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TEL2, an Essential Gene Required for Telomere Length Regulation and Telomere Position Effect in *Saccharomyces cerevisiae*

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The DNA-protein complexes at the ends of linear eukaryotic chromosomes are called the telomeres. In *Saccharomyces cerevisiae*, telomeric DNA consists of a variable length of the short repeated sequence $C_{1-3}A$. The length of yeast telomeres can be altered by mutation, by changing the levels of telomere binding proteins, or by increasing the amount of $C_{1-3}A$ DNA sequences. Cells bearing the *tel1-1* or *tel2-1* mutations, known previously to have short telomeres, did not respond to perturbations that caused telomere lengthening in wild-type cells. The transcription of genes placed near yeast telomeres is reversibly repressed, a phenomenon called the telomere position effect. The *tel2-1* mutation reduced the position effect but did not affect transcriptional repression at the silent mating type cassettes, *HMR*a and *HML*α. The *TEL2* gene was cloned, sequenced, and disrupted. Cells lacking *TEL2* function died, with some cells arresting as large cells with three or four small protrusions or "blebs."

Telomeres, the ends of linear eukaryotic chromosomes, are specialized chromosomal domains that are involved in the replication and protection of chromosomal termini (for reviews, see references 48 and 63). Telomeres associate with one another and with the nuclear envelope, and in organisms where they can be visualized, they can be packaged into heterochromatin (29, 63). Genes placed near telomeres are transcriptionally repressed (18, 28), similar to the reduced expression that occurs when euchromatic genes are placed near centromeric heterochromatin (for reviews, see references 18, 42, 48, and 56).

In most organisms, the chromosome end is capped by a simple, repeated DNA sequence; for example, wild-type yeast cells have ~350 bp of $C_{1-3}A$ DNA at the ends of each chromosome (for a review, see reference 63). These $C_{1-3}A$ sequences appear to be necessary and sufficient for telomere function in mitosis (38, 41, 49) and meiosis (60). Although the lengths of the terminal $C_{1-3}A$ tracts at different telomeres vary, average *Saccharomyces* telomere length appears to be regulated such that the length of the terminal $C_{1-3}A$ tracts is 350 ± 75 bp. When the amount of $C_{1-3}A$ sequences in wild-type yeast cells is increased ~30-fold by using high-copy plasmids, chromosomal telomeres double in length to 500 to 700 bp (46). Extra $C_{1-3}A$ DNA affects telomere length whether the extra tracts are introduced as telomeres or as internal tracts on a circular plasmid. When $C_{1-3}A$ DNA is introduced as the ends of linear plasmids, both plasmid and chromosomal telomeres lengthen (46). The fact that excess $C_{1-3}A$ sequences cause telomere elongation on both plasmids and chromosomes would lead one to predict that telomere length should increase without bounds. This expectation is based on the consideration that the elongation of chromosomal and plasmid telomeres introduces still more $C_{1-3}A$ sequences, which should cause even more lengthening. However, this bootstrap effect does not occur. Rather, telomeres reach a final equilibrium length which is stable as long as there is an excess of $C_{1-3}A$ sequences in the cell (46). This new equilibrium does not represent an upper limit to telomere length. Cells bearing certain mutations in DNA polymerase α can have $C_{1-3}A$ tracts as long as 2 kb (10), and cells bearing mutations that eliminate the carboxy terminus of the *Saccharomyces cerevisiae* telomere binding factor *RAP1* protein, or Rap1p, can have $C_{1-3}A$ tracts of 4 kb (*rap1^t* alleles [24]). These data suggest that wild-type yeast cells regulate telomere length in response to an excess of $C_{1-3}A$ sequences to achieve a new equilibrium state.

Additional data suggesting that yeast cells can regulate telomere length comes from experiments involving new telomere formation. When linear plasmids are formed in yeast cells by using short or long stretches of $C_{1-3}A$, the final length of $C_{1-3}A$ sequence on the plasmid termini is similar to that of the chromosomal telomeres of the host strain (37, 41, 46, 59, 61). This result is obtained even when the input plasmid starts with 250 to 350 bp of $C_{1-3}A$ sequence (46, 61). A hypothesis consistent with these data would be that yeast cells somehow measure the length of the terminal $C_{1-3}A$ tract from the boundary of the $C_{1-3}A$ and non-$C_{1-3}A$ sequences to the physical end and maintain this length within a certain range.

In *S. cerevisiae*, the average length of the telomeric $C_{1-3}A$ tract can be altered by mutations in several different genes. These mutations fall into two classes. Some cause telomeres to lengthen or shorten continuously. For example, the *cdc17-1* mutation, which alters the catalytic subunit of DNA polymerase α, causes telomeres to lengthen continuously when cells are grown at a semipermissive temperature (8, 10). The *est1-1* mutation causes telomeres to shorten continuously until cells die (31), as does deletion of *TLC1* (55). Other mutations cause cells to maintain telomeres at a new average length. For example, the recessive *tel1-1* and *tel2-1* mutations cause telomeres to be maintained at shorter lengths (32), while the

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TABLE 1. Yeast strains

Strain	Genotype	Reference or source
KR14-86	α *ade2-1 ade8-18 ura3-52 trp1Δ1 leu2-3,112 his4 cyh2*^{r}	46
KR36-6L	**a** *ade2*(*-1* or *-101*) *ade8-18 ura3-52 trp1Δ1 his3Δ leu2Δ-RC*	47
A_HF	**a** *ade1 ade2 URA1 ura3-a his7 lys2 tyr1 gal1*	A. Lustig
AJL210	α *ade1 ade2 URA1 ura3-b his7 lys2 tyr1 gal1 cdc27 tel1-1*	A. Lustig
AJL1291d	α *ade1 ade2 URA1 ura3-b his7 lys2 tyr1 gal1 tel2-1*	A. Lustig
A_HFA8L	**a** *ade1 ade2 ade8Δ-SX ura3-***a** *leu2Δ-RC his7 lys2 tyr1 gal*	A. Lustig
KR75-1D	α *ade1 ade2 ade8Δ-SX ura3-b leu2Δ-RC his7 lys2 tyr1 gal1 cdc27 tel1-1*	This work
KR75-2A	**a** *ade1 ade2 ade8-ΔSX ura3-***a** *leu2Δ-RC his7 lys2 tyr1 gal1 cdc27 tel1-1*	This work
KR76-2B	α *ade1 ade2 leu2Δ-RC his7 lys2 tyr1 gal1 tel2-1*	This work
KR76-2D	α *ade1 ade2 ade8Δ-SX ura3-***a** *ura1 his7 lys2 tyr1 gal1 tel2-1*	This work
KR78-6C	α *ade1 ade2 ade8Δ-SX ura3-***a** *leu2Δ-RC his7 lys2 tyr1 gal1 tel2-1*	This work
KR78-11D	α *ade1 ade2 ade8Δ-SX ura3-***a** *leu2Δ-RC his7 lys2 tyr1 gal1 TEL2*	This work
KR78-2B	**a** *ade1 ade2 ade8Δ-SX URA3 leu2-ΔRC his7 lys2 tyr1 gal1 tel2-1*	This work
KR90-1C	α *ade2*(*-1* or *-101*) *ade8-18 ura3-52 trp1Δ1 leu2-3,112 his3Δ cyh2*^{r} *TEL2*::*LEU2*	This work
1422-7D	**a** *ura3 leu2 his3 lys2 esp1-1*	J. McGrew and B. Byers
KR95^t	**a**/α *ade2-1*/*ade2-101 ade8-18*/*ade8-18 ura3-52*/*ura3-52 trp1Δ1*/*trp1Δ1 leu2-3,112*/*leu2-ΔRC his3Δ*/*HIS3 his4*/*HIS4 cyh2*^{r}/*cyh2*^{r} *tel2Δ*::*TRP1*/*TEL2*	This work
KR95-1A	**a** *ade2*(*-1* or *-101*) *ade8-18 ura3-52 trp1Δ1 leu2*(*-3,112* or *-ΔRC*) *his3Δ cyh2*^{r} *tel2Δ*::*TRP1*	This work
KR95-2A	**a** *ade2*(*-1* or *-101*) *ade8-18 ura3-52 trp1Δ1 leu2*(*-3,112* or *-ΔRC*) *his3Δ cyh2*^{r} *TEL2*	This work
KR95-2D	α *ade2*(*-1* or *-101*) *ade8-18 ura3-52 trp1Δ1 leu2*(*-3,112* or *-ΔRC*) *his3Δ cyh2*^{r} *tel2Δ*::*TRP1*	This work
KR97-56	**a** *ade2*(*-1* or *-101*) *ade8-18 ura3-52 trp1Δ1 leu2*(*-3,112* or *-ΔRC*) *his3Δ cyh2*^{r} *tel2Δ*::*TRP1 VIIL adh4*::*URA3-TEL*	This work

introduction of a large excess of $C_{1-3}A$ sequences (46) or overexpression of a deletion derivative of the *RAP1* protein containing the carboxy terminus, Rap1ΔBBp, causes telomeres to be maintained at about twice their normal length (14). The first group of mutations includes genes that might play a role in telomere replication: *CDC17* is the structural gene for a subunit of DNA polymerase α (8), *TLC1* encodes yeast telomerase RNA (55), and the *EST1* protein may play a role in telomerase activity (see references 30 and 31). The second group of mutations, *tel1-1* and *tel2-1*, cause perturbations that result in telomeres reaching a new steady-state length. Cells bearing the *tel1-1* mutation have terminal $C_{1-3}A$ tracts of 50 to 100 bp, while *tel2-1* cells have tracts of 100 to 150 bp (32). If the *TEL1* and *TEL2* genes were involved in regulating the number of telomere repeats, then mutations in these genes might set a new equilibrium telomere length. If *TEL1* and *TEL2* are regulators, then telomere length in *tel1-1* and *tel2-1* cells might be refractory to other perturbations that alter telomere length, for example, excess $C_{1-3}A$ or overproduction of Rap1ΔBBp.

