Swt1, a Novel Yeast Protein, Functions in Transcription*

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The conserved TREX complex couples transcription to nuclear mRNA export. Here, we report that the uncharacterized open reading frame YOR166c genetically interacts with TREX complex components and encodes a novel protein named Swt1 for “synthetically lethal with TREX.” Co-immunoprecipitation experiments show that Swt1 also interacts with the TREX complex biochemically. Consistent with a potential role in transcription as suggested by its interaction with TREX, Swt1 localizes mainly to the nucleus. Importantly, deletion of Swt1 leads to decreased transcription. Taken together, these data suggest that Swt1 functions in gene expression in conjunction with the TREX complex.

In addition to an impairment in transcription elongation, mutants of the THO complex show a transcription-dependent hyperrecombination phenotype (13, 14). This combined phenotype of transcription impairment and hyperrecombination is most likely caused by the accumulation of DNA–RNA hybrids during transcription, which is consistent with the hypothesis that THO functions in transcription elongation through cotranscriptional mRNA formation (6, 7, 15). Consistently, mutations in mRNA export factors such as SUB2, YRA1, MEX67, and MTR2 also lead to a similar transcription-impairment and hyperrecombination phenotype as mutation of THO (14).

Interestingly, the TREX complex is genetically linked to Rrp6, the nucleus-specific component of the exosome (7, 16). In addition to Rrp6, the nuclear exosome contains several 3′-5′-exoribonucleases and is involved in processing of small nuclear and nucleolar RNAs, rRNAs, and pre-rRNA spacers (17). Furthermore, it has been shown that the exosome plays a role in nuclear degradation of mRNAs of mutants with defects in 3′-end formation and/or polyadenylation (7, 16, 18). Interestingly, these mRNAs are retained at the site of transcription and Rrp6 is needed for this retention (19). Thus, it was proposed that the exoribonuclease Rrp6 functions in the quality control of mRNP formation by retention of incorrectly processed mRNAs and their degradation (Ref. 20 and references therein).

Here, we describe for the first time a function for the novel protein Swt1 in a transcription associated process. The open reading frame (ORF)3 YOR166c encoding this novel protein becomes essential in yeast strains that are compromised in TREX function and was thus named SWT1 (for synthetic lethal with TREX). In addition to this genetic relationship, Swt1 interacts biochemically with the TREX complex and RNA polymerase II. Even though Swt1 is not essential for cell viability, deletion of SWT1 leads to a decrease in transcription. Taken together, we propose that Swt1 is needed in conjunction with the TREX complex for efficient gene expression.

EXPERIMENTAL PROCEDURES

Yeast Strains and Plasmids—Strains RS453, JBa, JBa, Δtho2, Δhpr1, Δmft1, Δthp2, SUB2 shuffle, and plasmids pH74467Δ, pRS315-THO2, pUN100-HPRI, pRS315-MFT1, pRS315-THP2, pRS315-SUB2, pRS315-sub2–85, and pNOPPATA1L were described previously. Strains spt5Δ-194 and spt5Δ-242 were a gift of Dr. Grant Hartzog. The synthetic lethal (sl) screening

