Two WD Repeat-containing TATA-binding Protein-associated Factors in Fission Yeast That Suppress Defects in the Anaphase-promoting Complex*

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The general transcription factor IID consists of the TATA-binding protein (TBP) and multiple TBP-associated factors (TAFs). Here we report the isolation of two related TAF genes from the fission yeast Schizosaccharomyces pombe as multicopy suppressors of a temperature-sensitive mutation in the ubiquitin-conjugating enzyme gene ubcP4. The ubcP4 mutation causes cell cycle arrest in mitosis, probably due to defects in ubiquitination mediated by the anaphase-promoting complex/cyclosome. One multicopy suppressor is the previously reported gene taf72, whereas the other is a previously unidentified gene named taf73. We show that the taf73 gene, like taf72, is essential for cell viability. The taf72 and taf73 genes encode proteins homologous to WD repeat-containing TAFs such as human TAF100, Drosophila TAF80/85, and Saccharomyces cerevisiae TAF90. We demonstrate that TAF72 and TAF73 proteins are present in the same complex with TBP and other TAFs and that TAF72, but not TAF73, is associated with the putative histone acetylase Gen5. We also show that overexpression of TAF72 or TAF73 suppresses the cell cycle arrest in mitosis caused by a mutation in the anaphase-promoting complex/cyclosome subunit gene cut9. These results suggest that TAF72 and TAF73 may regulate the expression of genes involved in ubiquitin-dependent proteolysis during mitosis. Our study thus provides evidence for a possible role of WD repeat-containing TAFs in the expression of genes involved in progression through the M phase of the cell cycle.

The general transcription factor (TF) IID plays a critical role in transcription initiation of protein-coding genes by RNA polymerase II. TFIIID is a multiprotein complex comprising the TATA-binding protein (TBP) and multiple TBP-associated factors (TAFs), which have been well conserved from yeast to humans (1, 2). TBP specifically recognizes TATA elements, whereas certain TAFs directly interact with initiator or downstream promoter elements. In addition to a role in core promoter recognition, TAFs have been proposed to function as targets of activators. Subsets of TAFs have also been found in histone acetylase complexes distinct from TFIIID (3, 4).

To assess the requirement of individual TAFs for transcription in vivo, yeast (Saccharomyces cerevisiae) mutants have been used (5–13). Interestingly, inactivation of some TAFs results in cell cycle phenotypes. yTAF130/145 inactivation leads to G1 arrest, whereas inactivation of yTAF90 or yTAF150/TSM1 results in G2/M arrest (5, 7). Genome-wide expression analyses have identified sets of genes whose expression depends on yTAF17, yTAF23/25, yTAF60, yTAF61/68, yTAF90, or yTAF130/145 (14, 15). For example, upon inactivation of yTAF90, ~8% of all yeast genes show a significant decrease in expression.

yTAF90 (16, 17) and its human and Drosophila homologs, hTAF100 (18–20) and dTAF80/85 (21, 22), respectively, all contain WD repeats. The WD repeat is a conserved sequence motif usually ending with Trp-Asp (WD), and WD repeat-containing proteins are implicated in a wide variety of cellular functions (23). yTAF90 and hTAF100 are also components of histone acetylase complexes distinct from TFIIID such as the Spt-Ada-Gen5 acetyltransferase (SAGA) and TBP-free TAF1-containing (TFTC) complexes (24, 25). In addition, an hTAF100-related protein, PAF65, is present in the p300/CBP-associated factor (PCAF) and TFTC complexes (25, 26).

Ubiquitin-dependent proteolysis has been shown to play a key role in progression through the cell cycle (27). A ubiquitin-protein ligase complex known as the anaphase-promoting complex or cyclosome (APC/C) promotes the metaphase-to-anaphase transition and the exit from mitosis by mediating ubiquitination of anaphase inhibitors and mitotic cyclins, leading to their destruction by the 26 S proteasome (28). In the fission yeast Schizosaccharomyces pombe, the ubiquitin-conjugating enzyme UbcP4 seems to be involved in APC/C-mediated proteolysis (29). First, depletion of UbcP4, like mutations in APC/C subunit genes such as cut9, blocks the initiation of anaphase. Second, overexpression of UbcP4 suppresses a cut9 mutation. Finally, among the family of ubiquitin-conjugating enzymes, UbcP4 is most closely related to clam E2-C, Xenopus UbcX, and human UbcH10, all of which are involved in ubiquitination of mitotic cyclins.

