Published online December 22, 2004

6706–6715 *Nucleic Acids Research, 2004, Vol. 32, No. 22*
doi:10.1093/nar/gkh1000

Tfg3, a subunit of the general transcription factor TFIIF in *Schizosaccharomyces pombe*, functions under stress conditions

Makoto Kimura* and Akira Ishihama[1]

Gene Engineering Division, BioResource Center, RIKEN (Institute of Physical and Chemical Research), Tsukuba, Ibaraki 305-0074, Japan and [1]Nippon Institute for Biological Science, Ome, Tokyo 198-0024, Japan

Received August 31, 2004; Revised and Accepted November 25, 2004

ABSTRACT

TFIIF is a general transcription factor (GTF) that binds to RNA polymerase II (pol II) for subsequent recruitment of pol II to a promoter. TFIIF of *Saccharomyces cerevisiae* contains a small subunit, designated Tfg3, in addition to two conserved subunits, TFIIFα (Tfg1) and TFIIFβ (Tfg2). In this study, we characterized Tfg3 of *Schizosaccharomyces pombe*. Using Tfg3 fused to green fluorescent protein (GFP), we found that Tfg3 is located in nuclei, and it is assembled into the C-terminal domain phosphatase (Fcp1)/TFIIF/pol II complex via interactions with TFIIFα and TFIIFβ. As in the case of *S.cerevisiae*, Tfg3 in *S.pombe* forms part of another GTF, namely TFIID. The TFIID complex isolated from *S.pombe* that had been cultured at elevated temperatures included increased levels of Tfg3. The interaction of recombinant Tfg3 with TATA-binding protein (TBP), the central subunit of TFIID, was temperature-dependent. Moreover, a mutant of *S.pombe* that lacked the gene for Tfg3 was sensitive to a battery of stresses including temperature upshift. Starting from a mutant with *tfg3*$^-$ mutation, we isolated five species of multicopy suppressors. Expression levels of the suppressor genes were lower in the mutant cell than in wild-type cell at an elevated temperature. Taken together, we propose that Tfg3 is involved in transcriptional regulation under stress conditions, in particular, at high temperatures.

INTRODUCTION

The transcription factor TFIIF, which consists of α and β subunits, was originally isolated from mammalian cells as the pol II-associating proteins, RAP74 and RAP30 (1), and it was characterized as a member of GTFs (2). TFIIF was also purified from *Saccharomyces cerevisiae* as transcription factor g, which, in contrast, has three subunits: Tfg1, Tfg2 and Tfg3 (3). The two larger subunits, Tfg1 and Tfg2, are homologs of human TFIIFα and TFIIFβ, respectively (4). Although Tfg3 resembles the human leukemogenic proteins ENL and AF-9 (5), these latter proteins are not GTFs. To date, no counterpart of Tfg3 has been found in mammalian cells. The primary function of TFIIF is the recruitment of pol II to a promoter, leading to generate a preinitiation complex (PIC). In the sequential model of PIC assembly, the TFIID/TFIIA/TFIIB complex is first formed on a promoter and thereafter the pol II/TFIIF complex is recruited to this preformed PIC (6); finally, TFIIE and TFIIH associate to form the complete PIC (7,8). A recently proposed alternative model suggests that TFIIF is incorporated into the pol II holoenzyme, which then binds to a promoter as a preassembled complex (9,10). In addition, TFIIF has been suggested to function as an elongation factor (11).

In a previous study, we isolated another TFIIF complex from *Schizosaccharomyces pombe*, which contained Fcp1 and pol II (12). The C-terminal domain (CTD) of the largest subunit of pol II consists of heptapeptide repeats, which undergo reversible phosphorylation throughout the transcription cycle. The enzyme Fcp1 has been identified as the CTD-phosphatase. Thus, this Fcp1/TFIIF/pol II complex was considered to represent a complex for CTD dephosphorylation (12). In this complex, TFIIF might play a regulatory role for enhancement of the Fcp1–pol II interaction by binding to both pol II and Fcp1 (13). In this ternary complex, we identified an *S.pombe* homolog of Tfg3 (12). In *S.cerevisiae*, Tfg3 is included in three types of the transcription complex, i.e. TFIID with core promoter-binding activity (4), the chromatin-remodeling complex SWI/SNF (5) and the nucleosomal histone-acetyltransferase complex NuA3 (14). The *TFG3* gene was originally isolated as the actin non-complementing gene *ANC1* (15). However, little is known about the functions of Tfg3 in these various complexes. We have performed a detailed characterization of Tfg3 in *S.pombe* and the results herein described suggest that Tfg3 is involved in the regulation of transcription under stress conditions.

MATERIALS AND METHODS

Strains of *S.pombe*

Strains JY741 (*h*$^-$ *ade6-M216 ura4-D18 leu1*) and JY746 (*h*$^+$ *ade6-M210 ura4-D18 leu1*) were the parental strains used in this study. For the genetic manipulations, we used

*To whom correspondence should be addressed. Tel: +81 29 836 3612; Fax: +81 29 836 9120; Email: kimura@brc.riken.jp

standard methods and media (16,17). Strain JY741/f-rpb3 was described previously (12). Strains JY741/f-tfg3 and JY741/gfp-tfg3 were constructed by two-step gene replacement, as described elsewhere (18), by transforming JY741 cells with pUC-ftfg3-ura4 and pUC-GFPtfg3-ura4, respectively, after digestion of plasmids with XhoI. To construct strain JY741/tfg3::ura4, we digested pBS-tfg3::ura4 with NdeI and SalI, and introduced the fragment into JY741. Strain JY741/tfg3Δ was generated by growing JY741/tfg3::ura4 cells on a plate prepared with 5-fluoroorotic acid. Strain JY741/tfg3::LEU2 was constructed by transforming JY741 with a PCR-amplified DNA fragment that corresponded to nucleotide (nt) −895 to nt +2062 of the *tfg3* sequence in pBS-tfg3::LEU2. To generate the *tfg3*$^+$/*tfg3::ura4*$^+$ diploid strain, we used pBS-tfg3::ura4, digested with NdeI and SalI, for transformation of a diploid strain generated by mating JY741 and JY746. The designations of all the strains we constructed here were confirmed by PCR and Southern-blotting. Derivatives of JY741 that expressed FLAG-TAF72 and FLAG-TAF73 were a gift from Dr H. Mitsuzawa (19).

Plasmids

Plasmid pUC-ura4 is a derivative of pUC18 with the *ura4*$^+$ fragment at the HindIII site. Plasmids pUC-ftfg3-ura4 and pUC-GFPtfg3-ura4 were generated from pUC-ura4. Both contain a fragment of the genomic DNA of *S.pombe* from nt −898 to nt +2068 relative to the site of translation initiation of *tfg3*$^+$ between BamHI and SalI sites. Plasmids pUC-ftfg3-ura4 and pUC-GFPtfg3-ura4 include sequences that encode FLAG-tag and GFP, respectively, inserted between the first and the second codon of *tfg3*$^+$ at PCR-generated NheI and ApaI sites. Plasmid pET-GSTtfg3 is a derivative of pET21b (Novagen) that encodes a glutathione S-transferase (GST)–Tfg3 fusion protein between the NheI and XhoI sites of pET21b. Plasmid pET–GST–TFIIFβ/α-H is a derivative of pET21b that encodes the GST–TFIIFβ fusion protein under control of a T7 promoter, an internal T7 promoter and TFIIFα between the NheI and XhoI sites of pET21b. The expressed TFIIFα protein (TFIIFα-H) carries a C-terminal hexahistidine (His_6)-tag. The pET-H-TBP and pET-H-TFIIB plasmids are derivatives of pET21d that encode amino-terminal His_6-tagged TBP (H-TBP) and TFIIB (H-TFIIB), respectively, between the NcoI and EcoRI sites of pET21d. The cDNA inserts encoding TBP and TFIIB were cloned by PCR from an *S.pombe* cDNA library. Plasmid pET-TFIIAα/β-H was constructed by inserting a PCR-amplified cDNA that encoded the large subunit of TFIIA between the NdeI and XhoI sites of pET21b for expression of a C-terminal His_6-tagged protein (TFIIAα/β-H). The large subunit of *S.pombe* TFIIA corresponds to a fusion of mammalian subunits TFIIAα and TFIIAβ, and it is referred to herein as TFIIAα/β. Plasmid pET-tfg3-H is a derivative of pET21b that encodes Tfg3 between the NdeI and XhoI sites of pET21b as to express a C-terminally His_6-tagged Tfg3 (Tfg3-H) protein. Plasmid pET-fcp1-H which encodes C-terminally His_6-tagged Fcp1 (Fcp1-H) was described previously (12). Plasmid pBS-tfg3::ura4 and pBS-tfg3::LEU2, derived from pBluescriptSK$^+$ (Clontech), include the DNA sequence from nt −899 to nt −49 of *tfg3*$^+$ plus *ura4*$^+$ and an *LEU2* gene from *S.cerevisiae*, respectively, and the sequence from nt +1116 to nt +2068 of *tfg3*$^+$ between the BamHI and SalI sites of pBluescriptSK$^+$ pREP81-rpb4 and pREP81-tfg3, derived from pREP81 (20), include DNA sequences that encode Rpb4 and Tfg3, respectively, between the NdeI and BamHI sites of pREP81.