In this paper, we show that the telomeres of *tel1-1* and *tel2-1* cells did not lengthen either when additional $C_{1-3}A$ sequences were introduced into the cell or when the deletion derivative of RAP1 containing the C terminus, Rap1ΔBBp, was expressed. The *tel2-1* mutation also reduced telomere position effect (TPE) repression of *URA3* transcription but not silencing at the silent mating type cassettes. Both *tel1-1* and *tel2-1* cells with short telomeres grew slowly at 37°C on rich medium. The *TEL2* gene was cloned by suppression of this slow-growth phenotype, sequenced, and shown to be essential.

MATERIALS AND METHODS

Yeast strains and methods. The strains used in this study are summarized in Table 1. The wild-type strains KR14-86 and KR36-6L have been described previously (45, 46). The A364a background strains A_HF, AJL210, and AJL1291d were from A. Lustig. The *ura1* mutations in A_HF and AJL210 were reverted by selecting for uracil prototrophy, and the *ura3* mutations were independently selected on 5-fluoro-orotic acid (FOA) medium (31a). The *ura3-a* and *ura3-b* alleles were distinguishable by Southern blotting after digesting with *Pst*I and probing with the 1.1-kb *URA3 Hin*dIII fragment (data not shown). AJL210 and AJL1291d are progeny of the fourth backcross of the original *tel1-1* and *tel2-1* isolates (32) to an A364a wild-type strain.

The wild-type strain A_HF was converted to A_HFA8L by transformation with the plasmids pade8-ΔSX (from R. Wellinger) and pLUL. The pade8-ΔSX plasmid contains an in-frame deletion of the DNA between the internal *Sal*I and *Xho*I sites within the *ADE8* gene (62) and a 0.9-kb insertion of *Salmonella hisG* DNA (1) at the *Bgl*II site downstream of *ADE8*. This deletion greatly reduces *ADE8* gene function. The *leu2-ΔRC* allele (constructed with pLUL) contains a deletion of the DNA between the internal *Cla*I and *Eco*RV sites within *LEU2* that eliminates *LEU2* function. Strain A_HFA8L was crossed with strains AJL210 and AJL1291d to give the *tel1-1* strains KR75-1D and KR75-2A, the *tel2-1* strains KR76-2B, KR76-2D, and KR78-6C, and the wild-type strain KR78-11D.

Strains A_HFA8L, KR75-2A, and KR78-6C were converted to *URA3-TEL* strains by transformation with pADH4-UCAIV, which replaces the distal 17 kb of the left arm of chromosome VII with the *URA3* gene and a short tract of $C_{1-3}A$ which elongates in vivo to form a full-length yeast telomere (as in reference 18). KR97-56 bearing YRpH3T2 is similar to KR95-1A (see below) but also has *URA3* at the telomere of the left arm of chromosome VII. In all of these strains, the *URA3* gene is transcribed toward the telomere.

The KR112 (*tel2-1*/*tel2Δ*) diploid was constructed by mating KR95-2D bearing YEpT2 to KR78-2B. This diploid was cured of YEpT2 and grown for 100 generations to express the *tel2-1* phenotype. The KR112 diploid was used in the position effect test of *HML*α and *HMR***a** (see Table 3).

Chromosome stability was measured by our previously described assay for chromosome III (45). In this work, strains KR78-11D (*TEL2*) and KR78-6C (*tel2-1*) were separately mated to the *tel2Δ*::*TRP1* strain KR95-1A/YEpT2 to form the diploid strains KR109 (*TEL2*/*tel2Δ*) and KR107 (*tel2-1*/*tel2Δ*). The plasmid YEpT2 was cured from both diploids, and *cyh2*^{R}/*cyh2*^{R} versions of each diploid were made by patching each diploid onto yeast extract-peptone-dextrose (YEPD) medium containing cycloheximide and picking a single colony. The resulting cycloheximide-resistant (Cyh^R) diploids were transformed with pH4::CUC-2 to introduce the CYH2^s gene into one copy of chromosome III within *HIS4* (as described in reference 45). Two separate transformants of each diploid that had the *CYH2*^{s} gene integrated on the same chromosome as *MAT***a** were chosen, grown without YEpT2 for a total of 100 generations, and used to measure the rate of chromosome III loss by fluctuation analysis with 20 colony tests (45). The recombination rates for both diploids were 1.0×10^{-4} for both strains, and the loss rates are given in Results.

All yeast media have been previously described (66). Yeast transformation was performed by the lithium acetate method with single-stranded salmon sperm DNA as the carrier (52).

Introduction of excess $C_{1-3}A$ sequences and overexpression of the Rap1p C terminus. The amount of telomeric $C_{1-3}A$ sequences in cells was increased by using the circular plasmid YEpFAT7.5 (46). This plasmid was originally cloned in yeast cells and contained a $C_{1-3}A$ tract of ~450 bp. Upon propagation in *Escherichia coli*, the length of the $C_{1-3}A$ tract was reduced to ~270 bp (reference 16a and data not shown). The plasmid with this shorter $C_{1-3}A$ tract was used in all experiments in this study. For the experiments reported here, YEpFAT7 or YEpFAT7.5 was transformed into the yeast strains described in Results and Ura$^+$ transformants were selected. Individual transformants were selected and streaked for single colonies on medium lacking either uracil (YC-ura) or leucine (YC-leu). Transformants were serially streaked five times on either YC-ura or YC-leu so that each strain had grown for 100 consecutive generations on one of

these media. Cells treated in this manner were used to start 5-ml YC-ura or YC-leu liquid cultures, as appropriate, to prepare yeast DNA for analysis by Southern hybridization (as in reference 46).

Overexpression of the *RAP1* protein C terminus, Rap1ΔBBp, was accomplished by using YEpFAT4-ΔBB (14). This plasmid encodes a truncated *RAP1* gene in which codons 1 to 19 are fused to codons 499 to 827 by deletion of an internal *Bam*HI fragment (14). Cells were transformed with YEpFAT4 or YEp FAT4-ΔBB, and Ura^+ transformants were selected. Individual transformants were then grown for 40 consecutive generations on YC-ura or YC-leu to allow maximal telomere lengthening (14). Cells treated in this way were used to start 5-ml YC-ura or YC-leu liquid cultures, as appropriate, that were used to prepare DNA for Southern hybridization as described above.

Plasmid copy numbers were determined by preparing genomic DNA from yeast cells, digesting it with *Xho*I, *Sal*I, and *Nsi*I, and analyzing the products by Southern hybridization. The *LEU2 Eco*RV-*Acc*I fragment was used as a probe. All of the sequences in this fragment are present on both the plasmid and the chromosome. The restriction enzyme digest and a 20-fold dilution of it were loaded in adjacent gel lanes, and the intensities of the plasmid and chromosomal bands were compared. The fraction of cells with plasmid was determined by plating 200 CFU (determined by counting with a hemocytometer) of each strain onto complete medium and medium lacking leucine. The plasmid copy number in wild-type, *tel1-1*, and *tel2-1* cells bearing YEpFAT4, YEpFAT4-RAP1ΔBB, and YEpFAT7.5 grown on medium lacking leucine was approximately 25 to 40 per cell. This copy number is lower than expected for selection for the *leu2-d* allele, and the reason for this is unclear. The copy number of each plasmid in the *tel1-1* and *tel2-1* cells (25 to 45/cell) was as high or higher than that seen in the wild-type cell.

Re-replica plate assay for slow growth at 37°C. To score the weak temperature sensitivity of *tel2-1* strains, colonies or patches of cells were taken through two consecutive replica platings. First, patches of cells were grown on either rich medium or one that selects for a plasmid. A wild-type strain and a *tel2-1* strain were usually included as controls. Second, cells were replica plated to a YEPD plate by standard methods (34). Third, the new replica plate was immediately used as a new master plate. The new master plate would be replica plated to a YEPD plate that had been preincubated at 37°C for at least 2 h and to a second YEPD plate (usually at room temperature or 4°C). The last two YEPD plates are the re-replica plates. The re-replica plate, which was prewarmed to 37°C, was incubated at 37°C for one to 2 days, while the other plate was incubated at 30°C for the same length of time. No growth differences were observed with the standard replica plate assays.

The *tel1-1* cells in this work also bear the Ts^- *cdc27* mutation. Three *cdc27* mutation revertants were selected and tested by the re-replica plate test as described in Results.

