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A novel family of *TRF* (DNA topoisomerase I-related function) genes required for proper nuclear segregation

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ABSTRACT

We recently reported the identification of a gene, *TRF4* (for DNA topoisomerase related function), in a screen for mutations that are synthetically lethal with mutations in DNA topoisomerase I (*top1*). Here we describe the isolation of a second member of the *TRF4* gene family, *TRF5*. Overexpression of *TRF5* complements the inviability of *top1 trf4* double mutants. The predicted Trf5 protein is 55% identical and 72% similar to Trf4p. As with Trf4p, a region of Trf5p is homologous to the catalytically dispensable N-terminus of Top1p. The *TRF4/5* function is essential as *trf4 trf5* double mutants are inviable. A *trf4 (ts) trf5* double mutant is hypersensitive to the anti-microtubule agent thiabendazole at a semi-permissive temperature, suggesting that *TRF4/5* function is required at the time of mitosis. Examination of nuclear morphology in a *trf4 (ts) trf5* mutant at a restrictive temperature reveals the presence of many cells undergoing aberrant nuclear division, as well as many anucleate cells, demonstrating that the *TRF4/5* function is required for proper mitosis. Database searches reveal the existence of probable *Schizosaccharomyces pombe* and human homologs of Trf4p, indicating that *TRF4* is the canonical member of a gene family that is highly conserved evolutionarily.

INTRODUCTION

Chromosomes are involved in dynamic cellular processes, such as DNA replication, transcription, chromatin assembly and genetic recombination, that lead to the formation of local domains of torsional stress (reviewed in 1). In *Saccharomyces cerevisiae* DNA topoisomerases I and II (topo I and II) appear to act together during DNA replication as a swivel to prevent the formation of positive supercoils ahead of the DNA replication fork. In *S.cerevisiae* under conditions where both topo I and II are inactivated, DNA replication stops rapidly (2), with elongation of new DNA chains continuing for only a few thousand nucleotides (3). Similar results have been obtained for *Schizosaccharomyces pombe* (4).

Transcription can also lead to the formation of locally supercoiled domains in DNA. In the 'twin domain' model (5), movement of a transcription complex along the helical backbone generates positive supercoils ahead of the complex and negative supercoils behind the complex. It has been suggested that transcription is a major determinant of supercoiling *in vivo* (6). In *S.cerevisiae*, topo I and II appear to function together as a swivel for rRNA transcription and, to a lesser extent, for mRNA transcription (2). Transcription from a strong promoter can lead to hypernegative supercoiling of plasmids in *top1* mutants (6,7), suggesting that topo I normally removes negative supercoils formed during transcription. These results and others have led to the suggestion that another major role for topo I is to relieve torsional stress generated during transcription.

Despite the considerable evidence for the involvement of topo I in both DNA replication and transcription, topo I is not essential in either *S.cerevisiae* or *S.pombe*. Null mutations in the gene encoding the only type I DNA topoisomerase activity detectable in crude extracts cause only modest growth defects (4,8,9). Furthermore, the overall rates of both DNA and RNA synthesis are normal in *top1* mutants (2), supporting the suggestion that topo II activity can substitute for topo I activity in these crucial processes (2,4,10). In *S.pombe*, conditional *top2* mutants have been isolated that are inviable at the permissive temperature in combination with a *top1* null mutation (11). In *S.cerevisiae*, *top1* null mutants display a synthetic growth defect in combination with some *top2* alleles (10) and we have identified alleles of *TOP2* in a screen for *top1* synthetic lethal mutations (12). These results demonstrate that some of the functions of type I and type II topoisomerases overlap. It remains unclear, however, whether topo I has additional functions distinct from those of topo II. The biological function of a second type I topoisomerase, topo III, (13,14), remains unclear. Null mutations in *TOP3* result in hyper-recombination and slow growth (15,16), but *top1 top3* double mutants are viable. Association of Top3p with Sgs1p, a DNA helicase, has been proposed to result in formation of a eukaryotic reverse gyrase (17).

In order to investigate further the *in vivo* functions of topo I, we have perfomed a genetic screen to identify mutations affecting gene products that perform overlapping or dependent functions (18) with topo I and, thereby, to further elucidate which processes

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in the cell require topo I. We have identified four complementation groups of mutants with this phenotype called *TRF*, for DNA topoisomerase I-related function (12). The predicted Trf4 protein shares a region of homology with the N-terminus of Top1p.

