The Saccharomyces cerevisiae RuvB-like Protein, Tih2p, Is Required for Cell Cycle Progression and RNA Polymerase II-directed Transcription*

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Two highly conserved RuvB-like putative DNA helicases, p47/TIP49b and p50/TIP49a, have been identified in the eukaryotes. Here, we study the function of Saccharomyces cerevisiae Tih2p, which corresponds to mammalian p47/TIP49b. Tih2p is required for vegetative cell growth and localizes in the nucleus. Immunoprecipitation analysis revealed that Tih2p tightly interacts with Tih1p, the counterpart of mammalian p50/TIP49a, which has been shown to interact with the TATA-binding protein and the RNA polymerase II holoenzyme complex. Furthermore, the mutational study of the Walker A motif, which is required for nucleotide binding and hydrolysis, showed that this motif plays indispensable roles in the function of Tih2p. When a temperature-sensitive tih2 mutant, tih2–160, was incubated at the nonpermissive temperature, cells were rapidly arrested in the G1 phase. Northern blot analysis revealed that Tih2p is required for transcription of G1 cyclin and of several ribosomal protein genes. The similarities between the mutant phenotypes of tih2–160 and those of taf145 mutants suggest a role for TIH2 in the regulation of RNA polymerase II-directed transcription.

One of the common post-translational modifications of nuclear and cytosolic proteins in eukaryotes is the addition of N-acetylglucosamine residues O-linked (O-GlcNAc)1 to serine and threonine residues. A number of physiologically or structurally important proteins have thus far been shown to contain O-GlcNAc, including the largest subunit of RNA polymerase II (RNAP II) (1), transcription factors such as Sp1 and hepatocyte nuclear factor 1 (2–4), nuclear pore proteins (5, 6) and chromatin-associated proteins (7). To study nuclear factors involved in nuclear transport and other nuclear events, we previously performed an in vitro binding assay of a rat liver nuclear matrix fraction using a wheat germ agglutinin affinity column (8). Several O-GlcNAc-containing proteins such as nucleoporins as well as nonglycosylated proteins like importin β were isolated (9). Two RuvB-like proteins, p50 (9) and p47 (10), were also isolated by this method probably because of their interaction with O-GlcNAc-bearing proteins. RuvB is a prokaryotic DNA helicase, and its helicase activity and DNA binding affinity are enhanced by interaction with RuvA (11, 12). These two factors form a large motor protein complex to promote branch migration at Holliday junctions at the late stages of homologous recombination. The p50/p47 and RuvB proteins share highly conserved Walker domains (A and B), indicative of proteins that bind nucleotide triphosphates (10, 13). Based on this, p50 and p47 were proposed to be the eukaryotic homologues of the RuvB DNA helicase. Indeed, p50 and p47 are present in a wide range of eukaryotes ranging from yeast to mammals, suggesting that this basic helicase activity may be conserved among the eukaryotes.

In addition to recombination, transient unwinding of the DNA duplex is required in many cellular processes such as replication, transcription, and DNA repair. To accommodate these different aspects of DNA metabolism, cells typically encode multiple helicases, occasionally found in functionally distinct complexes. To date, at least 10 different DNA helicases have been identified in Saccharomyces cerevisiae (14–17). However, only a few DNA helicase genes are essential for viability, such as RAD3, SSL2, and DNA2. Functional analyses of RAD3, SSL2, and DNA2 indicate that they are indeed required for fundamental cellular functions. Rad3p and Ssl2p are essential components of TFIIH, which is required for transcriptional initiation and transcription-coupled repair (15, 16), whereas Dna2p is required for DNA replication (17).