Cloning the *TEL2* gene. KR78-6C cells (bearing the *tel2-1* mutation) were transformed (52) with a yeast genomic library cloned into YEp24 (9), and transformants were selected by plating on six YC-ura plates. Each plate contained approximately 1,300 transformants; to construct a pool of transformants, 2 ml of sterile H_2O was placed on the plate, the cells were suspended with a spreader, and the suspension was transferred to a sterile test tube. The plate was then washed with an additional 2 ml of sterile H_2O, which was subsequently added to the suspension. The six 4-ml suspensions were centrifuged to pellet the cells. The H_2O was removed, and the cells were resuspended in 5 ml of YC-ura liquid medium and grown overnight at 30°C. Each culture was vortexed vigorously for 15 s, and a 0.1-ml aliquot of cells from each suspension was diluted into 5 ml of YC-ura liquid medium. The six cultures were grown at 30°C for 2 days to saturation ($\sim 10^8$ cells per ml), approximately 10 generations. Each culture was diluted (0.05 ml) into 5 ml of fresh YC-ura medium and grown for 24 h at 30°C. This procedure was repeated six times. After the seventh consecutive culture, the cells were diluted into YEPD liquid medium prewarmed to 37°C and grown at 37°C for 3 days. Only one of the seven cultures grew to saturation. The number of CFU (single cells or budded cells) per milliliter in this dense culture was determined by counting with a hemocytometer, and 200 CFU from each culture was plated onto each of 10 YEPD plates that had been prewarmed to 37°C. The plates were incubated at 37°C for 2 days. Only 2 to 3 large colonies grew on each plate, 14 of which were analyzed for telomere length and temperature sensitivity. All 14 strains had telomeres of wild-type length and were Ts^+. The YEp24 library plasmid was isolated from three of these strains. All three strains contained one plasmid as determined by restriction enzyme mapping. This plasmid was designated YEpT2. These results were consistent with a single YEpT2 transformant being amplified in this pool and one positive transformant being obtained from the initial 7,800 transformants.

Recombinant DNA manipulations of the *TEL2* gene. YEpT2 contained a 7.8-kb genomic yeast DNA insert that has no *Xho*I sites. To localize the *TEL2* gene within this insert, YEpT2 was partially digested with *Rsa*I and the fragment corresponding to the size of the linearized plasmid was isolated by gel electrophoresis. The linearized plasmid was then ligated to an 8-bp *Xho*I linker, cut with *Xho*I, and religated under conditions which led to circularization of the plasmid; the circularized plasmid was then transformed into *E. coli* MC1066 (*r⁻ m⁺ pyr*::Tn*5 trpC9830 leuB600 lac*Δ*X74 strA galU galK*). Plasmids were isolated from Ura^+ *E. coli* transformants, and the *Xho*I linker insertions were mapped. Five insertions in different places in the insert and one internal deletion were isolated and separately transformed into KR78-6C (*tel2-1*) cells. Three isolates of the six types of yeast transformants were streaked for single colonies on YC-ura medium at 30°C. A single colony from each strain was picked and restreaked on YC-ura medium. This process was repeated three more times for each strain so that all *tel2-1* transformants had grown for 100 generations in the presence of the YEpT2-*Xho*I linker insertion plasmid. The strains were then tested for telomere length and temperature sensitivity. Two plasmids failed to complement the *tel2-1* mutation, an insertion plasmid and the internal deletion plasmid (which shared an end point with the noncomplementing insertion). The 3.2-kb *Nsi*I fragment (see below) was subcloned into the *Pst*I site of pVZ-1 (21), and the deletions were made by restriction enzyme digestion. The DNA sequence was determined by the dideoxy sequencing method (50) by using a Sequenase 2.0 kit (Amersham) with both single-stranded and double-stranded templates and by automated sequencing with dye terminators. The locations of the following restriction sites are based on the numbering in Fig. 4: *Sca*I, nucleotide 551; *Hin*dIII, 1665; *Pst*I, 1869; and *Xba*I, 2418.

The 3.2-kb *Nsi*I fragment, which lacked the *Xho*I linker insertion, was cloned into the *Pst*I site of pVZ-1 to form the plasmid pT2Na, which was used to construct several yeast vectors. YIpL2T2 is pT2Na plus the 3.0-kb *Bgl*II fragment containing the *LEU2* gene in the *Bam*HI site of pT2Na. The only *Xho*I site in this plasmid is upstream of the *LEU2* gene, and this site was used to target the plasmid to the *LEU2* locus by homologous recombination (43) (see Results). YRpL2T2 is a yeast replicating vector that is pT2Na plus a 2.6-kb *Sal*I-*Bgl*II fragment bearing the *LEU2* gene and the *Xba*I-*Nsi*I fragment of the yeast 2μm circle, which contains the origin of replication but not the 2μm REP3 plasmid stabilizing region (6). YRpH3T2 is a similar plasmid that contains the *HIS3* gene instead of the *LEU2* gene.

Two types of *tel2* gene disruption plasmids were constructed: a transplacement vector (44), pT2::TRP1, and an integration vector (43), YIpt2-ΔL2. pT2::TRP1 contains the 0.6-kb *Nsi*I-*Sca*I fragment upstream of the *TEL2* open reading frame (ORF), the *Xmn*I-*Bgl*II *TRP1* fragment from YCp19 (57), and the *Xba*I-*Nsi*I fragment downstream of the *TEL2* ORF with a *Bam*HI site 5′ to the *Xba*I site from the pVZ-1 polylinker. These fragments were cloned into the pVZ-1 *Pst*I site in the order (*Pst*I *Nsi*I)-(*Sca*I *Xmn*I)-*TRP1*-(*Bgl*II *Bam*HI)-*Xba*I-(*Nsi*I *Pst*I), where sites in parentheses are fusions that destroy both restriction sites. The integrating vector YIpt2-ΔL2 contains a 400-bp fragment of *TEL2* from the *Xho*I linker insertion to the *Pst*I site, both in the *TEL2* ORF, cloned into the integrating vector pRS305 (54) which had been cut with *Xho*I and *Pst*I. The only *Hin*dIII site in this plasmid is in the *TEL2* DNA, and the *Hin*dIII site is ~200 bp from both the *Xho*I site and the *Pst*I site.

Sequence similarity searches were performed with the BLAST program (2) on the National Center for Biotechnology Information (NCBI) servers. The *TEL2* DNA sequence was compared with the nonredundant DNA and protein sequences in the databases. The sequences of the predicted ORFs were also compared with the same database sequences. Separate comparisons were also made with the expression sequence tag (EST) database. No significant similarities were found between any of the database sequences and sequences encoded in the 3.2-kb *Nsi*I fragments, with the exception of the overlap with the *ESP1* sequence, as of 11 March 1996.

Construction of *tel2* disruption strains. The *tel2*Δ::*TRP1* allele was made by disrupting one copy of the gene in a diploid strain, sporulating the strain, and isolating a haploid strain with the mutant allele. The diploid KR95 was formed by mating KR14-86 to KR36-6L (45), selecting His^+ diploids, and then selecting for Cyh^R clones by patching an individual diploid colony on YEPD containing cycloheximide (10 μg/ml). KR95 was transformed with pT2::TRP1 that had been digested with *Sph*I and *Bam*HI. Trp^+ colonies were selected and analyzed by Southern hybridization to find a strain that had one wild-type copy of *TEL2* and one disrupted copy, KR95ᵗ. KR95ᵗ was transformed with YEpT2, induced to sporulate, and dissected to produce KR95-1A and KR95-2A (Table 1), both bearing YEpT2. Both strains were derived from 4-spored tetrads in which *MAT*, *trp1*, *his4*, and *his3* segregated 2:2.

The *tel2*-Δ*L2* disruption was constructed in KR95-2A bearing YEpT2. YIpt2-ΔL2 was linearized by digestion with *Hin*dIII, which cuts only in the *TEL2* DNA sequences, and used to transform KR95-2A cells, selecting for Leu^+ transformants. Transformants were screened for the ability to grow without YEpT2 by replica plating on FOA medium. Four of 100 transformants could not grow on FOA plates, and these four strains were transformed with YRpH3T2 to give His^+ Leu^+ strains. Three of the new strains could now grow on FOA plates or on FOA medium lacking leucine, indicating that the presence of YRpH3T2 resulted in the loss of the YEpT2 plasmid from these transformants. Southern analysis of these three strains indicated that they possessed the *tel2*-Δ*L2* disruption-integration at the *TEL2* locus. These strains were not able to grow without the presence of a normal *TEL2* gene on a plasmid in the cell.

Cloning the *tel2-1* gene. The *Nsi*I fragment containing the *tel2-1* gene was isolated by PCR with primers at each end of the sequenced *Nsi*I fragment (TEL2PCR5a CGGATCCAGTACTTGTGGACAGGTTGCC and TEL2 PCR3a CGGATCCATTCTCGATCCTTGCC) and genomic DNA from the *tel2-1* strain KR78-6C as the template, using 35 cycles of 1 min at 94°C, 1 min at 55°C, and 3 min at 72°C. The 3.2-kb fragment was digested with *Bam*HI and cloned into the *Bam*HI site of pVZ-1, and a single clone was sequenced on both strands from nucleotides 176 to 2820 of the *TEL2* sequence (see Results). The only difference detected was at nucleotide 1006, which was a G-to-A transition causing a serine-to-asparagine mutation in the predicted *TEL2* protein. A region

from nucleotide 870 to 1120 of the *TEL2* sequence (using the primers T2873S CGCCGGATCCGCGATGGACTGCAAGAG and T21120AS CGCCGAATT CCCCTAAAAACCCAGGTGG) was amplified by PCR in separate reactions with genomic DNA from four *tel2-1* strains (AJL1291d, KR76-2B, KR76-2D, and KR78-6C), two wild-type strains (A_HF and A_HFA8L), and three *tel1-1* strains (KR75-1D, KR75-2A, and AJL210) as templates. All amplification reactions for sequencing consisted of denaturation for 5 min at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C and one cycle of 6 min at 72°C. The PCR products from all of these reactions were sequenced directly, using the T2931S primer (GAACCGGGATGTTTGAC), by automated sequencing in the CCF automated sequencing core. Two separate, independent amplifications and sequencing reactions were performed for DNA from AJL1291d and A_HFA8L with the same results. All four *tel2-1* amplified products contained the G-to-A transition at nucleotide 1006 found in the original *tel2-1* clone, while the wild-type and *tel1-1* products contained the wild-type *TEL2* sequence (G at nucleotide 1006).

