads were washed with the same buffer and eluted with TEV protease. The TEV eluates were then incubated with calmodulin beads pre-washed with TAP buffer containing 2 mM  $\text{CaCl}_2$ , and calmodulin beads washed and eluted with calmodulin elution buffer (10 mM Tris-HCl at pH 8.0, 5 mM EGTA). Copurifying proteins were analyzed by SDS-PAGE and Coomassie staining, and were identified by mass spectrometry or Western blotting using antibodies directed against HA (Roche Applied Science), Npl3 (Siebel and Guthrie 1996), the N terminus ( $\gamma\text{N}$ -18, Santa Cruz Biotechnology), and the CTD of Rpb1 (8WG16, Covance).

#### RNA analysis

Total cellular RNA was isolated by acidic phenol extraction from cells grown in raffinose or shifted to 2% galactose for the indicated time points. The primer extension analysis was performed according to a standard method. Briefly, 1 pmol of  $^{32}\text{P}$ -labeled primer ( $4 \times 10^6$  counts per minute [cpm]/pmol) specific for *ACT1* mRNA, *GAL10* mRNA, or *SCR1* RNA (an RNAPIII transcript that served as a loading control) was mixed with 5  $\mu\text{g}$  of RNA, hybridized for 5 min at 37°C, extended by reverse transcriptase for 1 h at 42°C, and analyzed on a 6% urea/acrylamide sequencing gel. Quantification was performed with a STORM 860 and ImageQuant software (GE Healthcare). Analysis of splicing efficiency was performed by RT-PCR. Products were separated on 2% agarose gels, images were recorded digitally, and ratios between spliced and unspliced products were quantified using ImageJ (NIH).

#### In vitro transcription assays

Yeast nuclear extracts were prepared from 3 L of culture as described previously. Plasmid transcription was performed essentially as described (Ranish et al. 1999). Transcription reactions were carried out in a 25- $\mu\text{L}$  volume. The reaction mixture contained 200  $\mu\text{g}$  of nuclear extract, 150 ng of plasmid template containing the yeast *HIS4* or *SER3* promoter (sequence available on request) (Koschubs et al. 2009), 1 $\times$  transcription buffer (10 mM HEPES at pH 7.6, 50 mM potassium acetate, 0.5 mM EDTA, 2.5 mM magnesium acetate), 2.5 mM DTT, 192  $\mu\text{g}$  of phosphocreatine, 0.2  $\mu\text{g}$  of creatine phosphokinase, 10 U of Ribolock RNase inhibitor (Fermentas), 100  $\mu\text{M}$  nucleoside triphosphates (NTPs), and 200 ng of recombinant Gcn4. The reaction was incubated for 20 min at 18°C or 30°C or for 2, 5, 10, and 20 min at 30°C for the time-course experiment, and total RNA was isolated using the RNeasy MinElute Cleanup kit (Qiagen). Transcripts were analyzed by primer extension essentially as described (Ranish et al. 1999). Instead of the  $^{32}\text{P}$ -labeled lacI oligo, 0.125 pmol of a fluorescently labeled 5' Cy5-oligo was used. Quantification was performed with a Typhoon 9400 and ImageQuant software (GE Healthcare). For the add-back experiment, the Prp19 complex was purified to the TEV eluate using a *SYF1-TAP* strain by TAP as described above, except that the TAP buffer contained 500 mM NaCl and the HIS-tagged TEV protease was depleted using Ni-NTA agarose.

#### Statistical analyses

Data are means  $\pm$  SEM from  $n = 3$ . Asterisks indicate statistical significance (Student's *t*-test; [\*]  $P < 0.05$ ).

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