Recently, several groups have independently reported the identification of p50. First, a 49-kDa protein isolated from rat liver nuclear extracts was found to interact with TATA-binding protein (TBP) (13). The 49-kDa protein, referred to as TIP49a (TBP-interacting protein) was identical to p50 and was enriched in the testes. The recombinant TIP49a is an ATP-dependent DNA helicase (18). Meanwhile, a human homologue of p50, RUVBL1, was shown to co-purify with RNAP II holoenzyme, and the yeast counterpart was reported to be essential for viability (19). Furthermore, in a search for proteins that interact with β-catenin, two novel human proteins, 52- and 44-kDa, were detected (20). The 52-kDa protein corresponding to p50 (TIP49a, RUVBL1) was designated Pontin 52 and shown to bind to TBP directly. In addition, p50 was also isolated as...
NMP238, a ubiquitously occurring nuclear matrix protein (21). On the other hand, little has been described thus far for p47. In a recent study, a new RuvB-like DNA helicase termed TIP49b was demonstrated to associate with TIP49a (22). The protein sequence between p47 (mouse) and TIP49b (rat) varies by only 2 residues. Although the above reports indicate that p50 interacts with TBP, RNAPII, and histone core histones, or both, p50 and p47 may also provide helicase enzymatic activity in recombination events. The key question of what critical function(s) p50 provides in vivo that makes it essential for viability is not yet understood, but it is important to note that studies to date link p50 primarily to the machinery of gene transcription. Given the similarities shared by recombination, replication, and transcription events at the DNA helicase level, it is not surprising that p50 may participate in multiple cellular events. In this study, we undertook a genetic approach to study the in vivo role of Tih2p, the less well-studied yeast homologue of p47. Similar to the yeast homologue of p50, TIIH1 (TIP49a homologue), TIIH2 (TIP49b homologue) is also an essential gene. Whereas the sequence homology of Tih2p to RuvB suggests a role in recombination and DNA repair, our results indicate that Tih2p is essential for proper cell cycle progression and for the selective transcription of a subset of genes. The participation of Tih2p in these functions may explain its requirement for viability.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains, Media, and Plasmid Constructions**—Yeast strains used in this paper are listed in Table I. All strains were grown in rich medium (YPD) or selective medium (synthetic complete, synthetic dextrose, synthetic galactose) supplemented with the appropriate nutrients. Genetic manipulation of yeast cells was performed as described previously (23). The plasmid vector series was described previously (24). Details of primers used in this study are available on request. The TIIH2 knockout plasmid, pDIITIIH2, was constructed by PCR amplification of a pair of DNA fragments flanking the TIIH2 ORF, creating appropriate restriction sites at each end, and by subsequent ligation of these fragments into pRS303. To replace the entire TIIH2 ORF with HIS3, pDIIS-TIIH2 was linearized with BamH1 and transformed into a diploid strain, CRDFY (FY background, Table I, FY strains are generous gifts from F. Winston, Albert Einstein Medical School, Boston, MA). Disruption was verified by Southern blotting. The TIIH2 sequence was localized on an EcoRI-ClaI fragment was amplified by PCR of genomic DNA and ligated into pRS316 and pRS314 to obtain pRS316-TIIH2 and pRS314-TIIH2, respectively. To create pRS425-ADH1p-p47, the ORF of p47 was PCR-amplified from a yeast genomic DNA clone and ligated into pRS425-ADH1p between the Sau3AI and SphI sites of the plasmid. pRS425-ADH1p was constructed as described (34) using anti-HA 12CA5 monoclonal antibody or anti-TBP antibody or anti-yTAF II61 or anti-TAF II145 antibody. To express an N-terminally double hemagglutinin-tagged Tih2p that pRS316- K81R-TIIH2 was constructed similarly as pRS426- K81R-TIIH2, but not 37 °C were isolated. Plasmids harboring the mutated TIIH2 genes were recovered from these putative ts strains and re-introduced into the parental strain (CRPA1). Temperature sensitivity was reconfirmed by plasmid shuffling. The sequences of some of the mutant alleles were determined.