We report here the isolation of two related TAF genes, taf72 and taf73, from S. pombe as multicopy suppressors of a temperature-sensitive ubcP4 mutation. TAF72 and TAF73 proteins have homology to WD repeat-containing TAFs such as...
An analysis of described (32, 33). Standard methods were used for molecular genetic phenotype). Bar phenotype undergoing cytokinesis without chromosome segregation (a cut cell) and type (S. pombe were counted with a hemocytometer. Log-phase cultures in YE medium were shifted from 25 to 36 °C. Cells without chromosome segregation, and the phenylindole. The panel a, 6-diamidino-2-densed chromosomes. The closed arrowhead indicates a septated cell without chromosome segregation, and the open arrowhead indicates a cell undergoing cytokinesis without chromosome segregation (a cut phenotype). Bar = 10 μm.

hTAF100, dTAF80/85, and yTAF90. We show that both TAF72 and TAF73 are associated with TBP and other TAFs, whereas only TAF72 is associated with Gcn5, a putative histone acetylase. We also show that taf72 and taf73 suppress a mutation in the cut9 gene. These results suggest that TAF72 and TAF73 may regulate the expression of genes involved in progression through the M phase of the cell cycle.

EXPERIMENTAL PROCEDURES

Yeast Strains, Media, and Molecular Genetic Methods—The following S. pombe strains were used in this study: JY741 (h ade6-M216 leu1 ura4-D18), JY746 (h ade6-M210 leu1 ura-D18), ubcP4 (h ade6-M210 ura4 leu1 ubcP4-140::ura4) (this study), cut9 (h leu1 cate1-665; a gift from Mitsubishi Yagaida) (30), and mts2 (h leu1-32 mts2-1; a gift from Coljin Gordon) (31). S. pombe media were prepared as described (32, 33). Standard methods were used for molecular genetic analysis of S. pombe (32, 33).

Isolation of a ubcP4 Mutation—An XhoI-XbaI fragment containing the ubcP4 gene was cloned into the Sall/XbaI site of pUC19 ASS, a pUC19 derivative lacking the SspI site. A ura4 fragment was inserted into the SspI site located 440 base pairs downstream of the ubcP4 gene. A ubcP4::ura4 fragment was amplified by PCR in the presence of 0.5 mm MmcI and used to transform an S. pombe ura4 strain. Ura+ transformants selected at 25 °C were replica-plated onto EMM + Ade containing phloxine B, and the plates were incubated at 35 °C. Plasmids were recovered from white colonies grown at 35 °C and used to retransform the ubcP4 strain. Subcloning of insert from two plasmids (pSP1-7 and pSP1-19) resulted in 2.6-kb SacI-HindIII and 2.2-kb SalI-HindIII fragments capable of suppression (see Fig. 3A). Sequencing followed by data base searches using the BLAST program revealed that the former contained the taf72 gene (35) and the latter contained a related but previously unidentified gene. This gene was named taf73 and analyzed further. taf73 cDNA was amplified by reverse transcription-PCR, cloned into pGem-T (Promega), and sequenced. A 3′-portion of taf73 cDNA was also amplified by 3′-rapid amplification of cdNA ends and sequenced. Disruption of the taf73 Gene—A 3.0-kb genomic DNA fragment containing the taf73 gene was amplified by PCR and cloned into pGem-T to generate pGem-Ttaf73. The entire vector sequence flanked by 5′- and 3′-noncoding sequences of taf73 was amplified from pGem-T(taf73) by PCR, digested with XhoI and SmalI, and ligated with a 1.8-kb XhoI-Smal ura4 fragment to generate pGEM-Ttaf73::ura4. A 2.7-kb blunt-end taf73::ura4 fragment was amplified from pGem-Ttaf73::ura4 by PCR with Vent DNA polymerase (New England Biolabs) and used for the one-step gene disruption (36). After transformation, Ura+ colonies were screened for sensitivity to 5-fluoroorotic acid (Toronto Research Chemicals). Correct disruption was confirmed by PCR. Complementation by a taf73 plasmid (Table I) also confirmed correct disruption.