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3 The abbreviations used are: ORF, open reading frame; sl, synthetic lethal; FOA, 5-fluorotic acid; HA, haemagglutinin; TAP, tandem affinity purification.
strains \( \Delta hpr1 \) ade2 ade3 was generated by mating \( \Delta hpr1 \) to Jba, sporulation of diploids, and tetrad analysis. The sl screening strain \( \Delta yor166c \) ade2 ade3 was generated likewise after mating of \( \Delta yor166c \) to Jba. The strain \( YOR166c/\Delta yor166c \) (W303 background) was obtained from Euroscarf and sporulated to obtain haploid \( YOR166c \) disrupted cells. Strains \( \Delta dst1 \) and \( \Delta rtf1 \) were obtained from Euroscarf. Strains HPR1 \( \Delta yor166c \), MTF1 \( \Delta yor166c \), THP2 \( \Delta yor166c \), and SUB2 \( \Delta yor166c \) were generated by mating the respective knock-out strain containing a pRS316-based wild-type gene with \( \Delta yor166c \), sporulation of diploid cells, and selection of double disrupted strains carrying a pRS316-based wild-type copy of either HPR1, MTF1, THP2, or SUB2 after tetrad analysis. Strains \( \Delta hto2 \) YOR166c, \( \Delta dst1 \) YOR166c, \( \Delta rtf1 \) YOR166c, \( \Delta sp5\)–194 YOR166c, and \( \Delta sp5\)–242 YOR166c were obtained by mating the \( \Delta hto2 \), \( \Delta dst1 \), \( \Delta rtf1 \), \( \Delta sp5\)–194, or \( \Delta sp5\)–242 strains, respectively, with strain \( \Delta yor166c \) carrying pRS316-YOR166c, sporulation, and tetrad analysis. Strain SWT1-TAP was generated by integration of a TAP-tag C-terminal of the SWT1 gene with a TRP1 marker according to (21). The GAL1::TAP-SWT1 strain was made by integration of an N-terminal GAL1-driven TAP-tag with a TRP1 marker, and TAP-SWT1 was derived from GAL1::TAP1-SWT1 through recombination with Cre recombinase according to Puig et al. (21). To obtain the strain GAL1::TAP-SWT1 HPR1-HA, an HA-tag was integrated with the HIS3 marker C-terminal of HPR1 according to (22). Strains TAP-SWT1 HPR1 and SWT1-TAP HPR1 were obtained by mating the N- or C-terminally-tagged SWT1 strains with \( \Delta hpr1 \) carrying pRS316-HPR1.

Sl screening plasmids pHT4467\( \Delta \)HPR1 and pHT4467\( \Delta \)YOR166c were cloned by inserting the coding region of HPR1 and YOR166c, respectively, including 5' and 3' sequences into pHT4467\( \Delta \). Plasmid pUN100–267 was recovered from FOA-resistant sl \( \Delta hpr1 \) 267, transformed with a pUN100 genomic library to clone the complementing gene, and contains the YOR166c ORF. Plasmid pRS315-YOR166c was constructed by amplifying the YOR166c coding region plus about 300 bp 5' and 800 bp 3' creating XhoI and PstI sites and cloning into the same sites of pRS315. pRS314-YOR166c and pRS316-YOR166c were generated by subcloning the XhoI-PstI or XhoI-BamHI fragment of pRS315-YOR166c into the same sites of pRS314 and pRS316, respectively. pNOPPATA1L-SWT1, -SWT1-N+M, -SWT1-M, -SWT1-M+C, and SWT1-N+C were cloned by amplification of the whole ORF of SWT1 or the sequence coding for the domains (N+M, bp 1–900; M, bp 379–900; M+C, bp 379–1374; N+C, bp 1–378 fused to bp 901–1374) creating NcoI and BamHI sites and cloned into the same sites of pNOPPATA1L. Plasmids pRS315-DST1, pRS315-RTF1, and pRS315-SP5 were constructed by amplifying the respective ORF plus about 500 bp 5' and 300 bp 3' creating XhoI and BamHI sites and cloning into the same sites of pRS315. pRS313lacZ was a gift of Karin Breuning.

**Synthetic Lethal Screens and Defined Synthetic Lethal Interactions**—The sl screens with deletion alleles of \( \Delta hpr1 \) and \( \Delta yor166c \) were performed as described (10). To test sl interactions in a defined manner double deletion strains of YOR166c/SWT1 and the gene to be tested (geneX), which were covered by one of the genes on a URA3-plasmid (pRS316), were generated. Each strain was transformed with plasmid-encoded YOR166c/SWT1, geneX, an empty vector, or a plasmid encoding a mutant allele of geneX (in case of SUB2). Transformants were restreaked onto FOA-containing plates to shuffle out the URA3-plasmid. No growth indicates a synthetic lethal relationship between the tested alleles.

**Functionality of Swt1 Domains and TAP-tagged Swt1**—To test the functionality of domains of SWT1 plasmids expressing combinations of the domains (N+M, M, M+C, and N+C) under the control of the NOP1 promoter were transformed into different sl strains isolated in the sl screens, which depend on the expression of a functional SWT1 gene for growth and are covered by a SWT1, HPR1, or THO2 gene on a URA3-plasmid. Transformants were restreaked onto FOA-containing plates to shuffle out the URA3-plasmid. Growth indicates a rescue of the synthetic lethal phenotype and thus functionality of the SWT1 domain construct.