Recombinant proteins

Each recombinant protein was expressed and purified on a column of glutathione (GSH)-Sepharose 4B (Amersham) or of Ni^{2+}-NTA agarose (Qiagen) column essentially as described elsewhere (12). The GST–TFIIFβ/α-H protein, which was a complex of TFIIFα-H and GST–TFIIFβ, was purified by chromatograph on the two columns in succession.

GST-pull-down assay

Ten μg of individual recombinant proteins or 2 μg of FLAG-tagged pol II [f-pol II, purified as described in ref. (12)] in 125 μl of reaction buffer [0.3 M potassium acetate, 50 mM Tris-acetate, pH 7.8, 10% glycerol, 1 mM EDTA, 1 mM DTT, 0.5% Nonidet P-40, 0.5 mM phenylmethylsulfonyl fluoride (PMSF)] was incubated at the appropriate temperature for 30 min. Then, 10 μl of GSH–Sepharose 4B that had been equilibrated with the same buffer was added, and the mixture was incubated on ice for 10 min. The resin was washed four times with 1 ml of the same buffer, and the proteins were eluted with 25 μl of sample buffer for SDS–PAGE. One-fifth of the eluted sample was analyzed by SDS–PAGE.

Purification of FLAG-tagged complexes

FLAG-tagged complexes were purified from the 'First-extract' and the 'Nuclease-treated extract' of JY741/f-rpb3 and JY741/f-tfg3 as described previously (12). Briefly, frozen cells were disrupted with Cryopress (Microtech Nichion) and suspended in 1× cell weight of buffer B (0.1 M potassium acetate, 0.1 M Tris-acetate, pH 7.8, 20% glycerol, 1 mM EDTA, 1 mM EGTA, 2 mM DTT, 0.4% Nonidet P-40, 1 mM PMSF). The cell lysate was then centrifuged at 15 000 *g* for 10 min, and the soluble fraction was centrifuged again at 100 000 *g* for 2 h. The resulting supernatant was designated as the 'First extract'. The pellet of the first centrifugation was suspended in buffer C (50 mM potassium acetate, 50 mM Tris-acetate, pH 7.8, 10% glycerol, 0.5 mM EDTA, 0.5 mM EGTA, 1 mM DTT, 0.2% Nonidet P-40, 0.5 mM PMSF) and centrifuged at 15 000 *g* for 10 min. The pellet was suspended in buffer D (50 mM potassium acetate, 50 mM Tris-acetate, pH 7.8, 10% glycerol, 10 mM $MnCl_2$, 1 mM DTT, 0.2% Nonidet P-40, 0.5 mM PMSF) containing 10 μg/ml each of DNaseI and RNaseA. After incubation at 30°C for 20 min, the digested pellet was centrifuged at 100 000 *g* for 2 h, and the supernatant was designated as the 'Nuclease-treated extract'. For 1 ml of the First extract and the Nuclease-treated extract, 20 μl of FLAG-specific antibody conjugated M_2-agarose (Sigma) equilibrated with the buffer C was added and gently mixed for 2 h. The M_2-agarose was washed four times with 1 ml of buffer C, and the bound proteins were eluted with 1× resin volume of buffer C containing 100 μg/ml of FLAG-peptide (Sigma).

FLAG-tagged TFIID was isolated from the derivatives of JY741 that expressed FLAG-Taf72 and FLAG-Taf73 (19) by essentially the same method as the First extract preparation with the exception that the buffer contained 0.15 M potassium acetate.

Antibodies and western-blotting analysis

Preparation of anti-Tfg3, anti-Fcp1 and anti-pol II subunit antisera was described elsewhere (12,21). M_2 monoclonal antibody against FLAG was purchased from Sigma. Quantitative western-blotting analysis of Tfg3 was performed as described elsewhere (21) with purified GST–Tfg3 and H_6-tagged Tfg3 as standards. Briefly, the concentrations of recombinant proteins were determined from intensities of SYPRO Orange (Molecular Probes) staining of an SDS–PAGE gel, which contained varying amounts of standard BSA and the recombinant proteins. Whole-cell lysate of *S.pombe* was prepared from the culture of which cell number was counted. Using the recombinant proteins as standards, the concentration of Tfg3 in the lysate was determined by western-blotting with the anti-Tfg3 antiserum.

Fluorescence microscopy

Images of a single cell of strain JY741/gfp-tfg3 were collected and processed as described elsewhere (21) with a 100× objective.

Assay for $CdSO_4$ sensitivity

Strains JY741 and JY741/tfg3Δ were cultured in YE(+Ade +Ura) medium (16) at 30°C to the exponential-phase, and varying concentrations of $CdSO_4$ were added to the cultures. After incubation at 30°C for 48 h, aliquots of the cultures were plated. The numbers of colonies were counted after 4 days of incubation at 30°C.

Isolation of multicopy suppressors of the *tfg3*$^-$ mutation

Strain JY741/tfg3::LEU2 was transformed with an *S.pombe* genomic library that had been constructed with the multicopy plasmid pFL20 (22). Transformants were selected on EMM(+Ade) plates (16) at 30°C, replica-plated and incubated at 38°C. Plasmids were recovered from the colonies that grew up and used to re-transform JY741/tfg3::LEU2 cells. Six individual plasmids that suppressed the thermo-sensitive (*ts*) phenotype were sequenced. A search through the database of the *S.pombe* Genome Project (Sanger Institute) revealed that 13 genes in total were included in the clones. Each gene and its flanking regions were subcloned into pFL20 and re-introduced into JY741/tfg3::LEU2 cells. Five individually isolated plasmids supported cell growth at 38°C.

Analysis of mRNA

Strains JY741 and JY741/tfg3Δ were cultured in YE(+Ade +Ura) medium at 30°C to the exponential-phase and shifted to 38°C. Immediately before the temperature shift and 1 and 4 h after it, the cells were collected. Total RNA was isolated by phenol extraction method (23). For slot-blot analysis, 5 μg each of the total RNA was slot-blotted onto a Hybond-N^+ (Amersham Biosciences) membrane and cross-linked by UV irradiation. Poly (dT) was labelled with fluorescein using Gene Images 3′-Oligolabellimg Module (Amersham Biosciences) for a probe. Northern-blotting was performed using 10 μg each of the total RNA. After electrophoresis, RNA was transferred to a Hybond-N^+ membrane and cross-linked. Probes were prepared using SphI–SalI 1.0 kb fragment of SPCC1529.01, KpnI–KpnI 2.2 kb fragment of SPBC3B8.09, SpeI–ClaI 1.1 kb fragment of SPAC25H1.02, XbaI–EcoRV 1.7 kb fragment of SPBC1734.11 and EcoRI–EcoRI 0.5 kb fragment of SPAC30D11.13 by Gene Images Random Prime Labeling Module (Amersham Biosciences). Hybridization, washing and signal detection were performed as the manufacturer's instruction using Gene Images CDP-Star Detection Module (Amersham Biosciences).