Construction of S. pombe Strains Expressing Epitope-tagged TAF or Gen5—To construct S. pombe strains expressing FLAG or HA epitope-tagged TAF protein, DNA fragments that encode epitope-tagged TAF were amplified by PCR using primers with overlapping extension (37) and used to replace the chromosome segment by transplacement (38). Strains expressing FLAG-tagged TAF22 or TAF73, in which a FLAG epitope (DYKDDDDK) was inserted at the N terminus of the TAF22 or TAF73 protein, were constructed as follows. DNA fragments containing both a 5′-noncoding region and a 5′-portion of the coding sequence with the FLAG sequence immediately after the initiation codon were amplified by PCR and cloned into pBluescript SK (Stratagene) carrying a ura4 fragment. The resulting plasmids were linearized at a unique restriction site within the taf genes AuaII for taf72 and SpeI for taf73 (see Fig. 3A) and used to transform strain JY741. Integration into the taf locus on the chromosome results in a full-length taf gene with the FLAG sequence and a 3′-truncated taf gene that are separated by the ura4 plasmid sequence. Correct integration was confirmed by PCR. Recombination that occurs upstream of the FLAG sequence leaves only a FLAG-TAF gene on the chromosome. 5-Fluoroorotic acid-resistant segregants were screened by PCR for the presence of the FLAG sequence to obtain taf72FLAG strains. Similarly, an S. pombe strain ex-
pressing HA-tagged TAF73, in which three copies of an HA epitope (YPYDVPDYA) were inserted at the N terminus of the TAF73 protein, was constructed using strain JY746. This taf73 HA strain was crossed with the wild-type, taf72 FLAG, and taf73 FLAG strains described above to construct diploid strains expressing HA-TAF73; HA-TAF73 and FLAG-TAF72; and HA-TAF73 and FLAG-TAF73, respectively.

S. pombe strains expressing HA-tagged Gcn5, in which three copies of an HA epitope were inserted at the N terminus of the Gcn5 protein, were used as multicopy suppressors of ubcP4ts that encode WD repeat-containing TAFs. A genomic DNA fragment from two plasmids (pSP1-19 and pSP1-7) that suppressed the ubcP4-140 mutation resulted in the 2.2-kb SalI-HindIII fragment containing the taf73 gene and the 2.6-kb SacI-HindIII fragment containing the taf72 gene. Exons are indicated by black boxes. A, amino acid sequence alignment of WD repeat-containing TAFs and a related PAF. Sequences are from S. pombe (sp) TAF73 (DDBJ/EMBL/GenBank™ Data Bank accession number AB039954) and TAF72 (accession number AB001372), yTAF90 (accession number Z36067), hTAF100 (accession number U80191), PAF65b (accession number AF069736), and dTAF80 (accession number U06460). The alignment was generated with the ClustalW program. Identical and similar residues were shaded with the program Boxshade. The arrows below the sequences indicate WD repeats.

Fig. 3. Multicopy suppressors of ubcP4ts that encode WD repeat-containing TAFs. A, genomic DNA fragments capable of suppressing the ubcP4ts mutation. Subcloning of inserts from two plasmids (pSP1-19 and pSP1-7) that suppressed the ubcP4-140 mutation resulted in the 2.2-kb SalI-HindIII fragment containing the taf73 gene and the 2.6-kb SacI-HindIII fragment containing the taf72 gene. Exons are indicated by black boxes. B, amino acid sequence alignment of WD repeat-containing TAFs and a related PAF. Sequences are from S. pombe (sp) TAF73 (DDBJ/EMBL/GenBank™ Data Bank accession number AB039954) and TAF72 (accession number AB001372), yTAF90 (accession number Z36067), hTAF100 (accession number U80191), PAF65b (accession number AF069736), and dTAF80 (accession number U06460). The alignment was generated with the ClustalW program. Identical and similar residues were shaded with the program Boxshade. The arrows below the sequences indicate WD repeats.
were resuspended in 700 μl of buffer A (20 mM HEPES-KOH (pH 7.6), 150 mM potassium acetate, 20% glycerol, 0.1% Nonidet P-40, and 1 mM dithiothreitol) with 1 mM phenylmethylsulfonyl fluoride (or 4-(2-aminoethyl)benzenesulfonyl fluoride) and protease inhibitor cocktail. The cell lysates were recovered through centrifugation at 18,000 × g for 10 min. The protein concentration of the extracts was typically determined with anti-FLAG antibody, 300–500 μl of whole-cell extract was mixed with 10 μl of anti-TBP antibody, 200–300 μl of anti-FLAG antibody, 300–500 μl of whole-cell extract was mixed with 10 μl of anti-S. pombe TBP serum or preimmune serum and incubated for 1 h on ice, and then 40 μl of a slurry of protein A-Sepharose (Amersham Pharmacia Biotech) was added and incubated for 1 h at 4 °C on a rotating wheel. The beads were washed three times with 1 ml of buffer A, resuspended in 1.5× SDS gel loading buffer, and frozen at −80 °C. Proteins that had derived from 750 or 1500 μg of total protein were separated by 7.5 or 10% SDS-polyacrylamide gel electrophoresis, transferred to a polyvinylidene fluoride membrane, and probed with antibodies. Immune complexes were detected by chemiluminescence. In some cases, a blot was stripped before reprobing.