**RNA Preparation and Analysis**—Cells grown in YPD medium to the early exponential phase at 23 °C (A600 nm = 0.2) were harvested at the appropriate times after temperature shift by centrifugation, washed with medium, frozen in liquid nitrogen, and stored at −80 °C. Total RNA from cells was isolated by hot phenol extraction (30). Northern blotting was performed as described (31). Twelve µg of each RNA sample were subjected to electrophoresis in a 1.1% formaldehyde/agarose gel, followed by transfer to a nylon membrane (Hybond-N+, Amersham Pharmacia Biotech). All probes used for hybridization were generated by PCR amplification using genomic DNA as template and labeled using a random primer labeling kit (TaKaRa). Blots were stripped for reprobing by boiling and cooling to room temperature in 0.1% SDS. For slot blot analysis, 2 µg of RNA from each sample were used. Experiments were performed essentially as described (32).

**Whole-cell Extract Preparation and Immunoblot Analysis**—Protein extracts from yeast strains grown in YPD liquid culture prepared essentially as described (33) were separated on SDS-polyacrylamide gels, transferred to nitrocellulose membranes and probed with either anti-yTAF II145 antibody or anti-TBP antibody or anti-yTAF II61 or anti-yTAF II90 antibodies. Detection of the antibody signal was accomplished with the Amersham Pharmacia Biotech ECL Western detection kit.

**IgG Pull-down, in Vivo Labeling, and Immunoprecipitation**—Extracts were prepared by pelleting 10 µl of cells (A600 = 0.5) cultured in synthetic complete, washing once with lysis buffer (50 mM Tris-HCl, pH 8.0, 5 mM EDTA), and resuspending again in 200 µl of lysis buffer supplemented by protease inhibitors. 50 µg of each sample were resuspended in 1 ml of methionine/cysteine-free synthetic complete medium were resuspended in 1 ml of methionine/cysteine-free synthetic complete medium were resuspended in 1 ml of methionine/cysteine-free synthetic complete medium and labeled with 1 mCi of [35S]methionine/cysteine for 20 min at 30 °C. Cell extracts were prepared using glass beads. Immunoprecipitation was performed as described (34) using anti-HA 12CA5 monoclonal antibody or rabbit IgG was used, respectively.

**DNA Flow Cytometry**—1 × 107 cells growing exponentially in YPD at 23 °C were temperature-shifted to 37 °C and harvested at various time points. These samples were subjected to electrophoresis in a 1.1% formaldehyde/agarose gel, followed by transfer to a nylon membrane (Hybond-N+, Amersham Pharmacia Biotech). All probes used for hybridization were generated by PCR amplification using genomic DNA as template and labeled using a random primer labeling kit (TaKaRa). Blots were stripped for reprobing by boiling and cooling to room temperature in 0.1% SDS. For slot blot analysis, 2 µg of RNA from each sample were used. Experiments were performed essentially as described (32).
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RESULTS

TIH2 Is an Essential Gene—In our previous studies, two eukaryotic homologues of the bacterial RuvB DNA helicase, p50 and p47, were isolated. Searching through the S. cerevisiae Genome Data base (Stanford University), two ORFs designated as YDR190C and YPL235W were identified as the yeast homologues of p50 and p47, respectively. Sequence identities are as high as 70% between p50 and YDR190C and 69% between p47 and YPL235W (Fig. 1A). On the other hand, sequence identities between p50 and p47 and between YDR190C and YPL235W are only 43 and 40%, respectively. Therefore, we propose classifying these eukaryotic RuvB-like proteins into two subfamilies, i.e., the p50 and the p47 subfamily (Fig. 1A). It was of great interest to us what different eukaryotic RuvB-like proteins would encode two highly conserved subfamilies of RuvB-like proteins that are homologous to each other and of similar molecular size (50 and 51 kDa). To investigate the functions of these RuvB-like proteins, we undertook a genetic study of the less well studied p47 yeast homologue, YPL235W. For simplicity, we will refer to YDR190C (p50 homologue) as TIH1 and YPL235W as TIH2.