We report here the isolation of a *TRF4* homolog, *TRF5*. DNA sequence analysis reveals that the predicted Trf4 and Trf5 proteins are 57% identical and 72% similar over their entire lengths. Overexpression of *TRF5* complements the inviability of *top1 trf4* double mutants. A *trf4 (ts) trf5* double mutant is hypersensitive to the anti-microtubule agent thiabendazole at a semi-permissive temperature and grossly defective in nuclear division at a restrictive temperature, demonstrating that *TRF4/5* function is required for proper mitosis. Database searches reveal the existence of probable *S.pombe* and human and homologs of *TRF4/5*, indicating that this novel gene family is evolutionarily conserved.

MATERIALS AND METHODS

Microbial techniques

Yeast strains (Table 1) were transformed using the lithium acetate method (19). The *TRF5* gene was isolated from a genomic DNA library consisting of random *S.cerevisiae* DNA *Sau*3A fragments inserted into the *Bam*HI site of YEp24. *Escherichia coli* cells (strain DH5α) were transformed by electroporation (20). Small scale plasmid DNA preparations were made by the boiling lysis method (21) or using Qiagen columns (Qiagen, Chatsworth, CA).

Yeast strain construction and crosses

CY667. Strains CY726 and CY445 were crossed and sporulated. CY667 is a cold-sensitive (*trf4-1*), Leu$^+$ (*top1-7*::*LEU2*) spore from this cross.

CY726. Derived from CY429 following EMS mutagenesis.

CY855 and CY857. Plasmid pCB470 was digested with *Sac*I to release a *trf4-101*::*HIS3* fragment. This fragment was used to disrupt *TRF4* in the *TRF4/TRF4* diploid CY858. The resulting diploid was sporulated and several tetrads were dissected. One of the His$^+$, cold-sensitive spores was designated CY855; another cold-sensitive His$^+$ spore was designated CY857. Disruption at the *TRF4* locus in CY855 was confirmed by Southern blot analysis.

CY869. pCB469 was digested with *Not*I and *Xho*I to release *trf4-102*::*TRP1* and the resulting fragment was used to disrupt *TRF4* in CY184 to create CY869.

CY870, CY872 and CY874. Plasmid pSH5 was digested with *Bam*HI and *Pst*I to release a *trf5-3*::*LEU2* fragment. This fragment was used to disrupt *TRF5* in the *TRF5/TRF5* diploid (CY858). Disruption at the *TRF5* locus at one allele was confirmed by Southern blotting. The resulting diploid (CY870) was sporulated and several tetrads were dissected. One of the Leu$^+$ spores was designated CY874 (*trf5-3*::*LEU2*). A sister spore that is *TRF5*$^+$ was designated CY872.

CY908. Plasmid pSH5 was digested with *Bam*HI and *Pst*I to release a *trf5-3*::*LEU2* fragment. This fragment was used to disrupt *TRF5* in the *trf4-101*::*HIS3/trf4-101*::*HIS3* homozygous diploid (CY892) to generate CY908. Disruption of *TRF5* was confirmed by Southern blotting. The resulting diploid was sporulated and tetrads were dissected to test for synthetic lethality between the *trf4* and *trf5* mutations.

Table 1. Yeast strains

Strain	Genotype
CY143	MAT**a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 rDNA*::*URA3*
CY184	MATα *ade2-1 ura3-1 trp1-1 his3-11,15 leu2-3,112 rDNA*::*ADE2*.
CY185	MATα *top1-7*::*LEU2 ade2-1 ura3-1 trp1-1 his3-11,15 leu2-3,112 rDNA*::*ADE2*
CY429	MAT**a** *top1-7*::*LEU2 ade2-1 ura3-1 trp1-1 his3-11,15 leu2-3,112 ade3*::*hisG*
CY431	MAT**a** *top1-7*::*LEU2 ade2-1 ura3-1 his 3-11,15 trp1-1 leu2-3,112 ade3*::*hisG*/pBS3
CY445	CY429 switched to MATα with pCB10.
CY667	MATα *top1-7*::*LEU2 trf4-1 his3-11,15 trp1-1 ade2-1 ade3*/pBS3
CY726	MAT**a** *top1-7*::*LEU2 trf4-1 his3-11,15 trp1-1 ura3-1 ade2-1ade3*::*hisG*
CY855	MATα *trf4-101*::*HIS3 ura3-1 ade2-1 his3-11,15 trp1-1 leu2-3,112 rDNA*::*ADE2*
CY857	MAT**a** *trf4-101*::*HIS3 ura3-1 ade2-1 his3-11,15 trp1-1 leu2-3,112 rDNA*::*URA3*
CY858	Diploid from CY184 X CY143
CY869	MATα *trf4-102*::*TRP1 ura3-1 ade2-1 his3-11,15 trp1-1 leu2-3,112 rDNA*::*ADE2*
CY870	Diploid CY858 disrupted with *trf5-3*::*LEU2* at one allele
CY872	MAT**a** *ura3-1 ade2-1 his 3-11,15 trp1-1 leu2-3,112 rDNA*::*ADE2*
CY874	MATα *trf5-3*::*LEU2 ura3-1 ade2-1 his3-11,15 trp1-1 leu2-112,3 rDNA*::*ADE2*
CY892	Diploid from CY855 × CY857
CY908	*trf4-101*::*HIS3/trf-101*::*HIS3, TRF5+/trf5-3*::*LEU2*
CY924	MATα *trf4-101*::*HIS3 trf5-3*::*LEU2 ura3-1 ade2-1 his3-11,15 trp1-1 leu2-3,112 rDNA*::*ADE2*/pCB432
CY949	MAT**a** *trf4-101*::*HIS3 trf5-3*::*LEU2 ura3-1 ade2-1 his3-11,15 trp1-1 leu2-3,112 rDNA*::*URA3*/pCB639 (*trf4-ts2*)