RESULTS

Tfg3 of *S.pombe* is included in the Fcp1/TFIIF/pol II complex

In a previous study, we isolated the Fcp1/TFIIF/pol II complex from *S.pombe* JY741/f-rpb3 and JY741/f-fcp1, which expressed the FLAG-tagged Rpb3 (f-Rpb3) subunit of pol II and FLAG-tagged Fcp1 (f-Fcp1), respectively (12). After analysis of the polypeptide composition of this complex, we identified the *S.pombe* homolog of Tfg3, a subunit of *S.cerevisiae* TFIIF (Figure 1A). To confirm that Tfg3 is an intrinsic component of the *S.pombe* TFIIF complex, we introduced the sequence that encodes the FLAG-tag into the chromosomal *tfg3*$^+$ gene of strain JY741. Then we have purified the FLAG-tagged Tfg3 (f-Tfg3) and associated proteins from cell extracts using FLAG-specific monoclonal antibody-conjugated agarose. From the 'First-extracts', which contained non-DNA-bound pol II (12) from both JY741/f-rpb3 and JY741/f-tfg3 cells, we obtained complexes with essentially the same protein composition in both cases (Figure 1B, left), an indication that Tfg3 was tightly associated with the unbound complex. In contrast, in the case of the 'Nuclease-treated extracts', which contained engaged pol II (12), the yield of the immuno-purified pol II complex from JY741/f-tfg3 was lower than that from JY741/f-rpb3 cells (Figure 1B, right), indicating that only a fraction of the DNA-bound pol II is associated with Tfg3.

We examined the intracellular localization of Tfg3, by fluorescence microscopy, in the strain JY741/gfp-tfg3, which harbored a fusion gene for GFP–Tfg3 on its chromosome in place of the wild-type *tfg3*$^+$ gene. As in the case of GFP–Rpb1 (pol II subunit 1) and GFP–Rpb3 (pol II subunit 3) (21), GFP–Tfg3 was localized in the nucleoplasm but not in the nucleolus or the cytoplasm (Figure 2A). This observation agrees with the prediction that Tfg3 functions in nucleosomes as a part of the transcriptional machinery.

Tfg3 is a subunit of TFIIF

Mammalian TFIIF consists of two subunit components, TFIIFα and TFIIFβ (1,2). In contrast, TFIIF of *S.cerevisiae* is composed of three components, Tfg1 (TFIIFα homolog), Tfg2 (TFIIFβ homolog) and Tfg3 (3,4). To determine the molecular composition of TFIIF in *S.pombe*, we examined the binding of Tfg3 to a TFIIFα/β binary complex in a GST pull-down assay. We incubated Tfg3 with His_6-tag at the C-terminus (Tfg3-H) with either the GST–Tfg3 fusion protein or the GST–TFIIFβ/α-H complex that contained a GST-fused (at the N-terminus) β subunit and a His_6-tagged (at the C-terminus) α subunit. We found that Tfg3-H bound to GST–Tfg3 (Figure 1C), which suggested the formation of dimers (or oligomers) of Tfg3-H and GST–Tfg3. Tfg3-H also bound to the GST–TFIIFβ/α-H complex (Figure 1C).

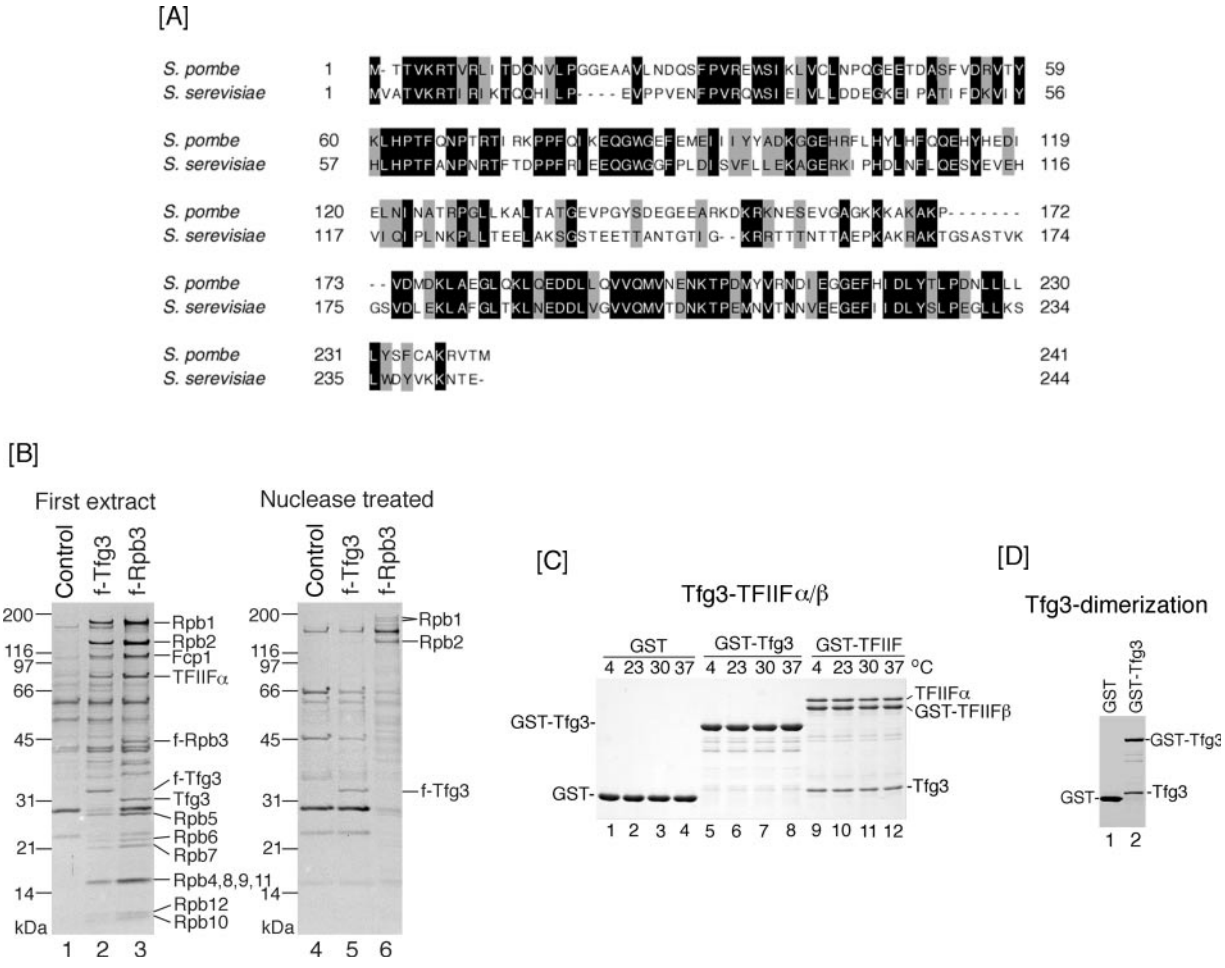

Figure 1. (**A**) Comparison of deduced amino acid sequences of Tfg3 from *S.pombe* (GenBank accession no. T38217) and *S.cerevisiae* (AAA61644). The sequences were aligned by ClustalW. Black boxes indicate identical residues; gray boxes indicate similar residues. (**B**) Proteins, isolated with FLAG-specific antibody-conjugated agarose from the 'First extract' and the 'Nuclease-treated extract' (12) of JY741 (Control), JY741/f-tfg3 (f-Tfg3) and JY741/f-rpb3 (f-Rpb3) cells, were fractionated by SDS–PAGE and silver-stained. Positions of molecular mass markers are indicated on the left. (**C**) The Tfg3–TFIIFα/β interaction. Tfg3-H was incubated with GST, with GST–Tfg3 and with GST–TFIIFβ/α-H at indicated temperatures and then mixtures were supplemented with GSH-Sepharose. The proteins bound to the resin were separated by SDS–PAGE, and the gel was stained with Coomassie brilliant blue (CBB). (**D**) Dimerization of Tfg3. Tfg3-H was mixed with GST and with GST–Tfg3 in denaturing buffer. After dialysis, proteins that bound to GSH–Sepharose were analyzed by SDS–PAGE.

Since the *tfg3*$^{-}$ strain had a *ts* phenotype (see below), we examined the dimerization of Tfg3 and the formation of a complex between Tfg3 and TFIIFα/β at various temperatures. At all temperatures tested, Tfg3-H bound to GST–Tfg3 and to GST–TFIIFβ/α-H. The binding of Tfg3-H to GST–Tfg3 was limited (Figure 1C), perhaps because both the GST–Tfg3 and the Tfg3-H formed dimers prior to mixing. To examine this possibility, we mixed Tfg3-H and GST–Tfg3 together and treated the mixture with guanidine hydrochloride. After dialysis to remove guanidine hydrochloride, we found that more Tfg3-H formed complexes with GST–Tfg3 (Figure 1D), confirming our hypothesis that Tfg3 forms dimers or multimers. Our observations indicated that Tfg3 is a component of TFIIF in *S.pombe* as it is in *S.cerevisiae*.