Since cell growth is dependent on a functional Swt1 in a \( \Delta hpr1 \) background, strains with a genomically N- or C-terminally TAP-tagged SWT1 were mated to a \( \Delta hpr1 \) strain carrying HPR1 on a URA3-plasmid and double mutants selected by tetrad analysis. Growth on FOA depends on functionality of the tagged SWT1 gene.

**Localization of Swt1 by Cell Fractionation**—Protein extracts from a SWT1-TAP strain in a Ficoll-containing buffer were separated into homogenate, post-nuclear supernatant, and nuclear pellet. The nuclear pellet was resuspended and separated on a sucrose density step gradient according to (23). Fractions were analyzed by SDS-gel electrophoresis and Western blotting using antibodies against protA (PAP, Sigma), HA (Roche Applied Science), Yra1 (9), Rpb1 (8WG16, Covance), nucleoporins (Mab414, Covance), Tim50 (24), and 3-phosphoglycerate kinase Pgk1 (Molecular Probes).

**Purification of TAP-Swt1**—Swt1 and associated proteins were purified by the TAP method (21). Copurifying proteins were analyzed by SDS-gel electrophoresis and Western blotting using antibodies directed against HA (Roche Applied Science), Sub2 (6), Yra1 (9), Mex67 (10), Npl3 (25), and the CTD of Rpb1 (8WG16, Covance).

**Analysis of Recombination Frequency**—Wild-type (W303), \( \Delta swt1 \), and \( \Delta hpr1 \) strains were transformed with plasmid pRS314-LY\( \Delta \)NS (26) and grown for 3 days at 30 and 37 °C, respectively. Nine independent transformants each were resuspended in \( \Delta hpr1 \) and plated on SDC(-Trp) and SDC(-Leu) plates, colonies counted after 3 days of growth, and the frequency of recombination calculated as Leu + recombinants over viable cells.

**\( \beta \)-Galactosidase Expression**—Cells were grown in synthetic medium containing 2% raffinose before either 2% galactose or 2% glucose were added. Cells were harvested after 8 h of logarithmic growth, washed twice with Z-buffer, and frozen in liquid nitrogen. Cells were lysed in Z-buffer by three freeze and thaw cycles, and the hydrolysis of \( \beta \)-nitrophenyl galactopyranoside was assayed as described (27). \( \beta \)-Galactosidase activity was calculated from six independent experiments and glucose values were subtracted from the galactose values.

**Northern Blot**—RNA was isolated from cells grown in raffinose or shifted to 2% galactose for 1 h by phenol extraction. RNA was separated by formaldehyde gel electrophoresis fol-
RESULTS AND DISCUSSION

The Uncharacterized YOR166c Interacts Genetically with TREX Components—To identify novel components that play a role in transcription elongation and/or mRNA export, we performed an sl screen with a deletion allele of HPR1, a gene encoding one of the four proteins of the THO complex. One of the sl candidates identified in this screen, sl Δhpr1 267, was complemented by a library plasmid (see Fig. 1A; +267) that contains seven full-length ORFs. Subcloning of individual genes showed that the uncharacterized gene YOR166c complements the sl candidate Δhpr1 267 (see Fig. 1A; + YOR166c). In addition, the coding region of Yor166c under control of the constitutive NOP1 promoter rescued the sl phenotype of sl Δhpr1 267 (see Fig. 1A; +NOP1::YOR166c). Thus, YOR166c interacts genetically with HPR1.

YOR166c is an uncharacterized, non-essential gene, knock-out of which leads to a temperature sensitive phenotype on minimal medium (see Fig. 5A). As a first attempt to characterize YOR166c we performed an sl screen with a deletion allele of YOR166c to identify genetic interactors of YOR166c and thus the context of its function. All seven sl candidates isolated in this screen were complemented by either THO2 or HPR1 (see Fig. 1B for one example each). This underlined the genetic interaction of YOR166c with HPR1 and showed that YOR166c also interacts genetically with another component of the THO complex, THO2, but unfortunately did not reveal any additional genetic interactors of YOR166c.