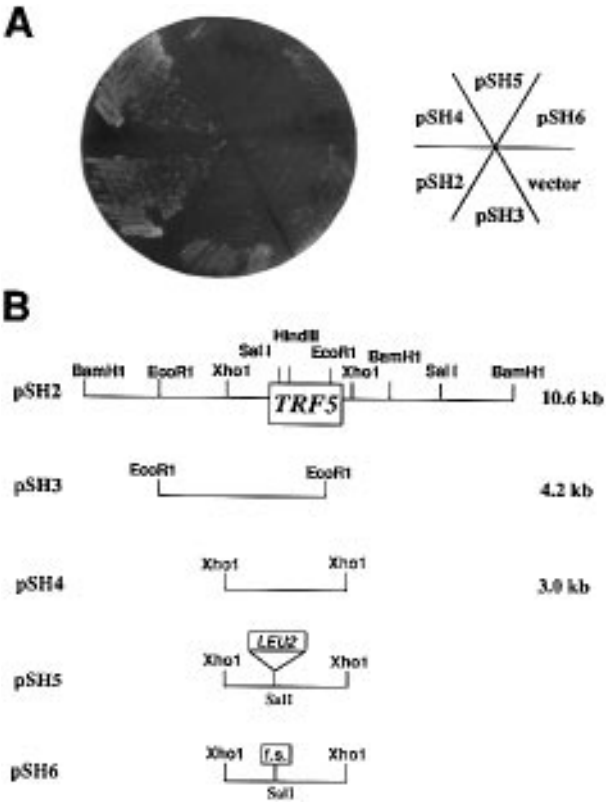

Figure 1. The *TRF5* locus. (**A**) Ability of *TRF5* region subclones to complement synthetic lethality of a *top1 trf4* double mutant. Each plasmid was transformed into a *top1-7*::*LEU2 trf4-1*/*pTOP1.URA3* yeast strain (CY726/pBS3). Transformants were grown non-selectively for pBS3 (*TOP1.URA3*) and then plated on 5-FOA medium, which provides a counterselection for *URA3*$^+$ cells, and examined for their ability to segregate away the *TOP1.URA3* plasmid and remain viable. (**B**) Restriction map of the *TRF5* locus and subclones.

Media and growth conditions

Yeast strains were routinely grown in YEP with 2% glucose (YPD). Thiabendazole (60 μg/ml) was added to YPD agar just prior to pouring the plates. Unless otherwise stated, cells were grown at 30°C.

DNA sequencing and mapping

A 3.0 kb fragment of genomic DNA containing *TRF5* was cloned into YEplac112 and sequenced by the chain termination method (22) using a Sequenase kit (US Biochemicals, Cleveland, OH) and [^{35}S]dATP. The 3.0 kb *Xho*I–*Xho*I insert from pSH4 was sequenced on both strands using synthetic primers. To map *TRF5*, this fragment was radiolabeled and hybridized to filters containing λ clones spanning the yeast genome (23).

Plasmid constructions (Table 2)

pSH2. pCB426 was digested with *Bam*HI and the 10.6 kb fragment carrying *TRF5* was ligated into the *Bam*HI site of YEplac112.

pSH3. pSH2 was digested with *Eco*RI and the 4.2 kb fragment was ligated into the *Eco*RI site of YEplac112.

pSH4. pSH2 was digested with *Xho*I and the 3.0 kb fragment was ligated into the *Sal*I site of YEplac112.

pSH5. The *LEU2* gene was excised from YEp13 by cutting with *Xho*I and *Sal*I and ligated into the *Sal*I site of pSH4.

pSH6. pSH4 was digested with *Sal*I and the ends were filled in with Klenow enzyme then religated to create a +4 frameshift (f.s.) mutation in *TRF5*.