The *tfg3*$^{-}$ mutation is associated with thermo-sensitive phenotype

To determine whether *tfg3*$^{+}$ is an essential gene, we constructed a diploid strain in which one copy of *tfg3*$^{+}$ was replaced by a *ura4*$^{+}$ marker gene. The *tfg3*$^{+}$/*tfg3::ura4*$^{+}$ diploid strain was induced to sporulate, and tetrads were dissected on a YE (+Ade +Ura) plate. Four spores from each tetrad grew (Figure 2B) and each tetrad contained two Ura^{+} and two Ura^{-} spores (data not shown), indicating that *tfg3*$^{+}$ was non-essential for cell growth under the conditions employed. In order to investigate the phenotype of *tfg3*$^{-}$ mutants, we constructed two haploid strains: JY741/tfg3::ura4, in which *tfg3*$^{+}$ was disrupted by *ura4*$^{+}$; and JY741/tfg3Δ, with the *ura4*$^{+}$ marker removed. Then we examined viability at various temperatures (Figure 2C). At 30°C, JY741/tfg3::ura4 grew faster than the parental strain JY741, presumably as a result of addition of the *ura4*$^{+}$ gene. At 38°C, however, the growth rate of both JY741/tfg3::ura4 and JY741/tfg3Δ was significantly reduced than JY741, and only small-sized colonies were detected after prolonged incubation, indicating that *tfg3*$^{+}$ was essential for cell growth at elevated temperatures.

We also examined the effects of overexpression of Tfg3 by expressing it on the *tfg3*$^{+}$ background via a multicopy plasmid in which the gene was under the control of a

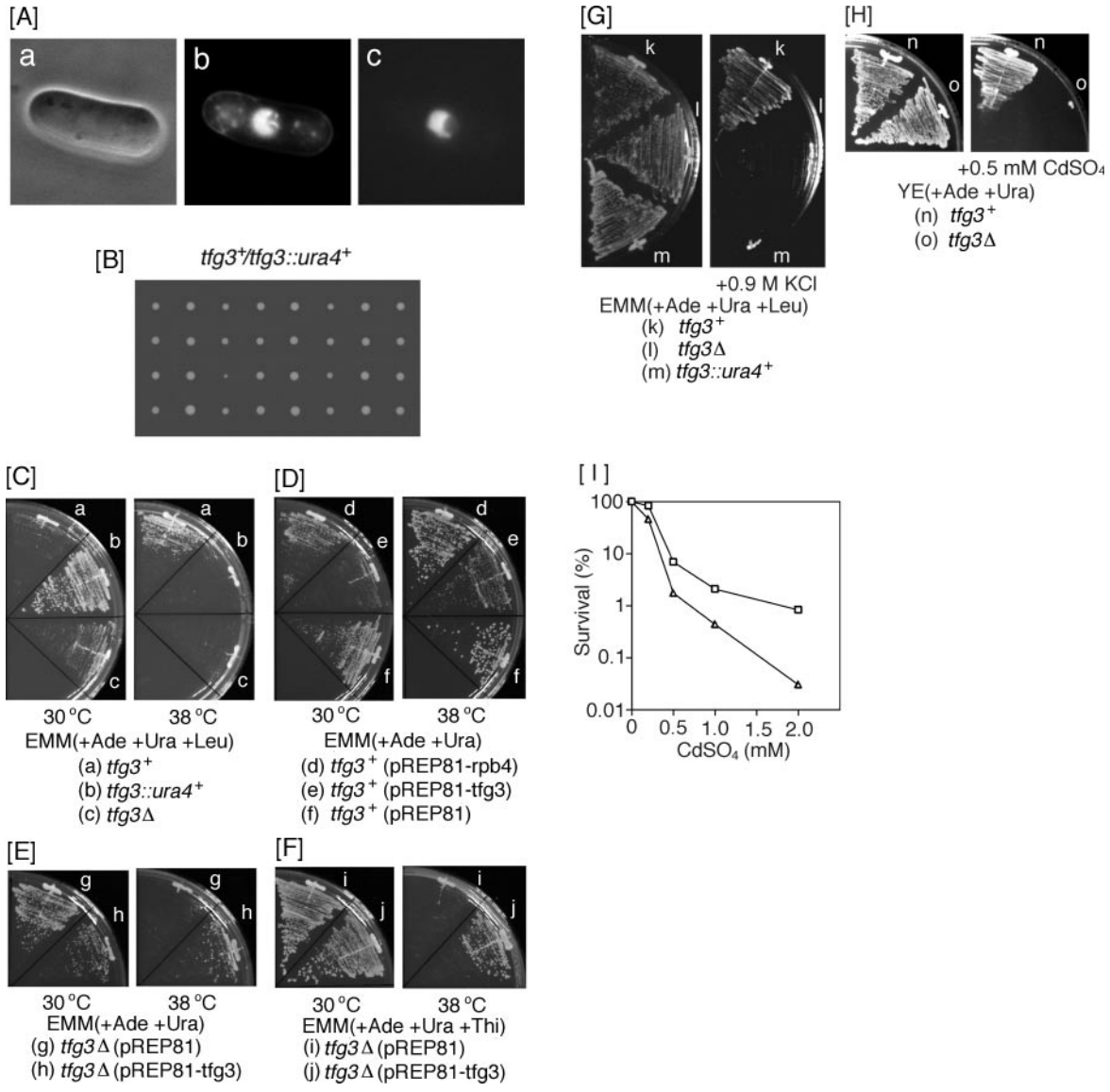

Figure 2. (**A**) Fluorescence micrographs of an *S.pombe* cell expressing the GFP–Tfg3 fusion protein. Phase contrast (a), 4′,6-diamidino-2-phenylindole staining (b) and green fluorescence (c) images of the same JY741/gfp-tfg3 cell. (**B**) Tetrad analysis of the *ura4-D18*/*ura4-D18 tfg3*$^{+}$/*tfg3*::*ura4*$^{+}$ diploid strain of *S.pombe*. Eight asci were dissected. Spores from the same tetrad are arrayed vertically. (**C**) Thermo-sensitivity of the *tfg3*$^{-}$ mutant. Derivatives of JY741 with the indicated *tfg3* alleles were streaked on the indicated plates and incubated at 30°C for 2.5 days or 38°C for 4 days. (**D**) Inhibition of cell-growth upon overexpression of Tfg3. Plasmids expressing Rpb4 (pol II subunit 4) or Tfg3 or the empty vector was introduced into JY741 and cell growth was examined as indicated. The plates were incubated for 4 days. (**E**) Rescue of the thermo-sensitivity of the *tfg3*$^{-}$ mutant by overexpression of Tfg3. Plasmids expressing or not expressing Tfg3 were introduced into the *tfg3*Δ strain and cell growth was examined. The plates were incubated at 30°C for 4 days or at 38°C for 11 days. (**F**) Effective reversal of the thermo-sensitivity of the *tfg3*$^{-}$ mutant by low-level expression of Tfg3. The same transformants as in (E) were grown under the conditions that repressed for the thiamine-repressible expression plasmid. The plates were incubated at 30°C for 4 days or 38°C for 8 days. (**G**) Sensitivity to osmotic-stress of the *tfg3*$^{-}$ mutant. Derivatives of JY741 with the indicated *tfg3* alleles were streaked on plates prepared with and without 0.9 M KCl and the plates were incubated at 30°C for 4 days. (**H**) Sensitivity to $CdSO_4$ of the *tfg3*$^{-}$ mutant. JY741 (*tfg3*$^{+}$)and its derivative with the *tfg3*Δ allele were streaked on plates prepared with and without 0.5 mM $CdSO_4$ and the plates were incubated at 30°C for 4 days. (**I**) Sensitivity to various concentrations of $CdSO_4$ of the *tfg3*$^{-}$ mutant. JY741 (squares) and its *tfg3*Δ derivative (triangles) were grown to the exponential-phase in liquid YE (+Ade +Ura) medium at 30°C, and exposed to the indicated concentrations of $CdSO_4$ for 48 h. Then, aliquots were taken and plated. The numbers of colonies were counted after 4 days of incubation at 30°C.

thiamine-repressible promoter (Figure 2D). Although no change was observed in cell morphology after the overexpression of Tfg3, it inhibited cell growth at both 30 and 38°C. These observations together suggested that the intracellular concentration of Tfg3 must be maintained at some optimum level for maximum growth. To examine our hypothesis, we expressed Tfg3 at various levels on the *tfg3*Δ background and monitored cell growth at 30 and 38°C. Confirming the results noted above, the basal-level expression of Tfg3 suppressed the *ts* phenotype (Figure 2F) better than induced-level expression (Figure 2E). Since the expression level of Tfg3 seemed to be critical for cell growth, we measured the intracellular level of Tfg3 directly, by quantitative western-blotting, in an exponential-phase culture of the parental strain

Table 1. Approximate intracellular levels of components of the Fcp1/TFIIF/pol II complex in *S.pombe*

Factor	Level (molecules/cell)
pol II[a]	10 000
Fcp1[b]	3500
Tfg3	20 000

Strain JY741 was grown in YE(+Ade +Ura) medium at 30°C. Cells at the exponential phase of growth were subjected to quantitative western-blotting analysis.
[a]Intracellular levels of pol II and all of its 12 subunits were reported previously (21).
[b]Ref. (12).