Physical and genetic mapping of the *TEL2* gene. Filters containing the ordered phage clones of the yeast physical map were provided by Linda Riles and Maynard Olson (42a). A 423-bp *Xho*I-*Pst*I fragment from the *TEL2* coding region was labelled with random hexamers (47) and used as probe according to the directions provided by L. Riles. Three overlapping phage clones were detected, placing *TEL2* on the right arm of chromosome VII, approximately 35 kb proximal to *SPT6*. Comparison of the restriction map of *TEL2* with the high-resolution *Eco*RI-*Hin*dIII double digest map provided by L. Riles indicated that *TEL2* is oriented in the chromosome such that transcription goes from the centromere toward the telomere.

The genetic map of *S. cerevisiae* (36) indicates that *TEL2* should be ~10 centimorgans (cM) from *ESP1*. The *TEL2* gene was marked by inserting the 2.2-kb *LEU2 Sal*I-*Xho*I fragment into the *TEL2 Xho*I linker insertion such that the *Xho*I site of *LEU2* was closest to the *TEL2* C terminus. This *TEL2*::*LEU2* insertion can provide full *TEL2* function in terms of cell growth and telomere length. A strain bearing this *LEU2* insertion, KR90-1C, was mated to a strain bearing a Ts^- lethal mutation in *ESP1*, 1422-7D (from J. McGrew and B. Byers). The resulting diploid was sporulated, and 182 randomly selected cycloheximide-resistant haploid spores were scored for mating type, temperature sensitivity, and leucine auxotrophy. Of the two classes of possible recombinant progeny, 1 Ts^+ Leu^- and 10 Ts^- Leu^+ spores were observed. The disparity between the two classes was too large to be due to random sampling error ($P < 0.01$). Assuming one recombinant class of spores is lost because of unknown differences between the parental-strain backgrounds, the distance (in centimorgans) between these markers is approximately equal to the yeast genetic map (36). The centimorgan-per-kilobase measurements in Results were obtained by using 4.5 to 6.1 kb as the distance between the *LEU2* insertion and the *esp1-1* mutation.

TPE assay. Assays were performed on haploid strains bearing *URA3-TEL* (A_HFA8L, KR75-2A, and KR78-6C) and diploid strains bearing one copy of *URA3-TEL* formed by mating either KR78-6C or KR78-11D to either KR97-56 or KR95-2A/*tel2-ΔL2*. In the case of the diploid strains, Southern hybridization was used to screen for loss of YRpH3T2. Plasmid-free cells were grown for 100 generations to allow full expression of the *tel2-1* mutant phenotype prior to TPE assays. Assays were performed by streaking cells for single colonies on YEPD medium at either 30°C (diploid strains) or 23°C (haploid strains). After single colonies had formed, one colony from each strain was picked and streaked for single colonies on synthetic complete (YC) medium, with incubation at either 30 or 23°C. For each strain, five single colonies were individually picked as agar plugs by using a Pasteur pipette, and each colony was placed in 1.0 ml of sterile H_2O. Each colony suspension was vortexed at the highest setting for 15 s. Tenfold dilutions of each suspension were made and plated (one plate per dilution) to determine the titers of cells that would grow on YC (viable cells), YC-ura (Ura^+ cells), and FOA (cells where *URA3* expression is repressed by TPE). The position effect for each colony is the percentage of cells that can grow on FOA medium. In all the strains used here, >90% of the viable cells were Ura^+. The level of position effect for each strain was determined by placing the values for each of the five colonies in order from highest to lowest and choosing the median value. In many cases, the median was also the mode and close to the average.

HML*α and *HMR*a position effect assays.** Silencing or position effect at *HML*α or *HMRa** was tested by monitoring sporulation of *MAT*Δ/*MAT*(**a** or α) diploids. The *TEL2*/*tel2*Δ *MAT*Δ/*MAT*(**a** or α) and *tel2-1*/*tel2*Δ *MAT*Δ/*MAT*(**a** or α) diploids were constructed by using KR108 (*TEL2*/*tel2*Δ) and KR112 (*tel2-1*/*tel2*Δ). The KR112 diploid was cured of YEpT2 and grown for 100 generations. Each diploid was then transformed with pKAN591 that had been digested to completion with *Pvu*II and *Hin*dIII, selecting for Leu^+ transformants. The plasmid pKAN591 is *MAT*α*x8* in pBR322 (58) containing the 2.2-kb *LEU2 Xho*I-*Sal*I fragment inserted at the *Xho*I linker (a gift from L. Breeden). This plasmid replaces *MAT***a** and *MAT*α with the *LEU2* gene. Transformants were tested for mating type to determine which copy of *MAT* had been eliminated, and the structures of the *mat*Δ::*LEU2*, *MAT***a** or *MAT*α, and *HML*α loci were confirmed by Southern hybridization. For the *TEL2*/*tel2*Δ KR108 strains, one *MAT***a**/*MAT*α diploid, one *mat*Δ/*MAT*α diploid, and one *MAT***a**/*mat*Δ diploid were tested. For the *tel2-1*/*tel2*Δ KR112 strains, one *MAT***a**/*MAT*α diploid, one *mat*Δ/*MAT*α diploid, and one *MAT***a**/*mat*Δ diploid were each transformed with YEp24 or pKR60. The presence of pKR60 in cells can derepress the position effect at the silent mating type cassettes (12) and telomeres (data not shown) (16b, 31a). The plasmid pKR60 is pCTC23 (12) with the *Bgl*II-*Xba*I *HIS3*-2μm ori portion removed and replaced with the *Bam*HI-*Xba*I *URA3*-2μm ori fragment from YEp24. This plasmid encodes a fusion of the *GAL4* protein (amino acids 1 to 147) with Sir4p (amino acids 1280 to 1394) expressed from the *ADC1* upstream activation sequence (UAS) and promoter (12).

The KR112 diploids bearing plasmids were grown overnight at 30°C in 5 ml of YC-ura liquid medium, diluted 1:4 with YEPD liquid medium, grown overnight at 30°C, and induced to sporulate. Briefly, an aliquot of 0.1 ml of cells was washed twice with sterile water and resuspended in 2 ml of 0.5% potassium acetate (KOAc) with 100 μg each of adenine, uracil, tryptophan, leucine, and histidine per ml. Cell suspensions were rotated for 5 or more days at 21°C to ensure complete sporulation. Aliquots of cells were viewed with a phase-contrast microscope at 400× magnification, and sporulated cells (tetrads, triads, and dyads) and mitotic cells were counted. The range of sporulation was 5 to 30% for unmodified KR108 diploids and <0.1% for the *mat*Δ/*MAT*(**a** or α) diploids (data not shown).

Nucleotide sequence accession number. The GenBank accession number for the *TEL2* sequence is U38538.

RESULTS

***TEL1* and *TEL2* functions are required for telomere length control.** Whereas wild-type cells have telomeres of 350 ± 75 bp, the *tel1-1* and *tel2-1* mutations cause cells to maintain telomeres with lengths of, respectively, 50 to 100 and 100 to 150 bp of $C_{1-3}A$ (32). If the *TEL1* and *TEL2* genes are involved in the regulation of telomere length, then these mutations might cause short telomeres by altering this regulation. For example, the *tel1-1* and *tel2-1* mutations may cause cells to ignore signals that would normally result in telomere elongation or to miscount the number of telomeric repeats at chromosome ends so that a shorter length than normal is maintained. Under either hypothesis, cellular perturbations that cause telomere lengthening in wild-type cells would not cause elongation in *tel1-1* or *tel2-1* cells.

Two types of perturbations that cause cells to increase their equilibrium telomere length are the introduction of long stretches of telomeric $C_{1-3}A$ DNA (46) and the overproduction of Rap1ΔBBp, a deletion derivative of the known yeast telomere binding protein Rap1p (14). In these in vivo experiments, telomere length is presumably altered because of competition with chromosomal telomeres for factors involved in telomere length regulation.

An excess of $C_{1-3}A$ sequences and the construct for producing large amounts of Rap1ΔBBp were introduced into cells on plasmids whose copy number could be controlled by nutritional selection, the YEpFAT plasmids. YEpFAT plasmids carry the promoter defective *leu2-d* allele and a selectable marker with a normal promoter, either *URA3* or *TRP1*. Plasmids can be maintained in multiple copies by selecting for the *URA3* or *TRP1* gene, and they can be maintained at higher levels by selecting for the *leu2-d* allele (see, e.g., reference 46 and references therein). YEpFAT plasmids bearing no insert (YEp FAT7 or YEpFAT4), long stretches of $C_{1-3}A$ (YEpFAT7.5), or the gene expressing RAP1ΔBBp (YEpFAT4-ΔBB) were introduced into congenic wild-type *tel1-1* or *tel2-1* cells. All transformants were grown under conditions that were previously shown to allow the maximum amount of telomere elongation in the presence of an excess of $C_{1-3}A$ sequences or extra *RAP1* protein C termini (14, 46). Telomere length was determined by Southern hybridization.