pCB432. The plasmid containing the library clone of *TRF4* (pCB427) was digested with *Sna*BI and partially with *Hin*dIII to release a 3.7 kb fragment that was subsequently inserted into the *Hin*dIII (left) and *Sma*I (right) sites of pRS316. The resulting construct was designated pCB432.

pCB469. pCB432 was digested with *Bam*HI, the first 288 amino acids of Trf4p were removed and the *TRP1* fragment was excised from pCB53 with a *Bam*HI/*Bgl*II digest and ligated to the deleted *TRF4* gene in pCB432, creating pCB469.

pCB635. A PCR product containing *TRF4* and 396 nt upstream of the ATG was cloned into the *Bam*HI site of pRS314.

pCB693. Identical to pCB635 (above) except that it carries the G317D mutation (*trf4-ts2*) in *TRF4*.

Precise mapping of the *TRF5* locus

CY726 (*top1-7*::*LEU2 trf4-1*) carrying pBS3 (*TOP1 URA3 ADE3 2u*) was transformed with one of several subclones (pSH2, pSH3, pSH4, pSH5 or pSH6), grown non-selectively for the *TOP1*-containing plasmid (pBS3) and plated on 5-fluorotic acid (5FOA) at 30°C. 5-FOA medium provides a *URA3*$^+$ counter-selection, so that cells that are unable to lose pBS3 will not grow on this medium. The 10.6 kb fragment containing *TRF5* (pSH2) permitted growth of Ura$^-$ segregants, indicating that *TRF5* complements the synthetic lethality of *top1 trf4* double mutants in addition to the *trf4-1 cs* phenotype. The subclone containing a 4.2 kb *Eco*RI fragment (pSH3) did not complement. However, a plasmid containing the 3.0 kb *Xho*I–*Xho*I fragment (pSH4) did, indicating that this fragment contained the *TRF5* gene.

Table 2. Plasmids

Plasmid	Genotype	Source
pSH2	*TRF5 URA3 2u*	This study
pSH3	*trf5* (*Eco*RI–*Eco*RI) *TRP1 2u*	This study
pSH4	*TRF5 TRP1 2u*	This study
pSH5	*trf5-3*::*LEU2 TRP1 2u*	This study
pSH6	*trf5 (f.s.) TRP1 2u*	This study
pCB53	*TRP1* in pUC18	Laboratory collection
pCB427	*TRF4 URA3 CEN* (library)	31
pCB432	*TRF4 URA3 CEN*	12
pCB469	*trf4–102*::*TRP1 URA3 CEN*	This study
pCB470	*trf4–101*::*HIS3 URA3 CEN*	This study
pCB635	*TRF4 TRP1 CEN*	This study
pCB693	*trf4-ts2 (G317D) TRP1 CEN*	This study
pBS3	*TOP1 ADE3 URA3 2u*	12
YEp13	*LEU2 2u*	Laboratory collection
YEplac112	*TRP1 2u*	32
pCB10	pGAL::*HO URA3 CEN*	33

Figure 2. Homology among Trf4p, Trf5p and an *S.pombe* homolog, SPAC12G12.13c, and putative human homologs. The alignment was generated using the GENWORKS sequence analysis program from Intelligenetics. Boxed residues are those that display a 'cost' of≤1. Not shown here is a 570 amino acid N-terminal extension in SPAC12G12.13c that is related to G-β subunits.

Isolation of a temperature-sensitive *trf4* allele

Plasmid pCB635 was mutagenized *in vitro* with hydroxylamine (24) and transformed into yeast strain CY924 (*trf5 trf4*/p*TRF4.URA3*) by selection for Trp^+. Transformants were grown non-selectively for the p*TRF4.URA3* plasmid and replica printed to 5-FOA plates at 24 and 37°C. Colonies able to give rise to 5-FOA-resistant segregants at 24°C but not at 37°C were identified. Plasmid pCB693 was recovered from 5-FOA-resistant segregants of *trf4 ts* candidates grown at 24°C and used to retransform CY924 to confirm that *trf4 ts* activity was plasmid associated. For one such isolate, the entire *TRF4* open reading frame was sequenced. The only mutation found was a G→A transition, resulting in a missense mutation of Gly317 to Asp (G317D or ts2).