JY741 that had been grown in YE medium at 30°C. As summarized in Table 1, we found a total of ~20 000 molecules of Tfg3 in a single cell of *S.pombe*, which, according to our previous calculations (12,21), contains ~10 000 molecules of pol II and ~3500 molecules of Fcp1.

The *tfg3*$^-$ mutation is associated with other stress-sensitive phenotypes

Next, we examined the growth of *tfg3*$^-$ strains under osmotic stress. When *S.pombe* cells that lacked Tfg3 were streaked on a plate that contained 0.9 M KCl, they did not grow. In contrast, *tfg3*$^+$ cells were able to grow (Figure 2G). We obtained essentially the same results when *tfg3*$^-$ strains were grown on a plate that contained 2 M glucose (data not shown). We also examined the sensitivity of *tfg3*$^-$ mutant to a heavy metal. When strains JY741 and JY741/tfg3Δ were streaked on a plate that contained 0.5 mM $CdSO_4$, only JY741 grew (Figure 2H), and when liquid cultures of these strains were exposed to various concentrations of $CdSO_4$, the mutant survived less than the wild-type (Figure 2I). In an experiment that the cultures of these strains were exposed to oxidative-stress, JY741/tfg3Δ showed slightly higher sensitivity to H_2O_2 than JY741 (data not shown). Thus, it appears that Tfg3 is needed for growth under various stress conditions.

Tfg3 is a TBP-associating factor (TAF)

Since Tfg3 was reported to associate with TBP and, thus, to be a TAF in *S.cerevisiae*, we examined the possible direct interaction between *S.pombe* TBP and Tfg3 by a GST-pull-down assay at various temperatures (Figure 3A). We found that Tfg3 formed stable complexes with TBP and that the TBP–Tfg3 interaction was clearly temperature-dependent, with the affinity being higher at higher temperatures. To examine the association *in vivo* of cellular Tfg3 with TFIID at various temperatures, we isolated TFIID from strains of *S.pombe* that contained the gene for FLAG-tagged Taf72 or Taf73 (19) using FLAG-specific antibody-conjugated resin. In agreement with our results *in vitro* (see Figure 3A), the isolated TFIID was associated with more Tfg3 when cells had been grown at a higher temperature (Figure 3C), even though the intracellular level of Tfg3 decreased in cells grown at the higher temperature (Figure 3B). Combined with the fact that *tfg3*$^-$ strains had a *ts* phenotype, these results suggested that Tfg3 functions as a TAF at higher temperatures.

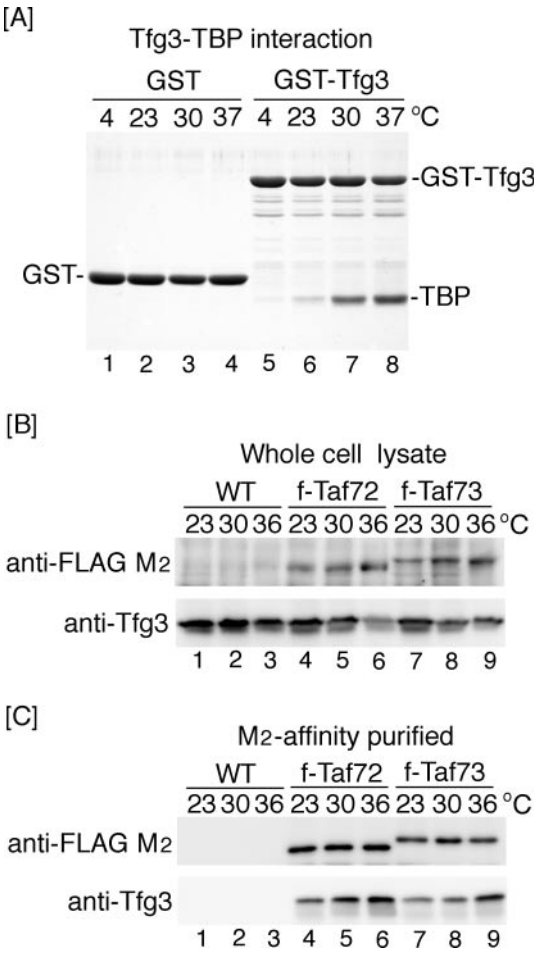

Figure 3. Tfg3 is a TAF. (**A**) The Tfg3–TBP interaction. H_6-tagged TBP was incubated with GST or GST–Tfg3 at the indicated temperatures and then treated with GSH–Sepharose. Proteins bound to the resin were analyzed by SDS–PAGE and staining with CBB. (**B**) Dependence on temperature of intracellular levels of Taf72, Taf73 and Tfg3. *S.pombe* JY741 and its derivatives carrying FLAG-tagged *taf72*$^+$ or *taf73*$^+$ were cultured at indicated temperatures. The whole-cell lysates were analyzed for levels of Tfg3 and FLAG-tagged TAFs by western-blotting with Tfg3-specific antiserum and FLAG-specific M_2 antibodies. (**C**) Dependence on temperature of incorporation of Tfg3 into TFIID. TFIID was purified with FLAG-specific M_2 antibody-conjugated agarose from the cells cultured as described in (B). The isolated proteins were analyzed by western-blotting as in (B).

Tfg3 interacts with TFIIB but not with pol II, Fcp1 or TFIIA

Tfg3 is assembled in the Fcp1/TFIIF/pol II complex (see above). We next examined whether Tfg3 interacts directly with Fcp1 or pol II by a GST-pull-down assay. We found that GST–TFIIFβ/α-H formed complexes with His_6-tagged Fcp1 (Fcp1-H) (Figure 4A) and with FLAG-tagged pol II (f-pol II) (Figure 4B) but, under the same conditions, neither Fcp1-H (Figure 4A) nor f-pol II (Figure 4B) formed stable complexes with GST–Tfg3. Neither the Tfg3–Fcp1 nor the Tfg3–pol II interaction was detected at higher or lower temperatures (data not shown). These observations indicate that Tfg3 is assembled into the Fcp1/TFIIF/pol II complex through molecular interactions with TFIIFα/β, but not with Fcp1 or pol II.