The results of this analysis of cells bearing plasmids encoding an excess of telomere DNA sequences and RAP1ΔBBp showed that the telomeres of wild-type cells elongated while those of cells bearing the *tel1-1* and *tel2-1* mutations did not (Fig. 1). The number of plasmids per cell was approximately the same in wild-type, *tel1-1*, and *tel2-1* cells (see Materials and Methods). These data indicate that *TEL1* and *TEL2* functions are required for telomere elongation in response to these per-

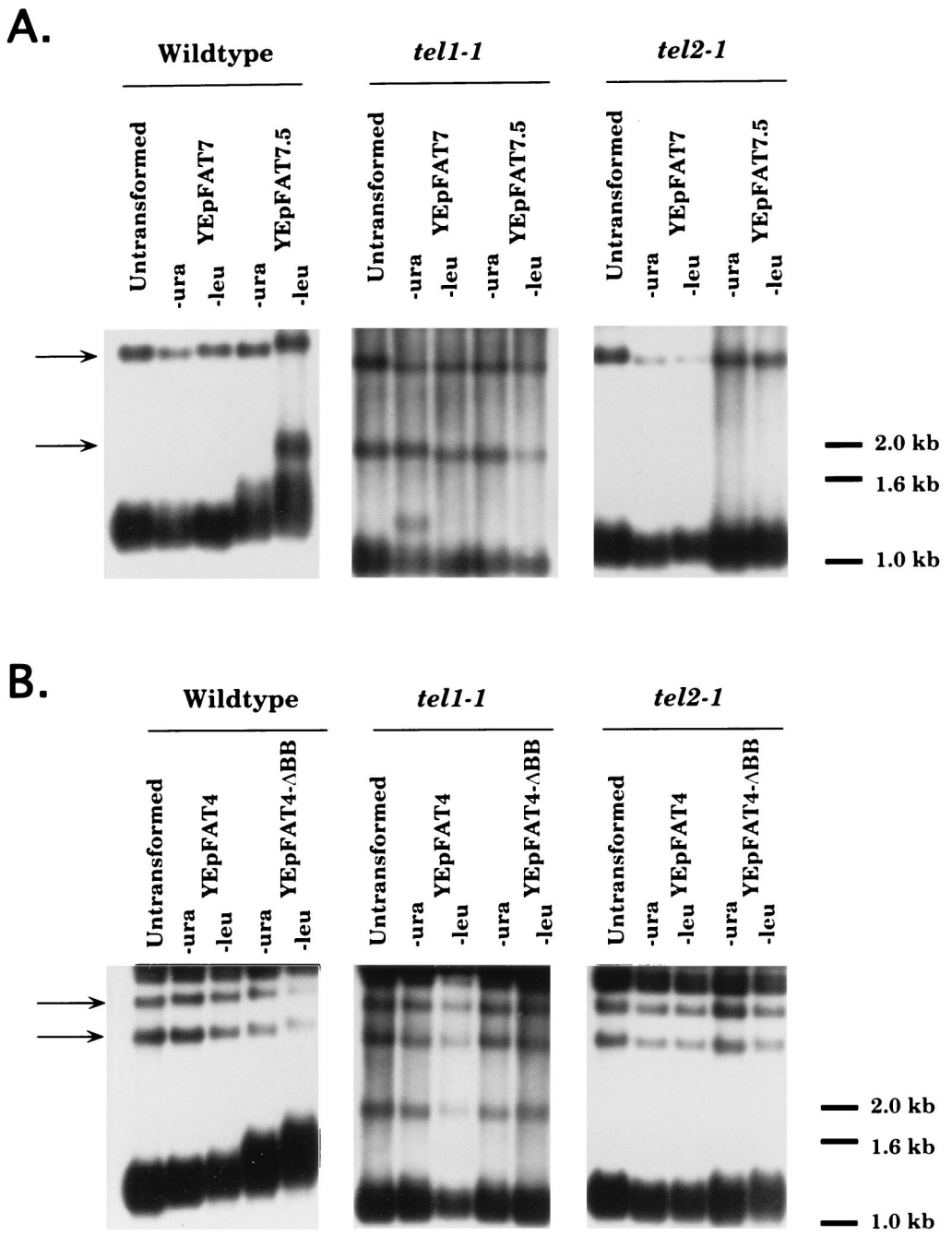

FIG. 1. Telomere elongation is perturbed in *tel1-1* and *tel2-1* cells. Wild-type (A_HFA8L), *tel1-1* (KR75-1D), and *tel2-1* (KR78-6C) cells were analyzed for telomere length in the presence of excess $C_{1-3}A$ sequences (A) and a plasmid expressing a deletion derivative of Rap1p (B). The excess $C_{1-3}A$ sequences were introduced by transforming cells with the circular plasmid YEpFAT7.5, which contains 270 bp of $C_{1-3}A$ sequences, and growing cells for 100 generations as described in Materials and Methods (46). Rap1ΔBBp was overproduced by transforming cells with YEpFAT4 bearing a truncated *RAP1* gene, *RAP1ΔBB* (see Materials and Methods) (14), and growing cells for 50 generations as described (Materials and Methods). In all panels, genomic DNAs were digested with *Xho*I and analyzed by Southern hybridization with $C_{1-3}A$ sequences as probe. The lowest band corresponds to the terminal restriction fragment of telomeres that contain a Y′ element, while the arrows indicate telomeres in different panels which did not hybridize to a Y′ probe and most likely are X telomeres with no Y′ elements (see reference 11).

turbations. These data are consistent with the hypothesis that *TEL1* and *TEL2* are involved in telomere length regulation.

A new phenotype associated with the presence of large amounts of Rap1ΔBBp was discovered during the course of these experiments. When wild-type, *tel1-1*, and *tel2-1* cells bearing YEpFAT4-ΔBB (Fig. 1B) were grown in YC-leu medium at 21°C, the numbers of *tel1-1* and *tel2-1* cells doubled every 5.3 and 5.4 h, respectively, while the wild-type cells required 10.3 h to double. The doubling times for all three cell types bearing the vector alone were ~5.4 h. Thus, large amounts of Rap1ΔBBp caused cold sensitivity in wild-type cells but not in *tel1-1* and *tel2-1* cells.

***TEL2* function is essential for viability in *S. cerevisiae*.** The original description of the *tel1-1* and *tel2-1* alleles suggested

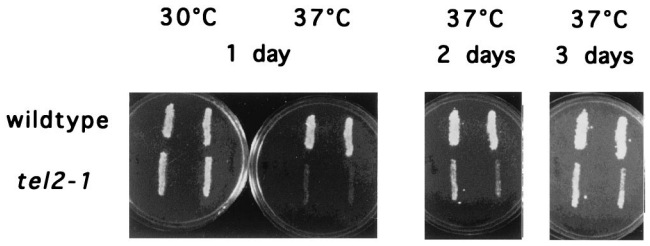

FIG. 2. *tel2-1* cells are impaired for growth at 37°C. Wild-type (A_HFA8L) and *tel2-1* (KR78-6C) cells were grown on a YEPD plate and tested by a re-replica plate assay (see Materials and Methods). The re-replica plates were grown at either 30 or 37°C for the time periods indicated.

that cells required up to 150 generations of growth for telomeres to attain their shortest lengths, a phenomenon known as phenotypic lag. There were no indications that these mutations had any effect on cell growth (32). During the course of this study, cells bearing the *tel2-1* mutation were found to have a growth disadvantage, a weak Ts^- phenotype, at 37°C on rich medium (YEPD; see Materials and Methods) (Fig. 2). The weak Ts^- phenotype cosegregated with the *tel2-1* mutation in four tetrads. This slow-growth phenotype was only observed in *tel2-1* cells that had short telomeres. When *tel2-1* spores are first isolated from tetrads, they give rise to cells with normal telomere lengths that gradually shorten as the cells go through repeated divisions (32). The slow growth at 37°C was detectable only after the *tel2-1* cells had grown for 80 to 100 generations, showing that shorter telomeres are associated with the slow-growth phenotype. The *tel1-1* mutation is linked to a Ts^- allele of *cdc27*. Three independent Ts^+ revertants were obtained and tested for the weak Ts^- phenotype (Fig. 2). These three *tel1-1* strains also showed the weak Ts^- phenotype of *tel2-1* cells (see Materials and Methods).

The *TEL2* gene was cloned by complementation of the weak Ts^- phenotype and then scoring for complementation of the short-telomere phenotype (assayed as in Fig. 2; see Materials and Methods). Cells bearing the *tel2-1* mutation were transformed with a yeast genomic library in the vector YEp24 (9). To ensure that the *TEL2* gene was expressed for an amount of time sufficient to reverse the *tel2-1* phenotype, the ~7,800 transformants were divided into six pools and grown for about 70 generations with selection for the library plasmids. Each pool was then challenged for rapid growth at 37°C in rich medium. One pool yielded many fast-growing colonies that also had normal-length telomeres. The library plasmid was isolated from three colonies from this pool. All three plasmids were identical by restriction enzyme mapping (data not shown).

The complementing library plasmid was designated YEpT2. The suppression of the *tel2-1* phenotypes was YEpT2 dependent: loss of the plasmid gave rise to strains that had short telomeres and grew poorly at 37°C. These results suggested that the complementing library plasmid contained the *TEL2* gene or a multicopy suppressor of the gene. When YEpT2 was retransformed into a *tel2-1* strain, the Ts^- and short-telomere phenotypes reverted after ~50 generations of growth in the presence of the plasmid.

YEpT2 contained a 7.8-kb insert of yeast genomic DNA. The region of the plasmid responsible for complementing the *tel2-1* mutation was localized by *Xho*I linker insertion mutagenesis (see Materials and Methods). Five *Xho*I linker insertions in the yeast genomic DNA were isolated. One of these insertions failed to complement the slow-growth and short-telomere phenotypes of the *tel2-1* mutation (Fig. 3 and 4). This insertion was near the center of a 3.2-kb *Nsi*I fragment. The 3.2-kb *Nsi*I fragment complemented the *tel2-1* mutation at one copy per cell when integrated at the *LEU2* locus (see Materials and Methods), suggesting that the *Nsi*I fragment contained the entire *TEL2* gene.

The sequence of the 3.2-kb *Nsi*I fragment revealed that the *Xho*I linker insertion disrupted a 688-amino-acid ORF (Fig. 4). The *Xho*I linker creates a frameshift after the first 20% of the ORF, so the majority of the protein would not be translated.