RESULTS

TRF5 overexpression complements a mutation in *trf4*

A 2μ yeast genomic library was screened for plasmids that suppressed the *cs* phenotype of a *trf4-1* mutant strain (CY667/pBS3) in order to clone the *TRF4* gene. In addition to *TRF4* (12), a clone containing a different 10.6 kb insert based on restriction mapping (pCB426) was identified. This gene was designated *TRF5*. Subsequently, *TRF5* was found to complement the synthetic lethality between *trf4* and *top1* based on its ability to allow a *top1 trf4* double mutant to survive following loss of a *TOP1.URA3* plasmid and, thereby, to give rise to 5-FOA-resistant segregants (Fig. 1A). Complementation of *top1 trf4* synthetic lethality was used to localize *TRF5* to a 3.0 kb region (Fig. 1B). A *Sal*I site within this 3.0 kb region was used to generate a *LEU2*

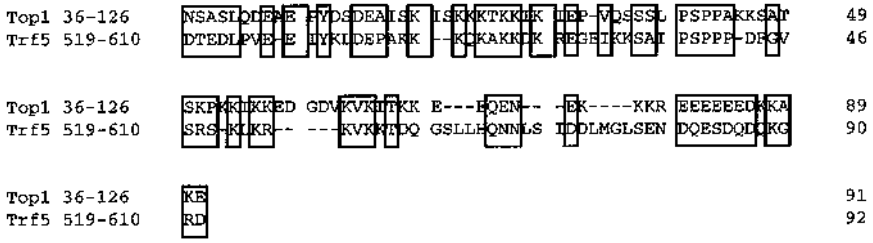

Figure 3. Homology between the Trf5p C-terminus and the Top1p N-terminus. The alignment was generated using the GENWORKS sequence analysis software from Intelligenetics. Boxed residues are those with a 'cost' of ≤3.

insertion mutation (pSH5) and a frameshift mutation allele (pSH6) in *TRF5*. Neither of these *trf5* alleles complemented the inviability of a *top1 trf4* double mutant (Fig. 1A). These results indicate that *TRF5* is contained within the 3.0 kb *Xho*I–*Xho*I fragment on pSH4. Hybridization of this region to filters containing DNA from contigs covering 90% of the yeast genome (23) revealed that *TRF5* mapped to the left arm of chromosome XIV ~40 kb centromere distal to *MET2*.

TRF4 and *TRF5* define a novel gene family

The 3.0 kb *Xho*I–*Xho*I fragment containing *TRF5* was sequenced on both strands (GenBank accession no. U47282). The predicted *TRF5* open reading frame encodes a 625 amino acid protein (Fig. 2) whose sequence is 57% identical and 72% similar to Trf4p. A search of the GenBank database reveals the existence of a clear *S.pombe* homolog of *TRF4/5* identified in the *S.pombe* genome project (Fig. 2). The predicted *S.pombe* homolog, called SPAC12G12.13c, is 33% identical and 56% similar to Trf4p over its C-terminal 683 amino acids. However, it also has a 570 amino acid N-terminal extension relative to Trf4p that is highly homologous to the G-β protein family and completely unrelated to Trf4p or Trf5p. The function of this protein in *S.pombe* is not known.

Short regions of possible homology to Trf4p were also found in two human expressed sequence tags (ESTs; Fig. 2). The first human EST (accession no. H85548) encodes a predicted peptide of 131 amino acids (H85548.pep in Fig. 2) that is 41% identical and 60% similar to a region of Trf4p. The second EST (accession no. H90950) encodes a predicted 130 amino acid peptide (H90950.pep in Fig. 2) that is 44% identical and 65% similar to a different region of Trf4p. The two EST peptides overlap but are not identical at the DNA sequence level, indicating that they are probably derived from different genes. In addition, two short predicted peptides (59 and 24 amino acids) derived from a *Drosophila* STS (accession no. G01479) are 42% identical and 61% similar to Trf4p (not shown). This sequence may traverse an intron/exon boundary, since it not derived from cDNA. The function of these putative *TRF4* homologs is not known. Examination of the Trf4p-related sequences with gcg programs such as MOTIFS and with BLOCKS motif databases also failed to identify meaningful signature regions.

In addition, limited sequence homology was also found between a 91 amino acid N-terminal region of *S.cerevisiae* Top1p and a 92 amino acid C-terminal region of Trf5p (Fig. 3). These regions are 33% identical and 58% similar. The alignment, while limited, is likely to be significant as it is 6.06 SD above the mean significance of 20 randomized alignments of the same two sequences using the gcg sequence analysis program BESTFIT. We previously reported

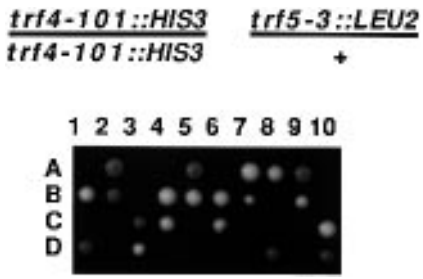

Figure 4. Synthetic lethality between *trf4* and *trf5*. A diploid strain (CY908) homozygous for *trf4-101*::*HIS3* and *TRF5*$^+$ was disrupted by transformation to Leu$^+$ with a fragment of pSH5 to generate a *trf5-3*::*LEU2* disruption at one of the *TRF5*$^+$ alleles. Disruption was confirmed by Southern blot analysis. The resulting strain was sporulated and tetrads 1–10 were dissected on YPD plates at 30°C to yield spores A–D.

homology between the N-terminus of Top1p and the N-terminus of Trf4p (12). Thus, a Top1p-related region exists at the N-terminus of Trf4p, but at the C-terminus of Trf5p. The N-terminus of Top1p is not required for catalytic activity (25) and may be involved in some other aspect of topo I function.