To assess in a defined manner the genetic interaction between YOR166c and THO2 or HPR1 and to test the likely assumption that YOR166c also interacts with the other two components of the THO complex, MFT1 and THP2, double knock-out strains of YOR166c and one of the four THO components were generated. Fig. 1C shows that deletion of YOR166c is synthetically lethal with a deletion of each of the four genes encoding a member of the THO complex. Thus, YOR166c interacts genetically with all four THO components.

To test whether the genetic interaction of YOR166c with the THO complex also extends to the other members of the TREX complex, we tested whether YOR166c shows a synthetic lethal phenotype with alleles of SUB2 and YRA1. Deletion of YOR166c is sl with the temperature sensitive allele of SUB2, sub2–85 (Fig. 1D). However, we could not detect any sl relationship between YOR166c and various alleles of YRA1 or MEX67 (data not shown). Based on its synthetic lethality with TREX components, we named the gene YOR166c SWT1 for sl with TREX. As implicated by its genetic interactions, Swt1 might play a role in transcription elongation and early steps of mRNA export and/or transcription-dependent recombination.

FIGURE 1. The non-characterized ORF YOR166c is sl with TREX components. A, identification of YOR166c in an sl screen with Δhpr1. The sl phenotype of sl Δhpr1 267 is rescued by plasmid-encoded HPR1 (+HPR1), the plasmid isolated by library transformation of sl Δhpr1 267 (+267), which contains the ORF YOR166c, plasmid-encoded YOR166c (+YOR166c), or NOP1::YOR166c driven YOR166c (+NOP1::YOR166c) but not an empty plasmid (+empty). B, isolation of THO2 and HPR1 in an sl screen with Δyor166c. sl Δyor166c 55 and 32, which were isolated in an sl screen with a deletion allele of YOR166c, are complemented by plasmid-borne THO2 (+THO2) and HPR1 (+HPR1), respectively, but not an empty plasmid (+empty). C, Δyor166c is sl with deletion alleles of the four components of the THO complex, THO2, HPR1, MFT1, and THP2. Double deletion strains of one of the THO complex components and YOR166c are complemented by either plasmid-encoded YOR166c (+YOR166c) or the plasmid-encoded THO member (+HPR1, +MFT1, +THP2, +THO2, respectively), but not an empty plasmid (+empty). D, YOR166c is sl with sub2–85. A double deletion strain of SUB2 and YOR166c complemented by URA3-plasmid encoded SUB2 was transformed with plasmids encoding SUB2, the ts allele sub2–85 and YOR166c, or with sub2–85 alone. Combination of Δyor166c with sub2–85 leads to an sl phenotype. All transformants shown in Fig. 1 were restreaked onto FOA-containing plates and incubated for 3 days at 30 °C.

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following standard procedures (28). For each sample 10 μg of RNA were loaded, transferred to positive TM membranes (MP biomedicals), and visualized by methylene blue. Membranes were hybridized to [32P]dCTP-labeled probes specific for lacZ or GAL1, which were randomly labeled (Stratagene Prime-It II) and purified (Bio-Rad Micro Bio-Spin 30). For the loading control the plasmid pG3T726SSH containing the 25S rDNA was used. The membrane was exposed to a storage phosphor screen and analyzed using a Storm imaging system (Molecular Dynamics).
To test whether this interaction is specific for the function of TREX or extends to other factors implicated in transcription elongation, we tested a possible sl relationship of SWT1 with DST1, RTF1, or SPT5. DST1 encodes yeast TFIIS, which is necessary for efficient transcription elongation as it cleaves the nascent mRNA on backtracked RNA polymerase II so the polymerase can resume RNA synthesis (Refs. 29 and 30 and references therein). Rtf1 is a component of the Paf complex, which is needed for correct histone modification and mRNA processing (Refs. 31 and 32 and references therein). Spt5 and Spt4 form a complex that is homologous to metazoan DSIF, a transcription elongation factor, which first acts negatively in its unphosphorylated form but also has a positive function in transcription elongation (Ref. 33 and references therein). As DST1 and RTF1 are non-essential genes, we used deletion alleles to test synthetic lethality with SWT1, whereas a ts and a cs allele of the essential SPT5 gene was used. SWT1 is not essential in a genetic background lacking DST1 or RTF1 or containing mutant alleles of SPT5 (data not shown). Although one cannot exclude that SWT1 interacts genetically with other alleles of DST1, RTF1, and SPT5 than the tested ones or with genes encoding other transcription elongation factors, the lack of genetic interactions with these three transcription elongation factors suggests that the genetic interaction between SWT1 and components of TREX is rather specific. This indicates that the function of Swt1 is closely related to TREX.