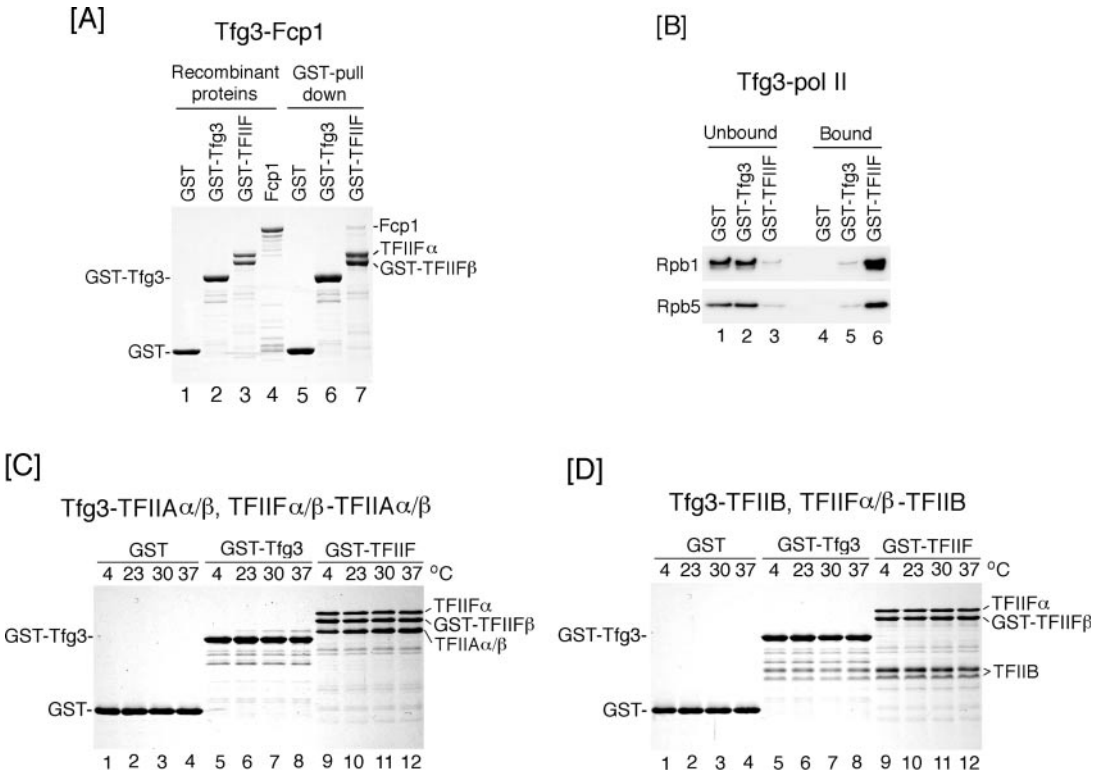

Figure 4. Protein–protein interactions involving Tfg3. (**A**) Tfg3–Fcp1 and TFIIFα/β–Fcp1 interactions. Fcp1-H was incubated with GST, with GST–Tfg3 and with GST–TFIIFβ/α-H and then treated with GSH–Sepharose. The proteins used (lanes 1–4) and those bound to the resin (lanes 5–7) were separated by SDS–PAGE and the gel was stained with CBB. (**B**) Tfg3–pol II and TFIIα/β–pol II interactions. Purified pol II was incubated with GST, with GST–Tfg3 and with GST–TFIIFβ/α-H, and then treated with GSH–Sepharose. The proteins bound (lanes 4–6) and not bound (lanes 1–3) to the resin were analyzed by western-blotting with Rpb1- and Rpb5 (pol II subunit 5)-specific antisera. (**C**) Tfg3–TFIIAα/β and TFIIFα/β–TFIIAα/β interactions. TFIIAα/β-H was analyzed by the same procedure as described in the legend to Figure 1C. (**D**) Tfg3–TFIIB and TFIIFα/β-TFIIB interactions. H-TFIIB was analyzed by the same procedure as in (C).

It has been reported that TFIIF interacts with TFIIA (24) and with TFIIB (25,26). We examined possible direct interactions of *S.pombe* Tfg3 with TFIIA and TFIIB by GST-pull-down assays. GST–Tfg3 did not bind to the recombinant large subunit of TFIIA, namely TFIIAα/β, whereas GST–TFIIFβ/α-H formed a stable complex with TFIIAα/β (Figure 4C). In contrast, TFIIB-H bound both to GST–TFIIFβ/α-H and to GST–Tfg3 at all temperatures tested (Figure 4D). Thus, it appeared that TFIIF associated with TFIIA via α/β subunits, while both TFIIFα/β and Tfg3 interacted with TFIIB.

Multicopy suppressors of the *ts* phenotype associated with the *tfg3*$^-$ mutation suggest a function for Tfg3

We isolated five species of multicopy plasmids that encoded suppressors of the *ts* phenotype associated with the *tfg3*$^-$ mutation from an *S.pombe* genomic library (Figure 5A and Table 2; see also Materials and Methods). SPCC1529.01 is a putative gene, whose identity was inferred from homology to the gene for a multidrug efflux transporter. SPBC3B8.09 encodes a homolog of *S.cerevisiae* Sas10, whose overproduction disrupts chromatin silencing (27). SPAC25H1.02 encodes a JmjC domain-containing protein. It has been proposed that proteins with the JmjC domain participate in transcriptional regulation by modulating the structure of the chromatin (28,29). SPBC1734.11 encodes a protein similar to heat-inducible molecular chaperone Ydj1 (Mas5) of *S.cerevisiae*, which is a homolog of DnaJ of *Escherichia coli* (30,31). SPAC30D11.13 encodes Hus5 (Ubc9), an ubiquitin-conjugating enzyme (32). This gene might also function in chromatin remodeling, since the ubiquitination of histones is one of the mechanisms that regulate gene silencing (33). In this screening, we failed to isolate the *tfg3*$^+$ gene, but this result is not unexpected since the overexpression of Tfg3 inhibited cell growth (see Figure 2).

To examine the effect of *tfg3*$^-$ mutation on transcription of these suppressor genes at a higher temperature, we analyzed the levels of total poly(A) RNA by slot-blotting and specific mRNA by northern-blotting in both strains JY741 and JY741/tfg3Δ. Total RNA was isolated from the cells cultured at 30°C, and the cells incubated at 38°C for 1 and 4 h after the temperature up-shift. The level of poly(A) RNA increased slightly after the temperature shift to almost the same extent for both strains (Figure 5B), indicating that the *tfg3*$^-$ mutation did not affect the transcription level after the temperature shift. However, the expression level of each suppressor gene was different between the two strains especially at the elevated temperature (Figure 5C). The heat-inducibility of the suppressor genes in JY741 was consistent with the result of systematic analysis of transcriptional response to environmental stress (see Table 2). SPCC1529.01 was induced in JY741 after 1 h of the temperature-shift, but this heat-induction was not prominent in JY741/tfg3Δ. Though SPBC3B8.09 was not heat-induced in JY741, the mRNA level was reduced in

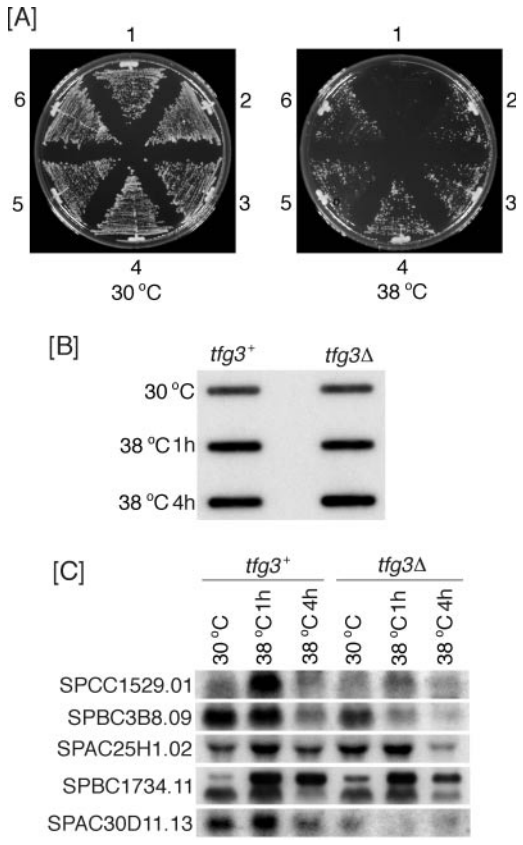

Figure 5. (**A**) Multicopy suppressors of the *tfg3*$^{-}$ mutation. Plasmids carrying various genes, which had been isolated as multicopy suppressors of the thermosensitivity associated with the *tfg3*$^{-}$ mutation (Table 2), were re-introduced into strain JY741/tfg3::LEU2 and then cells were streaked on EMM(+Ade) plates, which were incubated at the indicated temperatures for 4 days: 1, empty vector (pFL20); 2, vector carrying SPCC1529.01; 3, vector carrying SPBC3B8.09; 4, vector carrying SPAC25H1.02; 5, vector carrying SPBC1734.11; 6, vector carrying SPAC30D11.13. (**B**) mRNA levels in JY741 and its *tfg3*Δ derivative before and after a temperature-shift. JY741 (*tfg3*$^{+}$) and the *tfg3*Δ derivative were cultured in YE(+Ade+Ura) medium at 30°C to the exponential-phase and shifted to 38°C. Cells were collected immediately before the temperature-shift and 1 and 4 h after it. The isolated total RNA was slot-blotted on to a membrane and probed with fluorescein labelled poly(dT). (**C**) Northern-blot analysis of the isolated multicopy suppressor genes. Total RNA isolated in (B) was subjected to northern-blotting using probes specific to each isolated multicopy suppressor genes. The systematic names of genes are indicated at the left.