TRF4 and *TRF5* define an essential function that is required at the time of mitosis

A null allele of *TRF5* was constructed by inserting *LEU2* into *TRF5* to make *trf5-3*::*LEU2* (Fig. 1B). This construct was used to disrupt one allele of *TRF5* in a *trf4*/*trf4* diploid (CY892). Following sporulation, 40 tetrads were dissected. All tetrads segregated two viable and two inviable spores, and all viable spores were Leu$^-$, indicating that the *trf4 trf5* double mutant is inviable (Fig. 4). Because *trf4* shows synthetic lethality with *top1* (12), we tested for a genetic interaction between *trf5* and *top1*. However, in contrast to *top1 trf4* double mutants, the *top1 trf5* double mutant was viable (data not shown).

To examine the basis of the defect in cells deficient in *TRF4/5* function, we constructed a conditional allele of *trf4* by *in vitro* mutagenesis with hydroxylamine (see Materials and Methods). DNA sequence analysis showed the *trf4 ts* allele to be a change of Gly317 to Asp. The mutation lies in a region conserved among *TRF4*, *TRF5* and SPAC12G12.13c. The *trf4 (ts) trf5* double mutant grows somewhat more slowly than the wild-type at 29.5°C, but arrests growth at 37°C (Fig. 5). At a semi-permissive temperature of 32°C, the *trf4 (ts) trf5* double mutant displays an increased sensitivity to the anti-microtubule drug thiabendazole (Fig. 5), suggesting that the common *TRF4/5* function may occur at the time of mitosis.

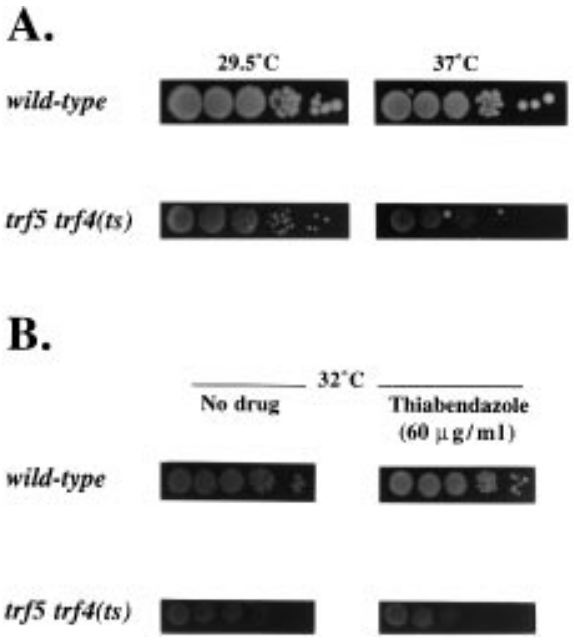

Figure 5. Temperature-sensitive growth of a *trf5 trf4 (ts)* double mutant and hypersensitivity to thiabendazole. Isogenic wild-type (CY184) or *trf4-101*::*HIS3 trf5-3*::*LEU2*/pCB693 strains were grown in liquid YPD medium at 29.5°C. The A_{600} was adjusted to 1.0 and serial 10-fold dilutions were made in sterile water. Aliquots of 5 µl of each dilution was spotted on YPD plates (**A**) and on YPD plates containing 60 µg/ml thiabendazole (**B**) and incubated at the indicated temperature for 2 days.

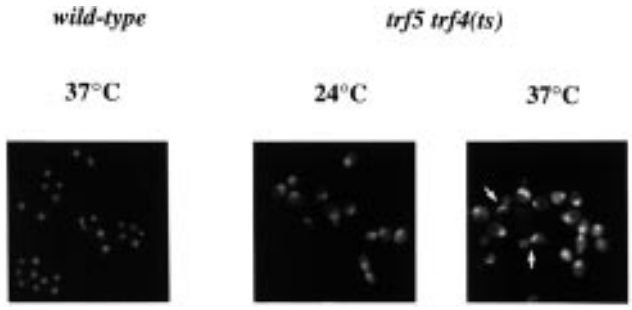

Figure 6. Nuclear morphology of wild-type and a *trf4 (ts) trf5* mutant. Liquid cultures of wild-type and a *trf4 (ts) trf5* mutant were grown in YPD at 24°C to mid-log phase and shifted to 37°C for 3 h. Cells were harvested, DAPI stained and examined under a Zeiss Axiophot fluorescence microscope with a 100× objective lens.