Domain Analysis of Swt1—Swt1 is a 458-amino acid protein that can be subdivided into an N-terminal domain, a middle domain, and a C-terminal domain according to its amino acid sequence and secondary structure predictions (see Fig. 2A). Whereas the N- and the C-terminal domain do not share any significant sequence homology to other known proteins or domains, the middle domain contains a PINc domain (Fig. 2B). PINc domains are a large family of predicted nucleotide-binding domains. Proteins containing such a domain are expected to be RNases based on their similarities to 5'-exonucleases (34, 35). In S. cerevisiae, seven proteins are classified as PINc proteins in the SMART data base. Fig. 2C shows a sequence alignment of all PINc-domain-containing proteins of S. cerevisiae; three of them have been characterized: Nmd4 is involved in nonsense-mediated mRNA decay, Rrp44 is a component of the exosome, and Nob1 is suggested to cleave the 20 S preribosomes. Recently, the remaining four uncharacterized PINc proteins including YOR166c/SWT1 were analyzed for a function in

![FIGURE 2. Functional analysis of Swt1 domains. A, domain organization of Swt1. Numbers on top indicate amino acids of Swt1. The dark gray domain is a region of low complexity. B, schematic of the PINc domain with conserved active site residues shown in black and lower conserved residues shown in dark gray (indicated by a black or gray star, respectively). C, multiple sequence alignment of all PINc-domain-containing proteins of S. cerevisiae. Sequences were aligned using ClustalW 1.8 and Boxshade and modified manually. Conserved active site residues are indicated with a black star and lower conserved residues with a gray star. D-F, three different candidates isolated in the sl screens with Δhpr1 and Δswt1, which depend on the presence of a functional SWT1 gene for growth, were transformed with constructs expressing combinations of Swt1 domains under the constitutive NOP1 promoter. A combination of two Swt1 domains gives a functional protein. Transformants were restreaked onto FOA-containing plates and incubated for 5 days at 30 °C.](image-url)
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pre-18 S rRNA cleavage (36). However, deletion of YOR166c did not effect ribosome biogenesis.

To assess which domains of Swt1 are essential for its function, domain deletion mutants were expressed under control of the constitutive NOP1 promoter. As deletion of SWT1 does not lead to a severe growth phenotype (see Fig. 5A), the functionality of these constructs was tested in the isolated sl strains (shown in Fig. 1), which depend on a functional Swt1 for viability. As expected these sl strains grow when transformed with a plasmid expressing Hpr1 or Tho2, respectively, or full-length Swt1 under control of the NOP1 promoter (positive controls), whereas these strains cannot grow when they contain a plasmid not coding for any of these genes (+ empty/negative control, Fig. 2, D–F). Growth of cells and thus functionality of Swt1 is also conferred by deletion mutants of Swt1 containing either the N and M domain (+N+M) or the M and C domain (+M+C; see Fig. 2, B–D). In contrast, any single domain is not functional (see Fig. 2, D–F; +M; for N and C domain alone; data now shown). Interestingly, fusion of the N domain to the C domain (+N+C), i.e. deletion of the M domain, leads to a functional protein, indicating that the PINc (= M) domain is not necessary for Swt1 function. This finding is surprising but consistent with our finding that heterologously expressed Swt1 does not show any detectable RNA binding or RNase activity in vitro.4 In addition, deletion of SWT1 is not sl with deletion of RRP6 (data not shown), the nuclear component of the exosome known to be needed for rRNA processing and degradation of aberrant mRNPs (19). However, Swt1 could need a binding partner that we have not yet identified so far to be catalytically active. Also, it has not yet been possible to demonstrate a nuclease activity of a eukaryotic PINc domain protein in vitro. Interestingly, a potential nuclease activity of Swt1 could be needed e.g. for the degradation of aberrant mRNPs. Taken together a combination of two of the three domains of Swt1 is functional suggesting a redundant function of the domains of Swt1.