Because TIH1 had been shown to be an essential gene (9, 19, 22), we first asked if TIH2 is also required for viability. The TIH2 gene was disrupted as described under “Experimental Procedures.” A \textit{CEN/ARS} plasmid containing the wild-type TIH2 gene (URA3, TIH2) was introduced into the TIH2/tih2::HIS3 diploid strain. One HIS3 URA3 progeny obtained from the tetrad analysis, designated CRPA1 (Table I), was transformed with either a pRS314-based plasmid (\textit{CEN/ARS}) containing wild-type TIH2 (TRP1, TIH2) or a control vector (pRS314) and was counter selected on 5-FOA medium. Only transformants containing the (TRP1, TIH2) plasmid were able to grow on 5-FOA medium, indicating that TIH2 is indeed required for mitotic vegetative growth (Fig. 1B).

To determine if Tih2p could be functionally replaced by the highly conserved mammalian p47, we constructed a multicopy plasmid overexpressing p47 from a yeast constitutive promoter (ADH1p). We found out that neither p47 nor p50 was able to rescue the tih2 \(\Delta\) disruptant, whereas a similar control vector overexpressing the yeast TIH2 gene did so as well as complementation with the endogenously expressed gene (Fig. 1B). Thus the yeast TIH2 gene cannot be complemented by p47, similar to the results obtained with p50, which was unable to replace the function of TIH1 in a tih1 \(\Delta\) disruptant (19, 22) (data not shown).

Tih2p Is Localized to the Nucleus—To probe the cellular location of Tih2p, a construct consisting of Tih2p with the green fluorescent protein fused to its N terminus (GFP-Tih2p) was introduced into the ADH1p expression vector. The parental null allele strain CRPA1 was transformed with this GFP-TIH2 fusion and tested for growth on 5-FOA medium. Transformants were found to grow normally, indicating that GFP-TIH2 was fully functional (Fig. 2A). Observation using a fluorescent microscope showed that this fusion protein was localized primarily to the nucleus (Fig. 2B), consistent with the fact that p47 was isolated from rat liver nuclear extract (10). Faint GFP fluorescence was also detected in the cytosol, probably because of overexpression. The cytosolic fluorescence was not caused by ATP binding/hydrolysis and are the region of Tih2p most homolo-
gous to the prokaryotic helicase, RuvB (9, 10, 35). To investigate whether the helicase domain of Tih2p contributes to its essential physiological role, the invariant lysine at position 81 so-called Walker A and B, TIH2 was shown (Fig. 3A). This indicated that K81R-temperatures (Fig. 3B, TIH2 failed to grow at different temperatures (Fig. 3B, only plates incubated at 30 °C are shown). This indicated that K81R-TIH2 alone is unable to support the growth of tih2 \(\Delta\) cells. Therefore, we concluded that the Walker A motif is essential for the function of Tih2p that contributes to cell viability.

Furthermore, when K81R-TIH2 was overexpressed under the control of the inducible GAL1 promoter (GAL1p) in wild-type cells, it exerted a dominant negative effect. These cells were normal when grown on glucose, but growth was signifi--
cantly retarded when grown on synthetic medium containing galactose as the sole carbon source (Fig. 3C). This result implies that the function of Tih2p may require the formation of self-complexes or complexes with certain cellular components in vivo.