To determine whether *TRF4/5* function is required at mitosis, a direct examination of nuclear morphology was performed on the *trf4 (ts) trf5* double mutant following a shift to a restrictive temperature (37°C) for 3 h (Fig. 6). Three hours after a shift to a restrictive temperature (37°C), 50% of the *trf4 (ts) trf5* cells that are undergoing nuclear division display an aberrant nuclear morphology in which the dividing nucleus is primarily within the mother cell (arrows in Fig. 6). This is similar to the so called 'cut' phenotype observed in certain *S.pombe* mutants that are defective in nuclear division, in which nuclear division is stalled and the nucleus is unequally distributed between mother and daughter cells (4). In addition, anucleate and binucleate cells resulting from a defective nuclear division are observed at greater frequency in *trf4 (ts) trf5* mutants than in the wild-type (Fig. 6, 37°C panel). Quantitation of the defects in nuclear divison showed that in *trf4 (ts) trf5* mutants shifted to 37°C for 3 h, 15 times more anucleate cells and 18 times more cut-like cells are observed than in an otherwise isogenic wild-type strain (Table 3). Some of the *trf4 (ts) trf5* defects are apparent even at the permissive temperature. For example, cells of the *trf4 (ts) trf5* mutant are considerably larger than *TRF*$^+$ cells even at 24°C. In addition, the *trf4 (ts) trf5* mutant shows six times more anucleate cells and six times more cut-like nuclei than the wild-type at 24°C. These results indicate that *TRF4/5* function is required for proper nuclear division.

DISCUSSION

We report the identification of a novel gene, *TRF5*, that is related to the *S.cerevisiae TRF4* (DNA topoisomerase I-related function) gene product. The *TRF4* and *TOP1* (encoding DNA topoisomerase I) gene products serve overlapping or dependent functions in *S.cerevisiae*, as the double mutant, but neither single mutant, is inviable (12). *TRF5* was isolated based on its ability to complement the cold sensitivity of a *trf4-1* point mutation and encodes a second member of the *TRF4* family. *TRF5* overexpression also complements the inviability of a *top1 trf4* double mutant and the predicted Trf5 protein has a region that is homologous to the N-terminus of Top1p. Putative *TRF4* homologs from *S.pombe*, *Drosophila* and human were identified in database searches. *trf4 trf5* double mutants are inviable, demonstrating that *TRF4/5* function is essential. A conditional *trf4 (ts) trf5* mutant was constructed and shown to be hypersensitive to the microtubule poison thiabendazole when grown at a semi-permissive temperature, as well as grossly defective in nuclear division at a restricitve temperature, indicating that *TRF4/5* function is required at the time of mitosis.

Table 3. Nuclear morphology

Morphology	Percentage of cells with each	
	24°C	37°C
Wild-type		
Normal	98	99
Anucleate	0.3	0.2
Binucleate	0.2	0.4
Cut-like	0.5	0.5
Total	529	440
trf4 (ts) trf5		
Normal	94	87
Anucleate	2	3
Binucleate	0.4	0.7
Cut-like	3	9.3[a]
Total	297	609

[a]50% of the cells that are in nuclear divison.

Both Trf4p and Trf5p show a region of limited but significant homology to the catalytically dispensable N-terminus of Top1p. This region is not conserved among type I DNA topoisomerases

and its role in Top1p function is not known. The high percentage of both positively and negatively charged residues in these regions (average 35%) may indicate that their function is to mediate interaction with chromatin, as similar regions are commonly found in HMG proteins (26).

The Trf4 family of proteins does not show significant overall homology with other proteins in the GenBank database (release 91) that would suggest a biochemical activity, with the exception of the *S.pombe* homolog, which shows a significant homology to G-β proteins via its 570 amino acid N-terminal extension. G-β family members encode proteins involved in many processes (27), including initiation of DNA replication (CDC4), chromosome separation (CDC20) and transcriptional repression (TUP1). A 40 amino acid repeated sequence containing consecutive Trp and Asp residues, termed the WD40 repeat, is found between five and eight times in most family members and appears to mediate protein–protein interaction (28).