Table 2. Multicopy suppressors of the *ts* phenotype associated with *tfg3*$^{-}$ mutation

Systematic name	Description of product	Induction by heat (39°C)[a]
SPCC1529.01	Multidrug efflux transporter[b]	+
SPBC3B8.09	Sas10 family[b]	−
SPAC25H1.02	JmjC domain protein	+
SPBC1734.11	DnaJ homolog[b]	++
SPAC30D11.13	Hus5, ubiquitin conjugating enzyme	±

[a]Ref. (37) and the *S.pombe* gene database (Sanger Institute).
[b]Inferred from homology.

JY741/tfg3Δ after the temperature-shift. The mRNA level of SPAC25H1.02 was less in JY741/tfg3Δ after 4 h at 38°C than in JY741. Two species of transcript were detected for SPBC1734.11 and the larger one was heat-induced. In this case, slightly less mRNA was detected in JY741/tfg3Δ after 4 h at 38°C. SPAC30D11.13 expressed less in JY741/tfg3Δ at 30°C and much less at 38°C than in JY741. All these indicated that Tfg3 functions at higher temperatures to regulate transcription in a gene-specific manner. None of our five suppressors of the *ts* phenotype associated with the *tfg3*$^{-}$ mutation suppressed the osmotic-stress-sensitive phenotype (see Figure 2G; data not shown). Thus it appeared that they suppressed the *tfg3*$^{-}$ mutation in a heat-stress-specific manner.

DISCUSSION

We report here that *S.pombe* Tfg3 is incorporated into the Fcp1/TFIIF/pol II complex via interaction with TFIIFα/β, but not with Fcp1 or pol II. We prepared the recombinant TFIIFα/β complex as GST–TFIIFβ/α-H because we were unable to express TFIIFα and TFIIFβ separately in *E.coli* as soluble proteins. When TFIIFα or TFIIFβ, prepared separately in denaturing buffer, was mixed with Tfg3 and then the mixture was dialyzed, Tfg3 associated with both TFIIFα and TFIIFβ (data not shown). In addition, like TFIIFα/β, Tfg3 also binds to TFIIB. Thus, we concluded that Tfg3 is the third subunit of *S.pombe* TFIIF as is the case in *S.cerevisiae*. The TFIIF in mammals, however, lack the corresponding subunit (or Tfg3 homolog). The assembly of Tfg3 into *S.pombe* TFIIF is rather unexpected because *S.pombe* is more similar, in many respects, to higher eukaryotes than *S.cerevisiae* [for example, see ref. (34)].

We found that Tfg3 of *S.pombe* formed dimers (or multimers). In human TFIIF, the α and β subunits form a hetero-tetramer (35). If TFIIFα/β of *S.pombe* is also a tetramer, the overall structure of TFIIF of *S.pombe* should be a hetero-hexamer or a larger complex, providing a wide surface for molecular contacts with other transcription factors. In fact, we found that Tfg3 of *S.pombe* was incorporated into TFIID as a TAF. In the *S.pombe* gene database (Sanger Institute), Tfg3 is denoted as Taf14 because of the strong homology to its counterpart in *S.cerevisiae*. In addition, Tfg3 of *S.cerevisiae* is known to be a component of the SWI/SNF (5) and NuA3 (14) complexes. Tfg3 is also involved in molecular interactions with TFIIB.

The *tfg3*$^{-}$ mutation was associated with stress-sensitive phenotypes, and the result of our tetrad assay under mild conditions indicated that *tfg3*$^{+}$ is a nonessential gene. Therefore, Tfg3 may be needed only under certain stress conditions. Since *S.pombe* contains a putative paralog of *tfg3*$^{+}$, namely SPAC17G8.07, which is probably an ortholog of YNL107W of *S.cerevisiae*, it is also possible that it supports cell growth via functional redundancy under mild conditions. The temperature dependence of the Tfg3–TBP interaction *in vitro* and the incorporation of Tfg3 into TFIID *in vivo* suggest that Tfg3 plays a regulatory role in transcription at elevated temperatures. In fact, the results of mRNA analysis from the *tfg3*$^{-}$ mutant after temperature up-shift indicated that Tfg3 functions in transcription regulation at higher temperatures in a gene-specific manner. The most plausible function of Tfg3 is to gather and organize various GTFs and chromatin-related factors into transcription apparatus, and to stabilize the transcription complex at higher temperatures.

We have isolated five species of multicopy suppressor genes of the *ts* phenotype associated with the *tfg3*$^{-}$ mutation. According to the analysis of expression pattern of these genes, the most probable mechanism of the suppression is that the multicopy plasmids compensated the expression levels of suppressor genes, which were reduced in the *tfg3*$^{-}$ mutant. However, because the overexpression of only one species of the genes was sufficient to suppress the *ts* phenotype, suppression mechanisms which include interactions of protein functions might be possible for some suppressors. Indeed, three suppressor genes encoded factors with chromatin-modulating function. SPBC3B8.09 is a homolog of *SAS10*. This gene in *S.cerevisiae* was originally isolated in a screening for genes whose overproduction disrupted gene silencing, which *SAS10* achieves in a sir- and silencer-independent manner (27). SPAC25H1.02 encodes a JmjC domain protein and is denoted as *jmj1* in the *S.pombe* gene database. It has been proposed that the JmjC domain plays a role in the regulation of chromatin remodeling (28,29). Several genes in the genome of *S.pombe* contain sequences that encode JmjC domains, e.g. *epe1*$^{+}$ and *lid2*$^{+}$. The *epe1*$^{+}$ gene was isolated in a genetic screening for mutations that promote silencing beyond the *IR-L* heterochromatin boundary (36). Epe1 counteracts transcriptional silencing via a negative effect on the stability of heterochromatin, and the JmjC domain is essential for its activity. The expression of *epe1*$^{+}$ was induced by temperature up-shift (37) and Lid2 in *S.pombe* appears to be related to the modification of histones (38). SPAC30D11.13, encodes the ubiquitin-conjugating enzyme Hus5 (Ubc9), and ubiquitination of histones is one of the mechanisms that regulate gene silencing in *S.cerevisiae* (33). A similar regulatory mechanism might exist in *S.pombe*, because two genes in *S.pombe*, *rhp6*$^{+}$ and *ubcX*$^{+}$, both of which encode ubiquitin-conjugating enzymes, were isolated in a screening for genes whose overproduction disrupted gene silencing (39).

The SPBC1734.11 gene encodes the homolog of Ydj1 (Mas5) in *S.cerevisiae*, which is the homolog of bacterial DnaJ (30,31), and both Ydj1 and DnaJ are heat-shock proteins. It has been reported that Ydj1 functions in the import of protein into mitochondria and to the endoplasmic reticulum. SPCC1529.01 encodes a putative multidrug efflux transporter and the expression of SPCC1529.01 is also heat-inducible (34). From the intracellular localization and the physiological roles of these two proteins, the suppression of *tfg3* mutation is not attributable to the direct interaction between Tfg3 and the SPBC1734.11 and SPCC1529.01 proteins.

Suppressors of the *tfg3* mutation herein isolated suppressed the *ts* phenotype, but none of these suppressed the high-osmolarity sensitivity of the *tfg3* mutant. One possibility is that Tfg3 is needed to stabilize the transcription complexes at elevated temperatures, but not under high-osmolarity conditions. Overall our findings suggested that Tfg3 of *S.pombe* functions in the regulation of transcription by connecting and organizing GTFs and chromatin-modulating factors under stress conditions in a gene-specific manner.