Antibodies—Rabbit anti-S. pombe TBP, anti-S. pombe TAF130, and anti-S. pombe PTRE6 polyclonal antibodies were kindly provided by Tetsuro Kokubo (Nara Institute of Science and Technology). Mouse anti-FLAG M2 monoclonal antibody and anti-FLAG M2-agarose were purchased from Sigma, and mouse anti-HA monoclonal antibody (16B12) was purchased from BÄBCO. Peroxidase-conjugated goat anti-rabbit IgG (Cappel) and peroxidase-conjugated goat anti-mouse IgG (Jackson Immunoresearch Laboratories) were used as secondary antibodies.

RESULTS

Isolation of Putative TAF Genes as Multicopy Suppressors of a ubcP4Δ Mutation—Depletion of the UbcP4 protein, an S. pombe ubiquitin-conjugating enzyme, blocks the initiation of anaphase in mitosis, suggesting a role of UbcP4 in cell cycle progression through mitosis (29). To confirm and extend this result, we isolated a temperature-sensitive mutation in the ubcP4Δ gene (designated ubcP4-140 or ubcP4Δ) as described under “Experimental Procedures.” A ubcP4-140 strain showed a rapid cessation of cell growth when the culture was shifted from 25 to 36 °C (Fig. 1A). After the shift to 36 °C, the following types of cells accumulated: metaphase-arrested cells with condensed chromosomes, septated cells without chromosome segregation, and cells undergoing cytokinesis without chromosome segregation (a cut phenotype) (Fig. 1B). At 6 h after the shift, ∼30% of the cells showed septation or cytokinesis without chromosome segregation. Thus, the ubcP4Δ mutation seems to block the initiation of anaphase, thereby causing uncoordinated mitosis where septation or cytokinesis occurs without chromosome segregation. This phenotype closely resembles those caused by mutations in the cut genes that encode components of APC/C such as cut9-665 (30) and cut4-533 (39). It is therefore most likely that UbcP4, in conjunction with APC/C, functions in ubiquitination of proteins required for progression through mitosis, including the anaphase inhibitor (securn) Cut2 and the mitotic cyclin Cdc13 (40).

The ubcP4-140 mutation was used for isolation of multicopy suppressors that enable growth at 35 °C (see “Experimental Procedures”) (Fig. 2). Screening of an S. pombe genomic library unexpectedly identified two related genes that encode proteins with homology to WD repeat-containing TAFs such as hTAF100, dtAF80/85, and yTAF90 (Fig. 3). One gene (represented by five clones) was found to be taf72Δ, a putative TAF gene isolated on the basis of sequence similarity (35). The taf72Δ gene encodes a protein of 643 amino acids with a predicted molecular mass of 72.4 kDa, but its association with TBP has not been demonstrated. The other gene (represented by two clones) was a previously unidentified gene, which has been named taf73Δ. A comparison between the genomic DNA and cDNA sequences of the taf73Δ gene revealed that there is a 56-base pair intron (nucleotides 1474–1529) with consensus sequences for splicing (41).

The taf73Δ gene encodes a protein of 642 amino acids with a predicted molecular mass of 72.3 kDa. Like other WD repeat-containing TAFs, TAF73 contains six WD repeats (23) in its C-terminal half (Fig. 3B). The TAF73 protein is 45% identical to TAF2, 35% identical to yTAF90, 30% identical to hTAF100, and 29% identical to dtAF80/85. Similarity was observed throughout the proteins, although the C-terminal regions show higher degrees of conservation (Fig. 3B). TAF72 is more similar to yTAF90, hTAF100, and dtAF80/85 than TAF73 is. We also compared TAF72 and TAF73 with PAF65, an hTAF100-related protein present in the human histone acetylase complexes PCAF and TPTC (25, 26). Both TAF72 and TAF73 are less related to PAF65 (27 and 22% identical, respectively)
levels were not affected by multicopy plasmids carrying the taf72+ or taf73+ gene. Transformants with pSP1 (Vector), pSP1–7 (taf72+), or pSP1–19 (taf73+) were sporulated and subjected to tetrad analysis.