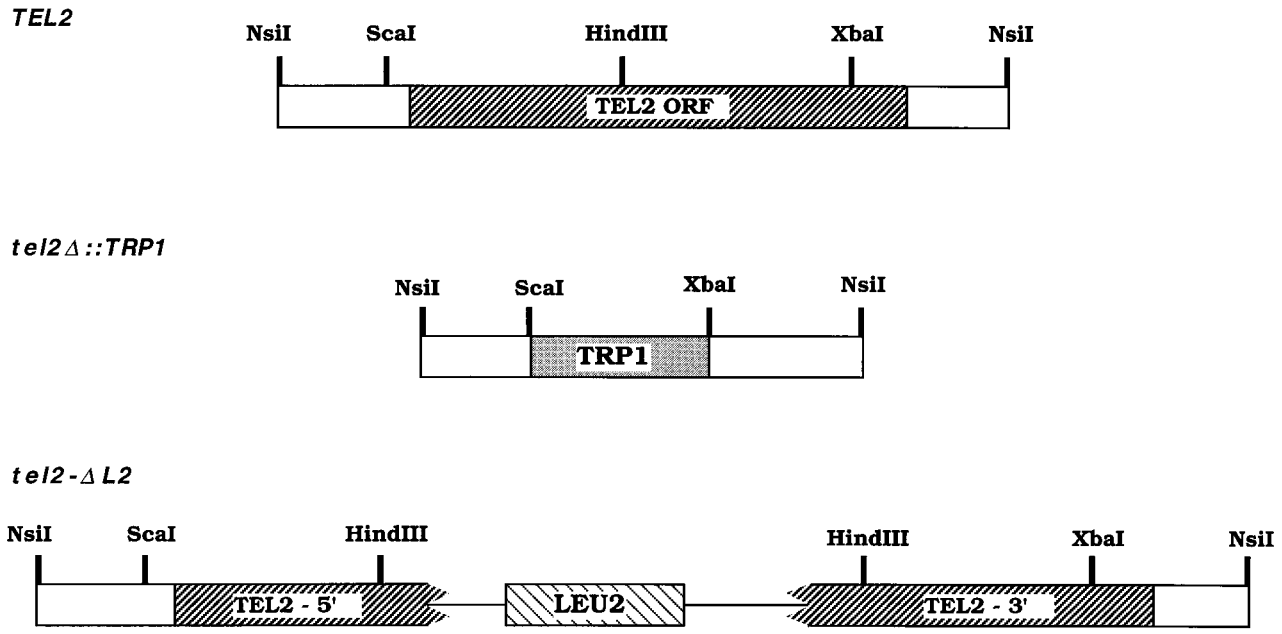

FIG. 3. *TEL2* gene disruptions. Diagrams of the wild-type *TEL2* gene and the *tel2*Δ::*TRP1* and *tel2*-Δ*L2* gene disruptions are shown. The *tel2*Δ::*TRP1* deletion removes all but the last 89 amino acids of the *TEL2* ORF and some upstream sequences. The *tel2*-Δ*L2* insertion does not remove any genomic sequences but creates a 3′ deletion followed by a 5.5-kb insertion and then a 5′ deletion of the *TEL2* gene (see Materials and Methods).

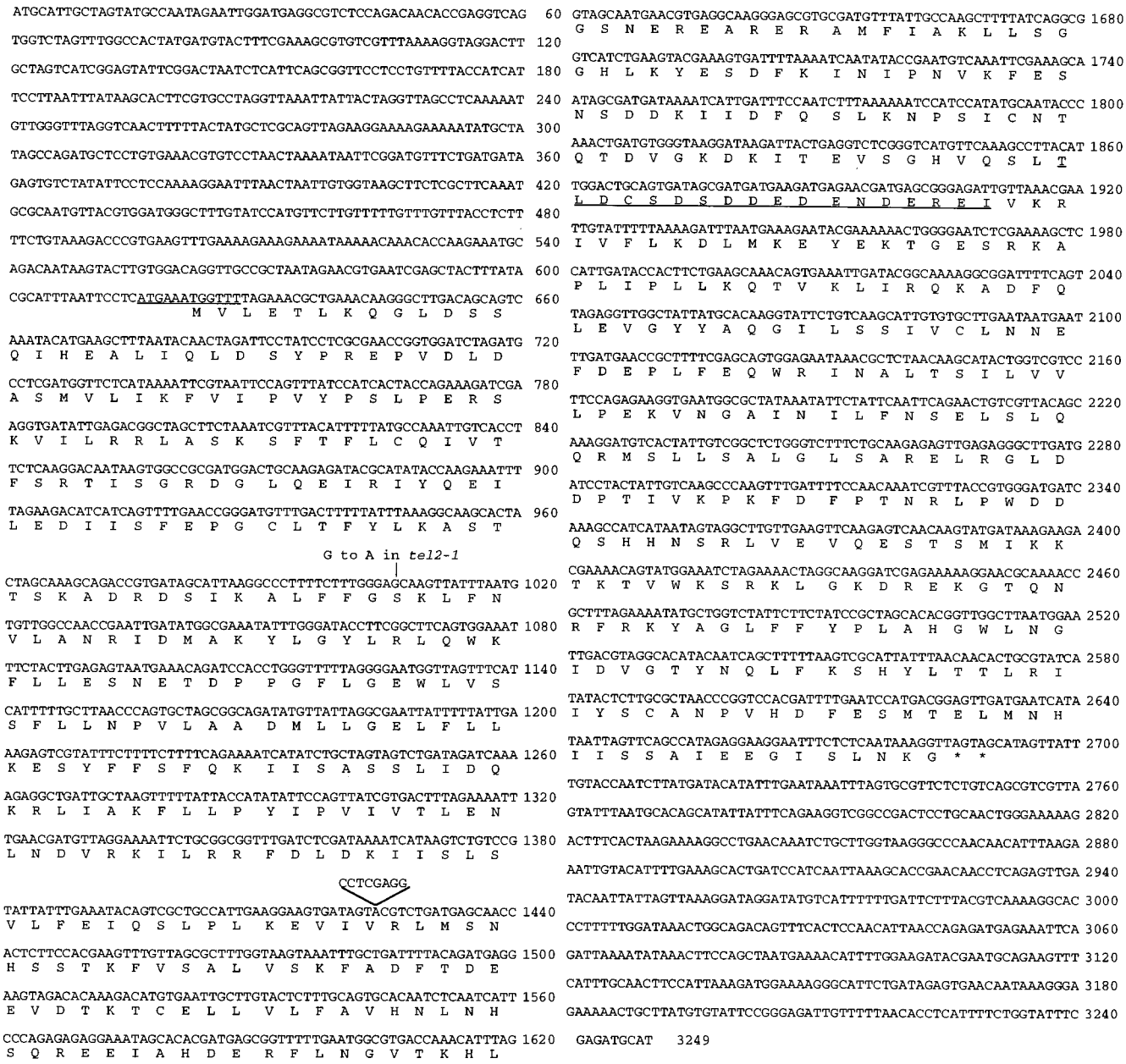

FIG. 4. Sequence of the *TEL2* locus. The DNA sequence of the *TEL2* locus and the amino acid sequence of the *TEL2* ORF are shown. The short ORF overlapping the *TEL2* ATG and the short stretch of amino acid sequence similarity between the *TEL2* and *ARD1* proteins are underlined. The site of the *Xho*I linker insertion in the *TEL2* ORF and the *tel2-1* mutation (G to A at nucleotide 1006, causing an S129N substitution) are indicated. The stars indicate termination codons. The *tel2-1* mutation was found in four of four *tel2-1* DNA samples tested (see text). The Tel2 protein is predicted to have a molecular mass of 79 kDa and a pI of 6.2.

Two disruptions in the 688-amino-acid ORF were constructed. The first disruption, *tel2*Δ::*TRP1*, replaced 1.9 kb of DNA with the *TRP1* gene (Fig. 3). This deletion removes 599 amino acids from the N terminus of the ORF (see below) (Fig. 4). Haploid cells bearing this disruption were unable to grow unless the ORF was provided in *trans* on a plasmid. The other disruption, *tel2-*Δ*L2*, placed 5.5 kb of plasmid DNA, including the *LEU2* gene, in the middle of the ORF without deleting any chromosomal DNA (Fig. 3). This disruption was also lethal (see Materials and Methods). These results show that the ORF that complemented the *tel2-1* mutation in single copy was also essential for viability of *S. cerevisiae*.