TRF4/5 function is required at the time of mitosis for proper nuclear division. A critical and poorly understood event in the chromosome cycle that occurs at mitosis is chromosome condensation. Thus, a plausible hypothesis is that *TOP1*, *TRF4* and *TRF5* are involved in mediating mitotic chromosome condensation. This model is consistent with the proposed mitotic function for *TRF4/5*. In addition, null mutations in *trf4* are inviable in combination with a mutation in *SMC1* (29), the yeast homolog of XCAP-C (30), a *Xenopus* protein that is required for mitotic chromosome condensation *in vitro* (I. B. Castaño and M. F. Christman, unpublished observations). Taken together, these results suggest that the *TRF4* gene family may encode proteins that mediate mitotic chromosome structure in some manner.

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REFERENCES

1 Wang,J.C. and Lynch,A.S. (1993) *Curr. Opin. Genet. Dev.*, **5**, 764–768.
2 Brill,S.J., DiNardo,S., Voelkel,M.K. and Sternglanz,R. (1987) *Nature*, **326**, 414–416.
3 Kim,R.A. and Wang,J.C. (1989) *J. Mol. Biol.*, **208**, 257–267.
4 Uemura,T. and Yanagida,M. (1984) *EMBO J*, **3**, 1737–1744.
5 Liu,L.F. and Wang,J.C. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 7024–7027.
6 Giaever,G.N. and Wang,J.C. (1988) *Cell*, **55**, 849–856.
7 Brill,S.J. and Sternglanz,R. (1988) *Cell*, **54**, 403–411.
8 Thrash,C., Voelkel,K., DiNardo,S. and Sternglanz,R. (1984) *J. Biol. Chem.*, **259**, 1375–1377.
9 Thrash,C., Bankier,A.T., Barrell,B.G. and Sternglanz,R. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 4374–4378.
10 Goto,T. and Wang,J.C. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 7178–7182.
11 Uemura,T., Morino,K., Uzawa,S., Shiozaki,K. and Yanagida,M. (1987) *Nucleic Acids Res.*, **15**, 9727–9739.
12 Sadoff,B.U., Heath-Pagliuso,S., Castaño,I.B., Yingfang,Z., Kieff,F.S. and Christman,M. F. (1995) *Genetics*, **141**, 465–479.
13 Wallis, J. W., Chrebet, G., Brodsky, G., Rolfe, M. and Rothstein, R. (1989) *Cell*, **58**, 409–419.
14 Kim, R. A. and Wang, J. C. (1992) *J. Biol. Chem.*, **267**, 17178–17185.
15 Cozzarelli, N. R. and Wang, J. C. (1990) *DNA Topology and its Biological Effects*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
16 Bailis, A. M., Arthur, L. and Rothstein, R. (1992) *Mol. Cell. Biol.*, **12**, 4988–4993.
17 Gangloff,S., McDonald,J.P., Bendixen,C., Arthur,L. and Rothstein,R. (1994) *Mol. Cell. Biol.*, **14**, 8391–8398.
18 Guarente,L. (1992) *The Molecular and Cellular Biology of the Yeast Saccharomyces*, Vol. 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. pp. 49–98.
19 Ito,H., Fukuda,Y., Murata,K. and Kimura,A. (1983) *J. Bacteriol.*, **153**, 163–168.
20 Dower,W.J., Miller,J.F. and Ragsdale,C.W. (1988) *Nucleic Acids Res.*, **16**, 6127–6145.
21 Holmes,D.S. and Quigley,M. (1981) *Anal. Biochem.*, **114**, 193–197.
22 Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463–5467.
23 Link,A.J. and Olson,M.V. (1991) *Genetics*, **127**, 681–698.
24 Sikorski,R.S. and Boeke,J.D. (1991) In Fink,C.G. and Guthrie,C.R. (eds), *In Vitro Mutagenesis and Plasmid Shuffling: From Cloned Gene to Mutant Yeast*. Academic Press, New York, NY, Vol. 194, pp. 302–319.
25 Bjornsti,M.A. and Wang,J.C. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 8971–8975.
26 Kruger,W. and Herskowitz,I. (1991) *Mol. Cell. Biol.*, **11**, 4135–4146.
27 Henikoff,S. and Henikoff,J.G. (1991) *Nucleic Acids Res.*, **19**, 6565–6572.
28 Komachi,K., Redd,M.J. and Johnson,A.D. (1994) *Genes. Dev.*, **8**, 2857–2867.
29 Strunnikov,A.V., Larionov,V.L. and Koshland,D. (1993) *J. Cell. Biol.*, **123**, 1635–1648.
30 Hirano,T. and Mitchison,T.J. (1994) *Cell*, **79**, 449–458.
31 Rose,M.D., Novick,P., Thomas,J.H., Botstein,D. and Fink,G.R. (1987) *Gene*, **60**, 237–243.
32 Gietz,R.D. and Sugino,A. (1988) *Gene*, **74**, 527–534.
33 Herskowitz,I. and Jensen,R.E. (1991) *Methods Enzymol.*, **194**, 132–146.