### TABLE I

| Plasmid       | No. of tetrads for the indicated no. of viable spores | No. of spores |
|---------------|-------------------------------------------------------|---------------|
|               | 0 | 1 | 2 | 3 | 4 | Ura+ | Leu+ |
| Vector        | 6 | 9 | 4 | 0 | 0 | 0   | 0   |
| taf73+        | 1 | 5 | 9 | 5 | 0 | 0   | 6   |
| taf72+        | 0 | 7 | 12| 0 | 0 | 0   | 11  |

*a* Ura+ spores are presumed to be Δtaf73::ura4+ segregants. All viable Ura+ spores were also Leu+.

*b* Leu+ spores are segregants carrying the plasmid.

For unknown reasons, the taf72+ plasmid increased the spore viability of taf73+ segregants.

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**Fig. 6. Association of TAF72 and TAF73 with TBP.** Immunoprecipitated proteins were separated by SDS-polyacrylamide gel electrophoresis, transferred, and probed as described under “Experimental Procedures.” A, co-immunoprecipitation of TBP with TAF72 or TAF73. Whole-cell extracts prepared from wild-type strain JY741 (WT; lane 1) and strains expressing FLAG-TAF73 (taf73FLAG; lane 2) or FLAG-TAF72 (taf72FLAG; lane 3) were used for immunoprecipitation (IP) with antibody to FLAG, followed by immunoblotting with antibodies to TBP, TAF73, and TAF130. B, co-immunoprecipitation of TAF72 or TAF73 with TBP. Whole-cell extracts prepared from strains expressing FLAG-TAF72 (taf72FLAG; lanes 1 and 2) or FLAG-TAF73 (taf73FLAG; lanes 3 and 4) were used for immunoprecipitation with preimmune serum (PI; lanes 1 and 3) or anti-TBP antibody (TBP; lanes 2 and 4), followed by immunoblotting with antibodies to TBP, FLAG, TAF130, and TBP6. C, co-immunoprecipitation of TAF73 with TAF72. Whole-cell extracts prepared from diploid strains expressing HA-TAF73 (taf73HA × WT; lane 1), HA-TAF73 and FLAG-TAF72 (taf72HA × taf72FLAG; lane 2), or HA-TAF73 and FLAG-TAF73 (taf73HA × taf72FLAG; lane 3) were used for immunoprecipitation with antibody to FLAG, followed by immunoblotting with antibodies to FLAG and HA.

than to hTAF100 (34 and 30% identical).

To test the possibility that the suppression of the ubcP4+ mutation by taf72+ or taf73+ results from increased in ubcP4 expression, we carried out Northern analysis. ubcP4 mRNA levels were not affected by multicopy plasmids carrying the taf72+ or taf73+ gene (Fig. 4). We speculate that the suppression results from increased expression of some other genes involved in APC/C-mediated proteolysis (see “Discussion”). Suppression of the ubcP4+ mutation seems to be specific to the taf72+ and taf73+ genes because no other TAF genes were isolated in our library screen.

**TAF72 and TAF73 Have Nonredundant Functions—**The taf72+ gene is essential for cell viability (35). To determine whether taf73+ is also an essential gene, we constructed a diploid S. pombe strain in which one copy of taf73+ was disrupted. The taf73+/Δtaf73::ura4+ cells were sporulated and subjected to tetrad analysis. Of 34 tetrads dissected, 0, 1, and 2 viable spores were observed for 2, 15, and 17 tetrads, respectively, and no tetrads with more than 2 viable spores were recovered (Fig. 5). Importantly, all the viable spores were Ura− and thus presumed to be taf73+. Microscopic observation of the 34 Δtaf73::ura4+ spores revealed that most spores germinated and divided three times before they ceased growing (no spores divided more than four times). In addition, Δtaf73::ura4+ haploid cells carrying a taf73 plasmid, pREP81(taf73cDNA), did not lose the plasmid under nonselective conditions. These results indicate that the taf73+ gene, like taf72+, is essential for cell viability. The Δtaf73 strain carrying the plasmid pREP81(taf73cDNA) grew even under conditions that repress taf73+ expression (i.e. in the presence of thiamine), indicating that residual expression allows cells to grow. Consequently, whether depletion of TAF73 causes a cell cycle phenotype remains to be determined.