ACKNOWLEDGEMENTS

The authors are grateful to Dr H. Mitsuzawa (National Institute of Genetics) for providing the FLAG-TAF strains of *S.pombe*, and to Dr Y. Nogi (Saitama Medical School) for providing the vectors. This work was supported by a Grant-in-Aid to M.K. from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

1. Sopta,M., Carthew,R.W. and Greenblatt,J. (1985) Isolation of three proteins that bind to mammalian RNA polymerase II. *J. Biol. Chem.*, **260**, 10353–10360.
2. Flores,O., Maldonado,E., Burton,Z., Greenblatt,J. and Reinberg,D. (1988) Factors involved in specific transcription by mammalian RNA polymerase II. RNA polymerase II-associating protein 30 is an essential component of transcription factor IIF. *J. Biol. Chem.*, **263**, 10812–10816.
3. Henry,N.L., Sayre,M.H. and Kornberg,R.D. (1992) Purification and characterization of yeast RNA polymerase II general initiation factor g. *J. Biol. Chem.*, **267**, 23388–23392.
4. Henry,N.L., Campbell,A.M., Feaver,W.J., Poon,D., Weil,P.A. and Kornberg,R.D. (1994) TFIIF-TAF-RNA polymerase II connection. *Genes Dev.*, **23**, 2868–2878.
5. Cairns,B.R., Henry,N.L. and Kornberg,R.D. (1996) TFG3/TAF30/ANC1, a component of the yeast SWI/SNF complex that is similar to the leukemogenic proteins ENL and AF-9. *Mol. Cell. Biol.*, **16**, 3308–3316.
6. Flores,O., Lu,H., Killeen,M., Greenblatt,J., Burton,Z.F. and Reinberg,D. (1991) The small subunit of transcription factor IIF recruits RNA polymerase II into the preinitiation complex. *Proc. Natl Acad. Sci. USA*, **88**, 9999–10003.
7. Buratowski,S., Hahn,S., Guarente,L. and Sharp,P.A. (1989) Five intermediate complexes in transcription initiation by RNA polymerase II. *Cell*, **56**, 549–561.
8. Flores,O., Lu,H. and Reinberg,D. (1992) Factors involved in specific transcription by mammalian RNA polymerase II. Identification and characterization of factor IIH. *J. Biol. Chem.*, **5**, 2786–2793.
9. Koleske,A.J. and Young,R.A. (1994) An RNA polymerase II holoenzyme responsive to activators. *Nature*, **368**, 466–469.
10. Kim,Y.J., Bjorklund,S., Li,Y., Sayre,M.H. and Kornberg,R.D. (1994) A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. *Cell*, **77**, 599–608.
11. Bengal,E., Flores,O., Krauskopf,A., Reinberg,D. and Aloni,Y. (1991) Role of the mammalian transcription factors IIF, IIS, and IIX during elongation by RNA polymerase II. *Mol. Cell. Biol.*, **11**, 1195–1206.
12. Kimura,M., Suzuki,H. and Ishihama,A. (2002) Formation of a carboxy-terminal domain phosphatase (Fcp1)/TFIIF/RNA polymerase II (pol II) complex in *Schizosaccharomyces pombe* involves direct interaction between Fcp1 and the Rpb4 subunit of pol II. *Mol. Cell. Biol.*, **22**, 1577–1588.
13. Archambault,J., Chambers,R.S., Kobor,M.S., Ho,Y., Cartier,M., Bolotin,D., Andrews,B., Kane,C.M. and Greenblatt,J. (1997) An essential component of a C-terminal domain phosphatase that interacts with transcription factor IIF in *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA*, **94**, 14300–14305.
14. John,S., Howe,L., Tafrov,S.T., Grant,P.A., Sternglanz,R. and Workman,J.L. (2000) The something about silencing protein, Sas3, is the catalytic subunit of NuA3, a yTAF(II)30-containing HAT complex that interacts with the Spt16 subunit of the yeast CP (Cdc68/Pob3)-FACT complex. *Genes Dev.*, **14**, 1196–1208.
15. Welch,M.D. and Drubin,D.G. (1994) A nuclear protein with sequence similarity to proteins implicated in human acute leukemias is important for cellular morphogenesis and actin cytoskeletal function in *Saccharomyces cerevisiae*. *Mol. Biol. Cell*, **5**, 617–632.
16. Moreno,S., Klar,A. and Nurse,P. (1991) Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. *Methods Enzymol.*, **194**, 795–823.
17. Alfa,C., Fabtes,P., Hyams,J., McLeod,M. and Warbrick,E. (1993) *Experiments with Fission Yeast: A Laboratory Course Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
18. Kimura,M., Ishiguro,A. and Ishihama,A. (1997) RNA polymerase II subunits 2, 3, and 11 form a core subassembly with DNA binding activity. *J. Biol. Chem.*, **272**, 25851–25855.
19. Mitsuzawa,H., Seino,H., Yamao,F. and Ishihama,A. (2001) Two WD repeat-containing TATA-binding protein-associated factors in fission

yeast that suppress defects in the anaphase-promoting complex. *J. Biol. Chem.*, **276**, 17117–17124.
20. Basi,G., Schmid,E. and Maundrell,K. (1993) TATA box mutations in the *Schizosaccharomyces pombe nmt1* promoter affect transcription efficiency but not the transcription start point or thiamine repressibility. *Gene*, **123**, 131–136.
21. Kimura,M., Sakurai,H. and Ishihama,A. (2001) Intracellular contents and assembly states of all 12 subunits of the RNA polymerase II in the fission yeast *Schizosaccharomyces pombe*. *Eur. J. Biochem.*, **268**, 612–619.
22. Losson,R. and Lacroute,F. (1983) Plasmids carrying the yeast OMP decarboxylase structural and regulatory genes: transcription regulation in a foreign environment. *Cell*, **32**, 371–377.
23. Kaiser,C., Michaelis,S. and Mitchell,A. (1994) *Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
24. Langelier,M.F., Forget,D., Rojas,A., Porlier,Y., Burton,Z.F. and Coulombe,B. (2001) Structural and functional interactions of transcription factor (TF) IIA with TFIIE and TFIIF in transcription initiation by RNA polymerase II. *J. Biol. Chem.*, **276**, 38652–38657.
25. Ha,I., Roberts,S., Maldonado,E., Sun,X., Kim,L.U., Green,M. and Reinberg,D. (1993) Multiple functional domains of human transcription factor IIB: distinct interactions with two general transcription factors and RNA polymerase II. *Genes Dev.*, **7**, 1021–1032.
26. Fang,S.M. and Burton,Z.F. (1996) RNA polymerase II-associated protein (RAP) 74 binds transcription factor (TF) IIB and blocks TFIIB-RAP30 binding. *J. Biol. Chem.*, **271**, 11703–11709.
27. Kamakaka,R.T. and Rine,J. (1998) Sir- and silencer-independent disruption of silencing in *Saccharomyces* by Sas10p. *Genetics*, **149**, 903–914.
28. Balciunas,D. and Ronne,H. (2000) Evidence of domain swapping within the jumonji family of transcription factors. *Trends Biochem. Sci.*, **25**, 274–276.
29. Clissold,P.M. and Ponting,C.P. (2001) JmjC: cupin metalloenzyme-like domains in jumonji, hairless and phospholipase $A_2\beta$. *Trends Biochem. Sci.*, **26**, 7–9.
30. Caplan,A.J. and Douglas,M.G. (1991) Characterization of YDJ1: a yeast homologue of the bacterial dnaJ protein. *J. Cell Biol.*, **114**, 609–621.
31. Atencio,D.P. and Yaffe,M.P. (1992) MAS5, a yeast homolog of DnaJ involved in mitochondrial protein import. *Mol. Cell. Biol.*, **12**, 283–291.
32. Al-Khodairy,F., Enoch,T., Hagan,I.M. and Carr,A.M. (1995) The *Schizosaccharomyces pombe hus5* gene encodes a ubiquitin conjugating enzyme required for normal mitosis. *J. Cell Sci.*, **108**, 475–486.
33. Sun,Z.W. and Allis,C.D. (2002) Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. *Nature*, **418**, 104–108.
34. Sunnerhagen,P. (2002) Prospects for functional genomics in *Schizosaccharomyces pombe*. *Curr. Genet.*, **42**,73–84.
35. Flores,O., Ha,I. and Reinberg,D. (1990) Factors involved in specific transcription by mammalian RNA polymerase II. Purification and subunit composition of transcription factor IIF. *J. Biol. Chem.*, **265**, 5629–5634.
36. Ayoub,N., Noma,K., Isaac,S., Kahan,T., Grewal,S.I. and Cohen,A. (2003) A novel jmjC domain protein modulates heterochromatization in fission yeast. *Mol. Cell. Biol.*, **23**, 4356–4370.
37. Chen,D., Toone,W.M., Mata,J., Lyne,R., Burns,G., Kivinen,K., Brazma,A., Jones,N. and Bahler,J. (2003) Global transcriptional responses of fission yeast to environmental stress. *Mol. Biol. Cell*, **14**, 214–229.
38. Roguev,A., Schaft,D., Shevchenko,A., Aasland,R., Shevchenko,A. and Stewart,A.F. (2003) High conservation of the Set1/Rad6 axis of histone 3 lysine 4 methylation in budding and fission yeasts. *J. Biol. Chem.*, **278**, 8487–8493.
39. Choi,E.S., Kim,H.S., Jang,Y.K., Hong,S.H. and Park,S.D. (2002) Two ubiquitin-conjugating enzymes, Rhp6 and UbcX, regulate heterochromatin silencing in *Schizosaccharomyces pombe*. *Mol. Cell. Biol.*, **22**, 8366–8374.