**Tih2p Interacts with Tih1p in Vivo**—The fact that p50 and p47 were each found in a large complex of similar size, 700 kDa, suggested that they might exist in the same complex (10). The dominant negative effect of K81R-Tih2p we observed also suggested that complex formation may be important to Tih2p function. Therefore we examined if Tih2p interacts with any other components in vivo. Wild-type Tih2 was modified with the hemagglutinin epitope to its N terminus (HA-Tih2), and the fusion is fully functional (Fig. 1B). A strain containing HA-Tih2 as the sole copy of Tih2 in vivo, was metabolically labeled with [35S]methionine/cysteine, and cell lysates were immunoprecipitated using the monoclonal antibodies 12CA5 against the HA epitope. In addition to HA-Tih2p, at least one major protein band of ~51 kDa was specifically detected (Fig. 3A). The size of the detected protein (51 kDa) was similar to that of Tih1p, a homologue of Tih2p. To investigate the possible interaction between Tih1p and Tih2p, Tih1p was tagged with IgG binding domains of the *Staphylococcus aureus* protein A to its N terminus (ProtA-Tih1). The resulting construct ex-
pressed by the constitutive nucleolar protein promoter (NOP1p) (36) was functional (data not shown). ProtA-Tih1p was detected by immunoblotting using rabbit IgG when introduced into a tih2 Δ strain harboring plasmid (TRP1, TIH2) (Fig. 4B, lanes 1 and 2). Cell lysates from tih2 Δ strains expressed both ProtA-Tih1p (URA3, ProtA-TIH1) and either nontagged Tih2p (TRP1, TIH2) or HA-tagged Tih2p (TRP1, HA-TIH2) were first precipitated using IgG-Sepharose followed by immunoblotting using anti-HA antibody (lanes 6 and 8). A specific band corresponding to HA-Tih2p was detected only in the strain expressing both tagged TIH2 (HA-Tih2p) and tagged TIH1 (ProtA-Tih1p) (compare the band positions in lanes 4 and 8). This result indicated that HA-Tih2p was selectively recovered by IgG-Sepharose precipitation from the strain also expressing ProtA-Tih1p. A tih2 Δ strain expressing ProtA-Tih2p as the sole copy of TIH2 was also constructed, and a pull-down of the lysates by IgG-Sepharose followed by silver staining revealed two major and several as yet unidentified minor bands. Microsequencing of the peptide making up the major band adjoining ProtA-Tih2p revealed that this protein was Tih1p (data not shown). Therefore, both Tih1p and Tih2p may interact directly to form a complex in yeast cells.

Growth Characteristics of a Temperature-sensitive Mutant, tih2–160—To determine the cellular role(s) of Tih2p, we isolated several ts tih2 alleles. On plates, all of the mutants exhibited a similar growth defect at the nonpermissive temperature of 37 °C. However, in liquid media their growth at 37 °C varied. We focused on analysis of one of the ts mutants, tih2–160, because it exhibited not only a very stringent ts growth on plates (no growth above 30 °C) but also a relatively rapid cessation of growth at 37 °C in liquid media (arrested within 4–5 h). Growth at 23 °C is also slower in this strain, but no other particular defects could be observed (Fig. 5A). Interestingly, tih2–160 cells incubated on plates at 37 °C regained viability and the capacity for growth when shifted down to 23 °C but not when shifted down to 30 °C (Fig. 5B). The amount of tih2–160 mutant protein detected in the cell was relatively unchanged even after 4 h of incubation at 37 °C suggesting that the mutant phenotype was because of loss of function and not degradation of the protein (data not shown).

Reversible G1 Arrest—When tih2–160 cells were shifted to 37 °C, we noticed that there was an accumulation of unbudded cells, indicative of a G1 phase cell cycle arrest (Fig. 6, B and C, upper panel). To delineate this cell cycle block more precisely, the arrested cells were analyzed using a fluorescence-activated cell sorter. As expected, tih2–160 cells showed a dramatic and rapid increase in the proportion of cells with a 1N DNA content (Fig. 6A). Nearly 70% of the cells were arrested within 3–4 h as unbudded cells, characteristic of the G1 phase, whereas the budding pattern of isogenic wild-type cells grown at 37 °C stayed relatively unchanged throughout the course (Fig. 6B). Consistent with the recovery of growth (Fig. 5B), the cell cycle block was released when the tih2–160 cells were shifted back to 23 °C (Fig. 6A) and the proportion of large budded cells increased to around 34% of the population, whereas that of unbudded cells dropped to ~40% (Fig. 6, B and C). This result indicated that Tih2p function is indeed required for proper cell cycle progression. The reversibility of this cell cycle arrest with temperature is consistent with the idea that the cell cycle arrest in tih2–160 cells is because of inactivation of tih2–160p at 30 °C and higher.