We next examined whether overexpression of TAF72 suppresses Δtaf73. The taf73+/Δtaf73::ura4+ diploid strain was transformed with multicopy plasmids carrying the taf72+ or taf73+ gene, sporulated, and subjected to tetrad analysis. As shown in Table I, tetrads with more than two viable spores were recovered from the diploid carrying the taf73+ plasmid, but not from the diploid carrying the taf72+ plasmid, indicating that overexpression of TAF72 did not suppress Δtaf73. Thus, TAF72 cannot substitute for TAF73.

**TAF72 and TAF73 Are Associated with TBP and Other TAFs—**To detect TAF72 and TAF73 proteins, we constructed S. pombe strains expressing FLAG-tagged TAF72 or TAF73, in which the wild-type gene on the chromosome was replaced by a gene encoding the epitope-tagged protein (see “Experimental Procedures”). Whole-cell extracts were prepared from these strains, along with wild-type strain JY741, which did not express any FLAG-tagged protein. Immunoblotting with anti-FLAG antibody detected FLAG-TAF72 and FLAG-TAF73 proteins, which were absent from the extract of the wild-type
hTAF250, dTAF230/250, and yTAF130/145, and PTR6 is a putative TAF that has homology to hTAF55 and yTAF67 (42). Immunoblotting with anti-TAF130 and anti-PTR6 antibodies showed that TAF130 and PTR6 were also co-immunoprecipitated with TAF72 and TAF73 (Fig. 6A, lanes 2 and 3). These results indicate that TAF72 and TAF73 are each present in a complex(es) with TBP, TAF130, and PTR6. Since TAF72 and TAF73 were associated with TAF130, a homolog of TFIIID-specific TAFs, it is most likely that TAF72 and TAF73 are components of the S. pombe TFIIID complex.

Conversely, we carried out immunoprecipitation with anti-TBP antibody. Immunoblotting with anti-FLAG, anti-TAF130, and anti-PTR6 antibodies revealed that TAF72, TAF73, TAF130, and PTR6 were co-immunoprecipitated with TBP (Fig. 6B, lanes 2 and 4). These TAFs were not immunoprecipitated when preimmune serum was used instead of anti-TBP serum (Fig. 6B, lanes 1 and 3). These results demonstrate that TAF72, TAF73, TAF130, and PTR6 are each associated with TBP and therefore, by definition, are TAFs.

**TAF72 and TAF73 Are Present in the Same Complex**—We next asked whether TAF72 and TAF73 are present in the same complex or in distinct complexes. To address this question, we constructed a diploid S. pombe strain expressing both FLAG-tagged TAF72 and HA-tagged TAF73. Immunoprecipitation with anti-FLAG antibody followed by immunoblotting with anti-HA antibody revealed that HA-TAF73 was co-immunoprecipitated with FLAG-TAF72 (Fig. 6C, lane 2). HA-TAF73 was not immunoprecipitated from the extract of a strain expressing HA-TAF73, but not FLAG-TAF72 (Fig. 6C, lane 1). These results clearly indicate that TAF72 and TAF73 are present in the same complex. We then used a diploid strain expressing both FLAG-tagged TAF73 and HA-tagged TAF73 to test the possibility that two copies of TAF73 are present in the same complex. HA-TAF73 was not co-immunoprecipitated with FLAG-TAF73 (Fig. 6C, lane 3), indicating that the complex contains only one molecule of TAF73.

**TAF72, but Not TAF73, Is Associated with Gcn5—**yTAF90, the S. cerevisiae homolog of TAF72 and TAF73, is also present in SAGA, a histone acetylase complex distinct from TFIIID (24). We tested the possibility that TAF72 and TAF73 are shared by TFIIID and other histone acetylase complexes. In S. pombe, a SAGA-like complex has not been characterized. However, data base searches revealed that the S. pombe genome contains genes that encode proteins homologous to SAGA subunits. For example, the SPAC1952.05 gene, which has been predicted by the S. pombe Genome Sequencing Project, encodes a 454-amino acid protein that is 53% identical and 69% similar to S. cerevisiae Gcn5 (439 amino acids), the histone acetylase subunit of the SAGA complex. As shown in Fig. 7A, there is a high degree of conservation between S. cerevisiae Gen5 and its putative S. pombe homolog except for the N-terminal region, which is dispensable for S. cerevisiae Gen5 function in vivo (43). We refer to this gene as gen5 and examine whether its product (Gen5) is associated with TAF72 and TAF73.