**Swt1 Is Mainly Localized to the Nucleus**—We wanted to determine the cellular localization of Swt1. To do this Swt1 was genomically tagged N- as well as C-terminally with the TAP tag. To assess whether these fusion proteins are functional, the TAP-SWT1 and SWT1-TAP strains were crossed to a HPR1 deletion strain, in which growth is dependent on a functional Swt1. N-terminally tagged Swt1 (TAP-Swt1) is functional, whereas Swt1-TAP is not (see Fig. 3A). However, expression of Swt1 from its endogenous promoter is too low for its detection by fluorescent microscopy (immunofluorescence using antibodies against the protA moiety of the TAP tag or green fluorescent protein-tagged Swt1). As an alternative to microscopy yeast cells expressing TAP-Swt1 from its endogenous promoter were fractionated on a Ficoll sucrose density step gradient. Yeast homogenates (H) were first separated into a nuclear pellet (NP) and a post-nuclear supernatant (PNS). The nuclear pellet was then loaded onto a Ficoll sucrose density step gradient. In this step gradient endoplasmic reticulum membranes concentrate at interphase II, mitochondria and heavy endoplasmic reticulum at interphase III, and nuclear proteins at interphase IV. The presence of different proteins in each fraction was followed by Western blotting. TAP-Swt1 is slightly concentrated in fraction IV and trails into lighter fractions (see Fig. 3B, upper panel). This distribution is similar to the mRNA export factor Yra1, which is nuclear at steady state but shuttles to the cytoplasm. Hpr1, as well as Rpb1, the largest subunit of RNA polymerase II, and nucleoporins are concentrated in fraction IV. In contrast, Tim 50, a mitochondrial protein, is concentrated in fraction II as expected. And Pgk1, the cytoplasmic 3-phosphoglycerate kinase, is concentrated in the supernatant (PNS) and absent from the gradient. The distribution of Swt1 on the gradient in comparison with other proteins of known localization indicates that Swt1 is a mainly nuclear protein but might shuttle between the nucleus and the cytoplasm.

**Swt1 Interacts Biochemically with the TREX Complex**—Based on its genetic interaction with the TREX complex as well as its mainly nuclear localization, we tested whether Swt1 also interacts with TREX components biochemically. To do this we purified functional N-terminally TAP-tagged Swt1 and assessed co-purifying proteins by Western blotting. Since the

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4 A. Kieser, unpublished results.
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The THO complex has been shown to play a role in transcription elongation and mRNA export. In addition, deletion of THO components leads to a transcription-dependent hyperrecombination phenotype (13). Based on its genetic and biochemical interaction with THO, deletion of SWT1 might cause hyperrecombination at transcribed loci, an mRNA export defect, or a reduction in transcription. Although we cannot exclude that Swt1 is necessary for the export of specific transcripts, loss of Swt1 function does not lead to a defect in nuclear export of bulk mRNA (data not shown). Also, in comparison with an isogenic wild-type strain the frequency of recombination in a Δswt1 strain is not significantly higher at 30 or 37 °C as assessed with a LEU2-based direct repeat reporter system (Fig. 5, B and C). Thus, deletion of SWT1 does not

Swt1 Is Needed for Efficient Transcription

A deletion of SWT1 causes a slow growth phenotype on synthetic medium at 37 °C. 10-Fold dilutions of Δswt1 cells carrying a plasmid encoding SWT1 (pRS315-SWT1) or an empty plasmid (pRS315) were spotted onto plates containing full medium (YPD) or synthetic medium (SDC) and incubated at the indicated temperatures for 2 or 3 days, respectively. B, scheme of the LEU2-based direct repeat reporter system. Recombination between the repeats leads to a functional LEU2 gene. C, loss of Swt1 does not cause hyperrecombination. The frequency of recombination of a Δswt1 strain is similar to a wild-type strain at 30 and 37 °C. As positive control the recombinant frequency of a Δhpr1 strain is shown (gray, right axis). D, deletion of SWT1 causes a decrease in β-galactosidase expression. Wild-type, Δswt1, and Δhpr1 cells carrying a plasmid encoding β-lactamase were grown at 30 and 37 °C (only wild-type and Δswt1) and β-galactosidase activity was measured. E, RNA levels of lacZ and GAL1 mRNA are lower in Δswt1 cells. Cells were grown as above, total RNA extracted, and Northern blots probed for lacZ, GAL1, and 25 S rRNA transcripts.