Inactivation of tih2–160p Abolishes Transcription of G1 Cyclin and Ribosomal Proteins—One explanation for the cell cycle arrest observed in tih2–160 cells is that Tih2p is required for the transcription of specific genes involved in the progression beyond the G1 phase. To test this hypothesis, tih2–160 cells and isogenic wild-type cells were analyzed for transcription of G1 cyclin. RNA was isolated at 0, 1, 2, and 4 h following incubation at the nonpermissive temperature (37 °C), and Northern blot analysis was performed. The result showed that in tih2–160 cells, transcription of the G1 cyclin CLN2 decreased dramati-
the large ribosomal subunit protein, RPL32, was significantly affected. In contrast, transcription of the ADH1 and actin (ACT) genes were unaffected even after 4 h of incubation at the nonpermissive temperature. Therefore, we concluded that Tih2p may be required for the transcription of a subset of genes, including those necessary for proper cell cycle progression. Taken together, these results are similar to those observed with taf145 mutants. yTAF145 is the yeast homologue of the higher eukaryotic protein TAF250 and is the only yTAFp known to contact TBP directly (38). Incubation of a temperature-sensitive yeast taf145 mutant for 4 h at its nonpermissive temperature results in G1 arrest, indicated by the accumulation of unbudded cells and in down-regulation of G1 cyclin and ribosomal protein genes transcription (32, 39). However, yTAF145 was found to be otherwise dispensable for global RNA polymerase II-directed transcription (32, 39). To investigate if Tih2p is required for global transcription, total RNA was prepared from tih2–160, an isogenic wild-type strain, and from the positive control RNAP II mutant, rpb1–1, and subjected to slot blot analysis using 32P-labeled oligo(dT) as a probe. As expected, inactivation of RNAP II resulted in a rapid loss of RNAP II-mediated transcription, whereas inactivation of tih2–160p by incubation at the nonpermissive temperature for 4 h had no significant effect on total poly(A)+ mRNA synthesis (Fig. 7B). Therefore, we concluded that global RNA polymerase II-directed transcription is unaffected in the tih2–160 mutant, similar to the result in taf145 mutants.

Immunoblot Analysis of yTAF145, TBP, and Other yTAFps Following Temperature Shift Inactivation of Tih2p—The mammalian p50/Pontin52/TIP49a was previously shown to interact with the TBP (13, 20), whereas the yeast homologue, Tih1p, was shown here to complex with Tih2p. Therefore, it is quite likely that p50 and p47 in mammalian cells and Tih1p and Tih2p in yeast form complexes that interact with their respective TBPs. If these interactions contribute to the stability of TBP, then the transcriptional defect observed upon Tih2p inactivation could be due in part to the disruption of these complexes and destabilization of TBP. Alternatively, another interpretation of the similarities between the tih2–160 and taf145 mutant phenotypes is that the defect conferred by tih2–160 mutation leads to ytafII145 degradation. To examine such possibilities, whole-cell extracts were prepared from tih2–160 cells incubated for 0, 1, 2, and 4 h at the nonpermissive temperature and subjected to immunoblot analysis using several antibodies.

In addition, to examine the integrity of the TFIID complex in tih2–160 mutant, antibodies against yTAFII61 and yTAFII90, which are present in both TFIID and Spt-Ada-Gcn5-acetyltransferase complex, were also tested (40).

In both wild-type and tih2–160 cells, TBP, yTAF145, yTAF161, and yTAF90 were detected at substantial levels even after 4 h of incubation at the nonpermissive temperature (Fig. 7C). This result argues against the possibility that inactivation of Tih2p leads to degradation of components of the TFIID or Spt-Ada-Gcn5-acetyltransferase complex and argues for a primary role of Tih2p in the transcription of these genes.

**DISCUSSION**

Two novel RuvB-like proteins, p50 and p47, were isolated from a rat liver nuclear extract using a wheat germ agglutinin-Sepharose affinity column (8). These proteins have highly homologous counterparts in a wide range of eukaryotes and can be classified into two subfamilies, i.e., the p50 and the p47 subfamily (Fig. 1A). The yeast counterpart of the p50 gene, designated TIIH1 in this study, has been previously shown to be an essential gene. Here, we showed that the yeast counterpart of the p47 gene, TIIH2, is also required for viability (Fig. 1B). Immunofluorescence studies showed that Tih2p is localized pri-
arily in the nucleus, consistent with the fact that it was isolated from a rat liver nuclear extract and suggesting that it participates in nuclear events (Fig. 2). To study the function of Tih2p in detail, we created several ts mutants and further examined the characteristics of one of them, tih2–160, which exhibited a relatively rapid cessation of growth at the nonpermissive temperature (Fig. 5).