We replaced the gen5 gene of the wild-type, taf72FLAG, and taf73FLAG strains with gen5HA, which encodes HA-tagged Gen5 protein, and prepared whole-cell extracts from the strains expressing HA-Gen5, HA-Gen5 and FLAG-TAF72, or HA-Gen5 and FLAG-TAF73. Immunoblotting with anti-HA antibody detected HA-Gen5 protein in the whole-cell extracts (Fig. 7B, lanes 1–3). Immunoblotting with anti-FLAG antibody followed by immunoblotting with anti-HA antibody revealed that Gen5 was co-immunoprecipitated with TAF72, but not with TAF73 (Fig. 7B, lanes 5 and 6). These results indicate that

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2 T. Kokubo, personal communication.
TAF72 is associated with Gcn5. Thus, it is likely that *S. pombe* has a SAGA-like complex that shares TAF72 with TFIID. Interestingly, in contrast to TAF72, TAF73 is not present in the putative SAGA-like complex. The association of Gcn5 with TAF72 is consistent with the prediction that the *gcn5* gene (SPAC1952.05) encodes a homolog of *S. cerevisiae* Gcn5.

**Overexpression of TAF72 or TAF73 Suppresses a cut9* Mutation**—The ubiquitin-conjugating enzyme UbcP4 seems to be involved in APC/C-mediated proteolysis during mitosis because deletion (29) or inactivation (see above) of UbcP4 results in an anaphase block similar to those caused by mutations in the APC/C subunit genes such as cut9-665 and because overexpression of UbcP4 suppresses the cut9-665 mutation (29). We asked whether *taf72* and *taf73* are able to suppress the cut9-665 mutation as well as the *ubcP4-140* mutation. As shown in Fig. 8A, multicopy plasmids carrying the *taf72* or *taf73* gene suppressed the temperature-sensitive growth of a cut9-665 mutant. This did not seem to result from an increase in UbcP4 expression because the *taf72* and *taf73* plasmids did not affect *ubcP4* mRNA levels (Fig. 4). We tested analogous suppression in *S. cerevisiae* using the TAF90 gene (the *taf72* and *taf73* homolog) and a mutation in the CDC16 gene (the *cut9* homolog). A multicopy plasmid carrying the TAF90 gene did not suppress the temperature-sensitive growth of a *cdc16-1* mutant at 30 °C on synthetic or complex medium (data not shown).

As described above, *taf72* and *taf73* are able to suppress mutations in both a ubiquitin-conjugating enzyme gene (*ubcP4*) and a ubiquitin-protein ligase complex subunit gene (*cut9*). To test specificity of suppression, we examined whether *taf72* and *taf73* suppress a mutation in another gene involved in ubiquitin-dependent proteolysis. The *mts2* gene encodes a homolog of the *S. pombe* hTAF250, dTAF230/250, and yTAF130/145. We demonstrated that TAF72, TAF73, and PTR6 has been shown to be associated with TBP. T. Kokubo and colleagues have identified another TAF gene, *taf130*, which encodes the *S. pombe* homolog of hTAF250, dTAF230/250, and yTAF130/145. In this study, we have isolated and characterized *taf72* and a new gene named *taf73*. We demonstrated that TAF72, TAF73, TAF130, and PTR6 are all associated with TBP. Thus, we conclude that these proteins are indeed TAFs. To our knowledge, this is the first report of biochemical characterization of TAFs in *S. pombe*. Data base searches revealed that the *S. pombe* genome contains many putative TAF genes, including those encoding homologs of human TAF150, TAF70/80, TAF31/32, TAF30, TAF28, TAF20, and TAF18 (data not shown). It is thus most likely that *S. pombe* has a TFIID complex(es) similar to those identified in human, *Drosophila*, and *S. cerevisiae*. The association of TAF72 and TAF73 with TAF130, a homolog of TFIID-specific TAFs, suggests that TAF72 and TAF73 are components of the *S. pombe* TFIID complex. It has been shown that there are multiple forms of TFIID complexes (45). We showed that TFIID in *S. pombe* contains both TAF72 and TAF73. It remains to be determined, however, whether *S. pombe* has multiple TFIID complexes.