FIGURE 5. Swt1 Is Necessary for Efficient Transcription. A, deletion of SWT1 causes a slow growth phenotype on synthetic medium at 37 °C. 10-Fold dilutions of Δswt1 cells carrying a plasmid encoding SWT1 (pRS315-SWT1) or an empty plasmid (pRS315) were spotted onto plates containing full medium (YPD) or synthetic medium (SDC) and incubated at the indicated temperatures for 2 or 3 days, respectively. B, scheme of the LEU2-based direct repeat reporter system. Recombination between the repeats leads to a functional LEU2 gene. C, loss of Swt1 does not cause hyperrecombination. The frequency of recombination of a Δswt1 strain is similar to a wild-type strain at 30 and 37 °C. As positive control the recombinant frequency of a Δhpr1 strain is shown (gray, right axis). D, deletion of SWT1 causes a decrease in β-galactosidase expression. Wild-type, Δswt1, and Δhpr1 cells carrying a plasmid encoding β-lactamase were grown at 30 and 37 °C (only wild-type and Δswt1) and β-galactosidase activity was measured. E, RNA levels of lacZ and GAL1 mRNA are lower in Δswt1 cells. Cells were grown as above, total RNA extracted, and Northern blots probed for lacZ, GAL1, and 25 S rRNA transcripts.

FIGURE 4. Swt1 Interacts with TREX Complex Components in Vivo. N-terminally TAP-tagged Swt1 expressed from the GAL1 promoter or a non-tagged negative control were purified from strains that also carry an HA-tagged version of Hpr1. The EGTA eluate after the TAP was separated on a 10% SDS-gel and analyzed by Coomassie staining (upper panel) and Western blotting with antibodies directed against the HA-tag of Hpr1, Sub2, Yra1, Npl3, Mex67, and the CTD of Rpb1 (lower panel).

FIGURE 3. Swt1 Is a Novel Protein with a Function in Transcription. A, deletion of SWT1 causes a slow growth phenotype on synthetic medium at 37 °C. 10-Fold dilutions of Δswt1 cells carrying a plasmid encoding SWT1 (pRS315-SWT1) or an empty plasmid (pRS315) were spotted onto plates containing full medium (YPD) or synthetic medium (SDC) and incubated at the indicated temperatures for 2 or 3 days, respectively. B, scheme of the LEU2-based direct repeat reporter system. Recombination between the repeats leads to a functional LEU2 gene. C, loss of Swt1 does not cause hyperrecombination. The frequency of recombination of a Δswt1 strain is similar to a wild-type strain at 30 and 37 °C. As positive control the recombinant frequency of a Δhpr1 strain is shown (gray, right axis). D, deletion of SWT1 causes a decrease in β-galactosidase expression. Wild-type, Δswt1, and Δhpr1 cells carrying a plasmid encoding β-lactamase were grown at 30 and 37 °C (only wild-type and Δswt1) and β-galactosidase activity was measured. E, RNA levels of lacZ and GAL1 mRNA are lower in Δswt1 cells. Cells were grown as above, total RNA extracted, and Northern blots probed for lacZ, GAL1, and 25 S rRNA transcripts.