These disruption mutations were tested for complementation with the recessive *tel2-1* mutation. Cells bearing the *TRP1* disruption and containing a plasmid bearing the 3.2-kb *Nsi*I fragment were mated to congenic *TEL2* and *tel2-1* cells (see Materials and Methods). Diploids that had lost the plasmid were isolated, and the strains were grown for 100 generations to overcome phenotypic lag. A *tel2-1*/*tel2-1* diploid was also constructed. All three diploids were tested for telomere length and growth at 37°C. We noted that telomeres in both wild-type and mutant diploid strains were slightly longer than telomeres in similar haploid strains (Fig. 5). However, the length difference due to the *tel2-1* mutations was maintained in both haploids and diploids (Fig. 5 and data not shown). Compared with the *TEL2*/*tel2-1* diploid, the *tel2-1*/*tel2*Δ::*TRP1* diploid had

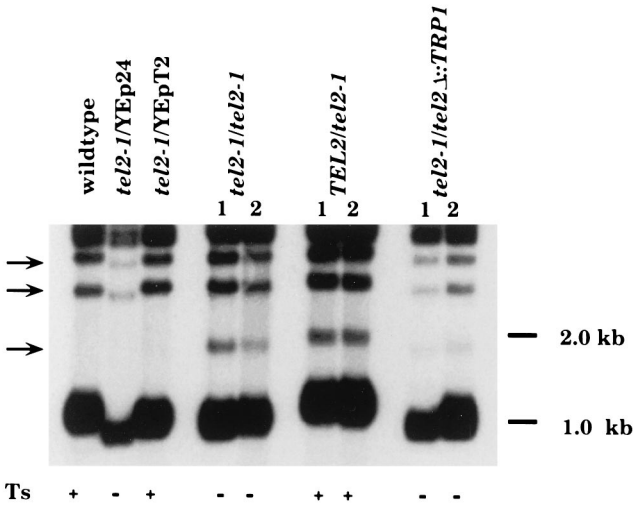

FIG. 5. The *tel2*Δ mutation fails to complement the *tel2-1* telomere length and temperature sensitivity phenotypes. Genomic Southern blots of the different diploids with $C_{1-3}A$ as probe are shown. Two different isolates of each diploid that were grown for 100 generations without a *TEL2* plasmid were analyzed. The genotype of each strain is indicated at the top of the lane and described in the text. The three lanes on the left are haploid strains. The temperature sensitivity of each strain in the re-replica plate assay is indicated at the bottom of its Southern blot lane. +, wild-type growth at 37°C in the re-replica plate assay (Fig. 2); −, slow *tel2-1* growth at 37°C. Arrows indicate X telomeres.

short telomeres and showed weak Ts^- growth, just as the *tel2-1*/*tel2-1* diploid did (Fig. 5). In contrast, the *TEL2*/*tel2*Δ::*TRP1* diploid was phenotypically wild type (data not shown). Identical results were obtained with a *tel2-1*/*tel2*-Δ*L2* diploid (data not shown). As shown below, the *tel2*Δ::*TRP1* allele also failed to complement the TPE phenotype of the *tel2-1* mutation (Table 2).

To confirm that the ORF in Fig. 4 encodes the *TEL2* gene, the 3.2-kb *Nsi*I fragment was isolated by PCR from the *tel2-1* strain KR78-6C. The entire ORF was sequenced on both strands, and the only difference between it and the *TEL2* sequence was the G-to-A transition at nucleotide 1006, encoding a serine-to-asparagine mutation at amino acid 129 (S129N). PCR primers flanking this mutation were used to amplify this region of DNA from four *tel2-1* strains (AJL1291d, KR76-2B, KR76-2D, and KR78-6C), two wild-type strains (A_HF and A_H FA8L), and three *tel1-1* strains (AJL210, KR75-1D, and KR75-2A) (see Materials and Methods) (Table 1), and the PCR products were sequenced. All four *tel2-1* strains contained the *tel2-1* mutation, A at nucleotide 1006 (Fig. 4), while the wild-type and *tel1-1* cells contained the *TEL2* sequence. These results, together with the *Xho*I linker insertion, single-copy suppression of *tel2-1*, and failure of the deletion to complement the *tel2-1* short-telomere length, weak Ts^-, and reduced TPE phenotypes, indicate that the 688-amino-acid ORF encodes the *TEL2* gene. Since lack of *TEL2* function is lethal, the *tel2-1* allele is not a loss-of-function mutation.

The *tel2*Δ::*TRP1* allele was used to test the effect of the *tel2-1* mutation on chromosome stability in yeasts. The rate of loss of yeast chromosome III was measured in *TEL2*/*tel2*Δ::*TRP1* and *tel2-1*/*tel2*Δ::*TRP1* diploids by fluctuation analysis (45) (see Materials and Methods). Loss rates of 6.1×10^{-4} loss events per cell division for the *TEL2*/*tel2*Δ::*TRP1* diploid and 15×10^{-4} loss events per cell division for the *tel2-1*/*tel2*Δ::*TRP1* diploid were found (with standard deviations for each fluctuation test of 2.0×10^{-4} and 7.0×10^{-4}, respectively; see Materials and Methods). Both loss rates were higher than our previously measured loss rates for chromosome III of 0.39×10^{-4} in a similar wild-type diploid (45), suggesting that having only one of the two copies of the *TEL2* gene in a cell may affect chromosome loss. Thus, when the *TEL2*/*tel2*Δ and *tel2-1*/*tel2*Δ strains were compared, the *tel2-1* mutation had, at best, a small effect on chromosome stability. A similar small effect on chromosome stability was recently demonstrated for the *tel1*Δ mutation (19).

As described above, disruption of the *TEL2* gene is lethal in a haploid cell. To determine whether cells lacking *TEL2* gene product die in an interesting way, a diploid strain bearing the *tel2*Δ::*TRP1* disruption and a normal *TEL2* gene was sporulated and the resulting tetrads were dissected. As expected, tetrads contained no more than two viable spores and all viable spores were Trp^-, confirming that the *TEL2* gene was essential. Microscopic examination of the *tel2*Δ::*TRP1* spores that failed to produce colonies revealed that these spores germinated and gave rise to microcolonies of two to eight cells. Many of these cells were large and misshapen.

To generate a larger population of cells lacking the *TEL2* gene, YRpH3T2, an unstable plasmid containing the *TEL2* gene, was introduced into a *tel2*Δ::*TRP1* strain. YRpH3T2 is expected to be lost at a high rate, thereby generating plasmid-free cells (39). The plasmid-free cells will continue to divide until they run out of *TEL2* gene product. Thus, the proportion of cells manifesting a phenotype due to a complete lack of Tel2 protein is expected to be small. Examination of cells from such a culture by phase-contrast microscopy revealed that a fraction of the cells had a novel phenotype. There were large spherical cells with up to four small "blebs" or "knobs" (Fig. 6). These blebs were smaller than the yeast buds typical of mutants that arrest as budded cells. The presence of multiple blebs is an unprecedented phenotype. When the *tel2*Δ::*TRP1* cells were stained with DAPI (4′,6-diamidino-2-phenylindole), a single nucleus was observed that was frequently associated with one of the blebs (data not shown). Since cells with small blebs were not detected in parallel cultures of congenic *TEL2* or *tel2-1* cells, they were most probably cells that had run out of *TEL2* gene product. Similar multiply budded cells have been seen in cells overproducing Las1p, an essential nuclear protein involved in cell morphogenesis (15), but those surface projections appear to be somewhat larger than the blebs in Fig. 6.

Structure and location of the *TEL2* gene. The *TEL2* ORF had no significant homology to any of the sequences in the NCBI Blast databases (see Materials and Methods). A short stretch of sequence similarity was found with the yeast gene *ARD1*, which encodes a component of the N-terminal acetylase and affects TPE (3, 40) (Fig. 4, underlined sequence). How-

TABLE 2. Reduction of TPE by *tel2-1*

Strain	Frequency of FOA^r	
	Median	Range
Haploid		
A_HFA8L (wild type)	1.0×10^{-2}	$(0.36–3.8) \times 10^{-2}$
KR78-6C (*tel2-1*)	8.9×10^{-4} (0.089)[a]	$(6.0–13) \times 10^{-4}$
Diploid		
TEL2/*tel2*Δ::*TRP1*	8.8×10^{-2}	$(8.6–14.3) \times 10^{-2}$
tel2-1/*tel2*Δ::*TRP1*	3.7×10^{-4} (0.0042)[b]	$(3.0–28) \times 10^{-4}$
tel2-1/*tel2*Δ-*L2*	1.2×10^{-3} (0.014)[b]	$(0.20–2.8) \times 10^{-3}$

[a] Value in parentheses is fold difference versus median frequency of FOA^r in wild-type haploid.

[b] Value in parentheses is fold difference versus median frequency of FOA^r in *TEL2*/*tel2*Δ::*TRP1* diploid.

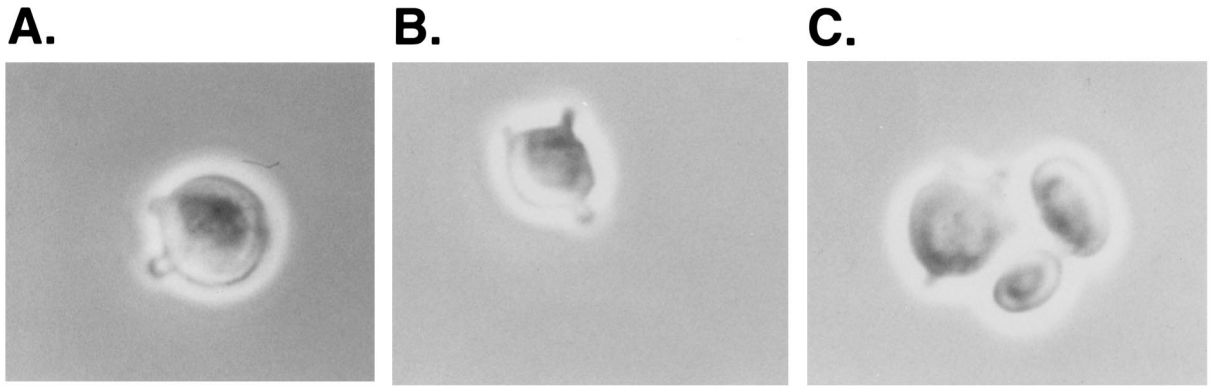

FIG. 6. Unusual cells found in *tel2*Δ cultures. Phase-contrast micrographs of cells from a culture of *tel2*Δ::*TRP1* cells bearing YRpL2T2, an unstable plasmid bearing the *TEL2* gene, are shown. A budded cell with wild-type morphology is shown in panel C next to a large cell with blebs. Large cells with blebs make up ~2% of the culture. Such cells are not seen in cultures of *TEL2* or *tel2-1* cells.

ever, the sequence similarity was limited to this short region. When this short region was used to search the databases, many proteins containing short stretches of acidic amino acids were found, but the extent of sequence similarity with these proteins was limited to this region, suggesting no overall functional similarity. *TEL2* showed no similarity to *LAS1*.