Homology to RuvB Suggests a Role for Tih2p in DNA Repair—The ruvB mutants were first isolated as UV- and mitomycin C-sensitive mutants (41). However, the tih2 mutants we have isolated exhibited only a very modest sensitivity to UV radiation, mitomycin C, or methyl methanesulfonate compared with other mutants known to be defective in DNA repair (data not shown). We reasoned that the function of Tih2p in DNA repair may be redundant. For example, the prokaryotic RecG gene encodes a Holliday junction-specific helicase and can partially replace the function of Ruv (42). Recombination and DNA repair in a recG mutant are only mildly affected but become severely defective when recG is combined with mutations in the ruv genes (43). If similar redundancy exists in the eukaryotic system, mutations in other genes may be required in order for the defect in DNA repair in tih2 mutants to be revealed. In this context, creating double mutants of TIH2 and its homologous gene, TIH1, could be interesting because they interact with each other (Fig. 4) and could be functionally synergistic.

Another observation that suggests a role for Tih2p in DNA repair is the fact that the tih2–160 mutant undergoes G1 arrest. It is possible that Tih2p is required for repairing DNA damage associated with replication, such as double strand breaks. Inactivation of tih2–160p may result in the accumulation of double strand breaks in replicated DNA, thereby activating cell cycle checkpoint genes leading to a G1 arrest. We tested whether the G1 arrest observed in tih2–160 cells requires the activation of the checkpoint control gene RAD9 (44). We created a double rad9Δ tih2–160 mutant and found that lethality was not enhanced compared with the tih2–160 single mutant, and the arrest was not Rad9-dependent (data not shown). Moreover, the double mutant exhibited reversibility of growth arrest similar to that observed in the tih2–160 single mutant (data not shown, Fig. 5) indicating good viability. Therefore, a role of Tih2p repairing DNA damage associated with replication cannot be observed in this study.

A Regulatory Role for Tih2p in RNAP II-mediated Transcription—The ability of the yeast cell to progress through the mitotic cell division cycle requires the coordinate and highly regulated transcription of several genes (45). The G1 cell cycle arrest observed at the nonpermissive temperature in tih2–160 mutants may be because of temperature-dependent conformational changes in tih2–160p that impair its ability to interact with gene-specific transcription factors necessary for cell cycle progression. Our hypothesis that Tih2p plays a role in transcription is supported by our preliminary observation that Tih2p was coimmunoprecipitated with yeast TBP and by several recent reports identifying the p50 family members, TIP49a, Pontin 52, and RUVBL1, which interact with general transcription factors TBP (with TIP49a and Pontin52) and

![Fig. 6. tih2–160 cells incubated at the nonpermissive temperature exhibited a reversible G1 phase cell cycle arrest as large unbudded cells. A, fluorescence-activated cell sorting analysis of the DNA content of TIH2 and tih2–160 strains at the indicated times following a shift from 23 to 37 °C and ultimately to 23 °C. B, the proportion (in percentages) of unbudded, small budded, and large budded cells of TIH2 and tih2–160 strains corresponding to the times indicated in A. C, phase contrast micrographs of tih2–160 cells after 3.5 h at 37 °C (top) followed by a shift back to 23 °C for 2.5 h (bottom). Scale bar, 3 μm.](image-url)
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RNA polymerase II holoenzyme (with RUVBL1) (13, 19, 20). Furthermore, it is known that transcription factors such as Sp1, hepatocyte nuclear factor 1, and RNA polymerase II holoenzyme are modified by O-GlcNAc and can be purified via wheat germ agglutinin-Sepharose column chromatography (1–4). Therefore, these transcriptional machinery components may mediate the binding of p50/p47 to the wheat germ agglutinin column.