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abundance of Swt1 expressed from its endogenous promoter is very low (e.g. about 300 times less than Sub2; data not shown), it was necessary to overexpress Swt1 from the strong GAL1 promoter. A strain that does not express any TAP-tagged protein was used as negative control. Swt1 does not purify very well but can be seen in the EGTA eluate of the purification as a Coomassie-stainable band (Fig. 4, upper panel). The TREX components Hpr1 (as a representative subunit of the THO complex), Sub2, and Yra1 co-purify with Swt1 but are absent from the mock purification (Fig. 4, lower panels). In contrast, Npl3, an abundant shuttling SR protein with a function in mRNA export, and Mex67, the large subunit of the mRNA exporter Mex67-Mtr2, do not co-purify with Swt1 (Fig. 4). Thus, Swt1 specifically interacts with TREX components in vivo. To test whether the interaction of Swt1 with TREX depends on the presence of RNA, the lysate was treated with RNase prior to the purification. Hpr1, Sub2, and Yra1 still co-purify with Swt1 under these conditions, indicating that the interaction between Swt1 and TREX is independent of RNA. The substoichiometric association of TREX with Swt1 might be due to the overexpression of Swt1, which is about 300 times more abundant at steady state when expressed from the GAL1 promoter as when expressed from its own promoter (data not shown). Taken together Swt1 interacts in vivo with the TREX complex and thus might associate with the elongating transcription machinery. This is consistent with the finding that Swt1 also interacts with RNA polymerase II (Fig. 4). Unfortunately, the pulled down amounts of RNA polymerase II were too low to determine the phosphorylation state of its CTD. Thus, instead of a direct function in transcription Swt1 could associate with a fraction of TREX that is not associated with elongation, e.g. recycle TREX from transcription complexes after the message has been released.
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lead to a hyperrecombination phenotype. Third, we tested whether deletion of SWT1 causes a defect in transcription. Since it is known that deletion of HPR1 causes a severe reduction in transcription of the bacterial lacZ gene (37), we chose this reporter. ∆swt1 cells grown at 30 °C show a decrease in expression of β-galactosidase of ~50% as compared with an isogenic wild-type strain (Fig. 5D). Thus, Swt1 is needed for efficient expression of the lacZ gene at a temperature, where deletion of this gene does not cause an impairment of growth (Fig. 5A). In comparison with cells lacking Swt1, deletion of HPR1 leads to a more severe reduction of β-galactosidase expression (Fig. 5D). Since ∆swt1 cells grow more slowly at 37 °C on minimal medium (Fig. 5A), we assessed whether expression of lacZ is even more compromised in ∆swt1 cells at 37 °C. As can be seen in Fig. 5D, β-galactosase expression is reduced to about 15% in ∆swt1 cells grown at this temperature. Since ∆swt1 cells do not display an obvious mRNA export defect (see above), this reduction in β-galactosidase expression is most likely not caused by compromised mRNA export.

To test directly whether deletion of Swt1 leads to a decrease in the amount of lacZ mRNA, we performed Northern blots. As can be seen in Fig. 5E the amount of lacZ mRNA in ∆swt1 cells is reduced at 30 °C and even further reduced at 37 °C as compared with an isogenic wild-type strain. Thus, Swt1 is needed for efficient accumulation of the lacZ transcript. As a second example, we assessed the amounts of GAL1 mRNA. As reported previously, expression of GAL1 is not as greatly reduced in ∆hpr1 cells as is the expression of lacZ (38). Cells lacking Swt1 show lower levels of GAL1 mRNA already at 30 °C, and the amount of GAL1 mRNA is further reduced at 37 °C as compared with an isogenic wild-type strain (Fig. 5E). Taken together, Swt1 is needed for efficient transcription of the lacZ reporter gene, the natural GAL1 gene, and most likely other genes.

Here, we describe for the first time a function for the uncharacterized ORF YOR166c, which, based on its synthetic lethal interaction with TREX, was named SWT1. Swt1 localizes mainly to the nucleus, interacts with TREX and RNA polymerase II in vivo and thus might be present at the transcribed gene. Importantly, deletion of SWT1 leads to a reduction in transcript levels of the lacZ reporter as well as the endogenous GAL1 gene. Thus, Swt1 is necessary for high transcript levels of highly expressed genes. Alternatively to a direct function in transcription, Swt1 might be needed for a process downstream of transcription, e.g., for the recycling of TREX from the transcription machinery. Interestingly, Swt1 contains a PINc domain, and the amount of Swt1 in cells is quite low (data not shown; Swt1 was also not visualized and thus not quantified by Ghaemmaghami et al. (39)) indicating that Swt1 could have a catalytic function. Thus, Swt1 could also be involved in the turnover of transcripts, e.g., of aberrantly formed mRNPs. However, the PINc domain is not essential for Swt1 function in vivo as assessed by rescue of the synthetic lethal relationship with THO mutants by a Swt1 allele lacking the PINc domain. Alternatively to a catalytic function, Swt1 could be necessary for the transcription and also nuclear export of only a small subset of genes. Further studies will be needed to elucidate the molecular function of this new player in gene expression.

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