Our results have implications for the stoichiometry of WD repeat-containing TAFs in the TFIID complex. We showed that TAF72 and TAF73 are present in the same complex. Unlike *S. pombe* TFIID, the human, *Drosophila*, and *S. cerevisiae* TFIID complexes contain a single species of WD repeat-containing TAF: *hTAF100*, *dTAF80/85*, and *yTAF90*, respectively. We speculate that these TAFs might be present in two copies in TFIID.

Subsets of TAFs have been found in histone acetylase complexes distinct from TFIID (3). WD repeat-containing TAFs are present in non-TFIID complexes such as SAGA and TFTC (24, 25). We showed that TAF72 is associated with Gcn5, a homolog of the histone acetylase subunit of the *S. cerevisiae* SAGA complex. In contrast to TAF72, TAF73 is not associated with Gcn5. It seems that TAF72 is present in both TFIID and SAGA-like complexes, whereas TAF73 is present only in TFIID.

**TAF and Cell Cycle**—The *taf72* and *taf73* genes were isolated as multicopy suppressors of a mutation in the ubiquitin-conjugating enzyme gene *ubcP4*. *taf72* and *taf73* also suppressed a mutation in the ubiquitin-protein ligase complex subunit gene *cut9*. Overexpression of TAF72 or TAF73 from the expression vector pREP1 carrying the *taf72* or *taf73* cDNA inhibited cell growth (*taf73* was more inhibitory than *taf72*) (data not shown). We think that this is due to interference of TFIID function. In contrast, suppression was observed for multicopy plasmids carrying the genomic DNA fragment of...
taf72Δ or taf73Δ, and these plasmids did not inhibit cell growth (probably because of lower expression levels). Thus, it is likely that moderate overexpression leads to suppression, but higher expression is deleterious to the cell.

We infer that moderate overexpression of TAF72 or TAF73 might lead to an elevated level of TFIIID or other TAF-containing complexes, which in turn results in increased expression of certain genes involved in APC/C-mediated proteolysis. Transcription might be generally increased upon overexpression of TAF72 or TAF73. Alternatively, overexpression of TAF72 or TAF73 might affect the transcription of only a subset of genes. We favor a model in which TAF72 and TAF73 are specifically required for the expression of a subset of genes involved in cell cycle progression through mitosis, including those involved in APC/C-mediated proteolysis, because yTAF90, the S. cerevisiae homolog of TAF72 and TAF73, is not generally required for transcription (15, 46), and a taf90Δ mutation causes cell cycle arrest at G2/M (7).

Genome-wide expression analysis was carried out with yTAF90 (15). The yTAF90-dependent genes include APC2, an APC/C subunit gene, which might explain the G2/M arrest phenotype caused by the taf90Δ mutation. Since yTAF90 is shared by TFIIID and SAGA, the transcription defects caused by yTAF90 inactivation should reflect yTAF90 function in both TFIIID and SAGA. Unlike yTAF90, TAF73 is not present in SAGA. Therefore, TAF73 will provide a good model for understanding the in vivo function of WD repeat-containing TAFs in TFIIID.

As discussed above, TAF72 and TAF73 may, directly or indirectly, regulate the expression of genes involved in APC/C-mediated proteolysis. Northern analysis indicated that ubcP4 mRNA levels did not increase upon overexpression of TAF72 or TAF73. cut9Δ or other APC/C subunit genes may be regulated. A multicopy suppressor of the cut9Δ mutation, hcn1Δ, has been reported that encodes a protein homologous to the S. cerevisiae APC/C subunit Cdc26 (47). Since Cut9 function is regulated by the protein kinase A pathway (47), possible candidates include genes involved in this pathway. It should be noted that the expression of TAF72- or TAF73-dependent genes is not necessarily cell cycle-regulated. In fact, genome-wide expression analyses in S. cerevisiae showed that the expression of most of the genes involved in APC/C-mediated proteolysis is not cell cycle-regulated, although some (for example, APC1 and CDC20) show cell cycle fluctuation (48, 49). In contrast, the expression of many APC/C subunit genes (APC4, APC5, APC9, APC11, CDC16, CDC23, CDC26, and CDC27) and APC/C activator genes (CDC20 and CDH1/HCT1) is induced through sporulation (meiosis) (50).

Our results provide evidence for a possible role of WD repeat-containing TAFs in the expression of genes involved in progression through the M phase of the cell cycle. Genes that require TAF72 or TAF73 function remain to be identified. Conditional lethal mutations would be useful to analyze the cell cycle phenotype and gene expression upon inactivation of TAF72 or TAF73.

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