Several yeast TAFs have been shown to be required for progression through the cell cycle: mutation of TAF145 leads to G1 arrest (see below), functional inactivation of yTAF190 by ts mutations or depletion leads to arrest at the G2/M phase of the cell cycle (31), and ts mutants of TSM1/TAF150 arrest at the nonpermissive temperature as large-budded cells with a 2N DNA content (46). Northern blot analysis of several RNA transcripts in tih2–160 cells suggests that transcription of G1 cyclin and ribosomal protein genes are down-regulated at the nonpermissive temperature. Other genes like actin, alcohol dehydrogenase, and the constitutive G1 cyclin CLN3 (Fig. 7) as well as those transcribed by RNA polymerase I and III (data not shown) are unaffected. Because this down-regulation occurs even when the levels of other TAF proteins and TBP are normal, we propose that Tih2p has a primary role in the regulation of transcription.

Tih2p May Function Similar to yTAFII145 to Regulate Transcription—yTAFII145 is the only yTAFII known to contact TBP directly (38), and its higher eukaryotic homologue dTAF230/hTAF250 is required for reconstitution of TFIIIB activity in vitro (47). However, yTAFII145 is dispensable for global RNA polymerase II-directed transcription (32). Given the phenotypic similarities between taf145 and tih2 mutants, it is likely that Tih2p, or maybe a complex containing both Tih1p and Tih2p, is required for a mechanism of transcriptional regulation similar to that involving yTAFII145. yTAFII145 is required to mediate stimulation of RNA polymerase II by transcriptional activators, leading to the initiation of transcription (48). Similarly, Tih2p (Tih1p-Tih2p complex) may also act as a bridge for TBP and gene-specific activators to activate transcription. Consistent with this idea is the observation that Pontin52/p50 bridges β-catenin/lymphocyte enhancer factor-1 and TBP to form a multiprotein complex that is believed to transcriptionally activate target genes of Wnt/Wg signaling (20).

Functional Comparison to Other Helicases—Recently, p50 and p47, isolated as TIP49a and TIP49b, have been shown to be ATP-dependent DNA helicases of opposite polarity (18, 22), and they exist in the same complex. This observation is reminiscent of the TFIIH complex, which also contains two DNA helicases, ERCC3/XPB/Ssl2p and ERCC2/XPD/Rad3p, mediating opposite polarity of unwinding (49). Both Ssl2 and Rad3 are essential genes and are required for gene transcription as well as DNA repair. Mutation of the Walker A domain causes lethality in Ssl2Δ cells but not in rad3Δ cells (50, 51). Together with results obtained in vitro using purified mammalian proteins (52), it appears that transcription initiation requires the helicase activity of Ssl2p but not that of Rad3p. On the other hand, both helicase activities are essential for DNA repair. The fact that a K81R mutation in Tih2p generates a lethal allele indicates that the Walker A domain of Tih2p performs an essential cellular function. A similar mutation introduced into the Walker A domain of Tih1p, K84R-Tih1p, also generates a lethal phenotype (data not shown). Taken together with the effect of Tih2p on transcription, these results suggest that Tih1p and Tih2p may be a novel class of helicase involved in transcriptional regulation via ATP utilization.

Recently, the mini-chromosome maintenance proteins, which are ATP-dependent helicases and are required for initiation of DNA replication (53), were reported to co-purify with RNA polymerase II and general transcription factors in high molecular weight holoenzyme complexes isolated from Xenopus oocytes and HeLa cells (54). The result suggests that mini-chromosome maintenance proteins function as components of the RNA polymerase II transcriptional apparatus. In yeast and mammalian cells, mini-chromosome maintenance proteins function as replication licensing factors but are far more abundant than the replication origins to which they bind (55). This abundance of mini-chromosome maintenance proteins may be now partly explained by their additional function in transcription. In yeast, abundance of Tih1p is about twice as much as Mcm3p (56). Although our data here suggest a relationship between Tih2p and the transcriptional machinery, other cellular functions of Tih1p, Tih2p, and hence the Tih1p-Tih2p complex are likely to be revealed by further biochemical and genetic analysis or interaction screening.

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