The *TEL2* ORF did have structural similarity to the *EST1* gene. The *EST1* locus contains four short overlapping ORFs of 3 to 18 amino acids within the 80 bp upstream of the first ATG of the *EST1* ORF (31). The *TEL2* locus has one 4-amino-acid ORF that overlaps the ATG of the *TEL2* ORF (Fig. 4). Small ORFs in the 5′ region of yeast genes are rare. In two other genes where they occur, *GCN4* and *CPA1*, these small ORFs are involved in translational control of expression (for a review, see reference 22). The *TEL2* gene has a high proportion of rare amino acid codons, like the *EST1* gene, suggesting that the *TEL2* and *EST1* gene products are present at low levels in vivo.

The *TEL2* gene was placed on the yeast physical and genetic maps. A 443-bp fragment from the *TEL2* ORF was used to probe the ordered phage clone set that constitutes the yeast physical map (42a). The *TEL2* gene mapped to the right arm of chromosome VII, ~35 kb proximal to *SPT6*, near *CLY8*, and ~10 cM distal to *ESP1*. Transcription of the *TEL2* gene is oriented toward the telomere.

The amount of meiotic recombination between *TEL2* and *ESP1* per kilobase of DNA was 1.5 to 1.6 times higher than expected (see Materials and Methods). While searching for homology to the 138-amino-acid ORF, the first 727 bp of the *TEL2* sequence was found to overlap the *ESP1* sequence (33). The *esp1-1* mutation, which resides in the last 1.4 kb of the 4.5-kb *ESP1* ORF (32a), was mapped against a marked *TEL2* gene (see Materials and Methods). On the basis of our data and the yeast genetic map (36), the amount of recombination was 1.6 to 2.1 cM/kb compared with the average of 0.67 cM/kb for chromosome VII. Therefore, the region between *TEL2* and *ESP1* appears to be a hotspot for meiotic recombination.

The *tel2-1* mutation reduces the position effect at telomeres but not at the silent mating type cassettes. When a gene is placed near a telomere in *Drosophila melanogaster* or *S. cerevisiae*, the expression of that gene is repressed in a manner that is very similar to position effect repression by heterochromatin (18, 28). To determine if the *tel1-1* or *tel2-1* mutation affected other aspects of telomere function besides length, the levels of TPE were examined. In yeast cells, TPE on a *URA3* gene is usually quantitated by determination of resistance to the drug FOA. Cells that express the *URA3* gene are sensitive to FOA, while cells that lack *URA3* expression are resistant to FOA (FOA^R). In wild-type cells, when the only copy of *URA3* in the cell is near a telomere, a fraction of the cells give rise to FOA^R colonies (18). The fraction of cells that exhibit TPE varies from strain to strain, ranging from 10^{-2} to 9×10^{-1} (18, 63) (data not shown).

To determine if the *tel2-1* mutation altered TPE, the *URA3* gene was placed at the left telomere of chromosome VII in the congenic strains A_HFA8L (wild type) and KR78-6C (*tel2-1*) (see Materials and Methods) and the fraction of FOA^R cells was determined for each (Table 2). The fraction of FOA^R *tel2-1* cells was 10 times lower than that in the congenic wild-type strain (Table 2). In a parallel experiment, A_HFA8L and KR78-6C were mated to *tel2*Δ strains to produce *TEL2*/*tel2*Δ::*TRP1*, *tel2-1*/*tel2*Δ::*TRP1*, and *tel2-1*/*tel2*-Δ*L2* diploids, each bearing one copy of *URA3* at VIIL (see Materials and Methods). These diploids were assayed for TPE. The fractions of FOA^R *tel2-1*/*tel2*Δ::*TRP1* and *tel2-1*/*tel2*-Δ*L2* diploids were, respectively, ~200 and ~70 times lower than that of the wild-type diploid (Table 2). Thus, the ORF disruption mutations failed to complement the reduced-TPE phenotype of the *tel2-1* mutation. In contrast, strains bearing the *tel1-1* mutation have been reported to exhibit normal TPE levels (18), a result confirmed in this study with a congenic *tel1-1* strain (data not shown). Since *tel1-1* cells have even shorter telomeres than *tel2-1* cells, the reduction in TPE in *tel2-1* cells is unlikely to be a secondary consequence of short telomeres.

The yeast silent mating type cassettes *HML*α and *HMR***a** contain copies of the *MAT* locus that are transcriptionally silenced (reviewed in reference 27). Many of the *trans*-acting factors required for silencing at *HML*α and *HMR***a** are also required for TPE (3). The diploid strains described above allowed us to test whether the *tel2-1* mutation allowed expression from the silent mating type cassettes.

*MAT***a**/*MAT*α diploids can sporulate to form tetrads, but diploids that are not heterozygous at *MAT* cannot. In order for a *MAT*Δ/*MAT*(**a** or α) diploid to sporulate, information from *HMR***a** or *HML*α must be expressed. *TEL2*/*tel2*Δ and *tel2-1*/*tel2*Δ diploids were transformed with a plasmid to delete either *MAT***a** or *MAT*α (see Materials and Methods). The *tel2-1*/*tel2*Δ *MAT*Δ/*MAT*(**a** or α) diploids were then transformed with YEp24 or pKR60. The plasmid pKR60 is a 2μm-*URA3* plasmid bearing a *GAL4-SIR4* gene fusion whose expression derepresses the silent mating type cassettes (12) and allows *MAT*Δ/*MAT*(**a** or α) diploids to sporulate (see below and Ma-

TABLE 3. Effect of the *tel2-1* mutation on *MAT*Δ/*MAT*(**a** or α) diploid sporulation

tel2-1/*tel2*Δ diploid	No. of spore figures[a]	No. of cells counted	Fraction sporulated
*MAT***a**/*MAT*α			
YEp24	20	1,112	0.018
pKR60[b]	10	1,161	0.0086
*MAT***a**/*MAT*Δ			
YEp24	0	10,392	$<9.6 \times 10^{-5}$
pKR60[b]	27	876	0.031
*MAT*Δ/*MAT*α			
YEp24	0	10,486	$<9.5 \times 10^{-5}$
pKR60[b]	37	792	0.047

[a] Sum of dyads, triads, and tetrads.
[b] Presence of this plasmid derepresses silencing at telomeres and *HM* loci.

terials and Methods). YEp24 is a similar vector with no insert. These diploids were sporulated in liquid medium, and the numbers of tetrads were counted (Table 3). The *MAT***a**/*MAT*α *tel2-1*/*tel2*Δ diploids sporulated such that 1 to 5% of cells were tetrads, triads, or dyads. Different *TEL2*/*tel2*Δ diploids showed 5 to 30% sporulation under these conditions (see Materials and Methods). No sporulated cells were observed in the *tel2-1*/*tel2*Δ *MAT*Δ/*MAT*(**a** or α) diploids bearing YEp24. However, these cells were able to sporulate if silencing at *HML*α or *HMR***a** was relieved by the presence of pKR60. These results indicate that the position effect at *HMR***a** or *HML*α was relieved in fewer than 1% of *tel2-1*/*tel2*Δ cells. As the *tel2-1*/*tel2*Δ diploid relieved TPE by approximately 100-fold (Table 2), these data suggest that the *tel2-1* mutation alters silencing only at telomeres or has a far greater effect at telomeres than at other loci.

DISCUSSION

The *tel2-1* mutation affected telomeres in three ways. First, *tel2-1* cells maintained shorter-than-normal telomeric tracts (32). Second, the telomeres of *tel2-1* cells remained short when cells carried plasmids that contained $C_{1-3}A$ DNA or expressed a deletion derivative of Rap1p (Fig. 1), both of which caused telomere lengthening in congenic wild-type cells (Fig. 1). In addition, the *tel2-1* mutation prevented the slow growth at 21°C caused by expression of large amounts of the carboxyl terminus of the *RAP1* protein. Third, the *tel2-1* mutation reduced TPE but had no detectable effect on silencing of *HML*α or *HMR***a**. The *tel2-1* mutation and overexpression of a portion of the *TLC1* gene are the only perturbations to date that have been found to alter TPE without affecting the *HM* loci (3, 25, 55). Thus, since the absence of Tel2p affects various aspects of telomere behavior, it is likely to play an important role in telomere structure and function. The *TEL2* gene, which encodes a 688-amino-acid protein with no striking similarity to any of the sequences in the NCBI database, was essential for growth. Cells that ran out of *TEL2* gene product arrested with a novel phenotype, large round cells with small blebs.

Although the multiple-telomere phenotype of *tel2-1* cells suggests that the *TEL2* protein (Tel2p) plays a direct role at telomeres, the rapid-death and small-bleb phenotypes of *tel2* null alleles suggest that Tel2p probably has other functions in the cell besides those at telomeres. Failure of telomere replication is expected to result in the gradual shortening of terminal $C_{1-3}A$ sequences until telomere function is lost. Cellular DNA polymerases require an RNA primer from which to initiate synthesis. The gradual loss of telomeric DNA due to the failure to replace the RNA primer would lead one to predict that many generations would be required before some chromosomes would lose telomere function and be lost, leading to cell death. For example, cells lacking yeast telomerase RNA or bearing the *est1*Δ mutation go through 40 to 100 cell divisions before they die (31, 55). However, *tel2-1* and *tel1-1* cell telomeres shorten until they reach a new equilibrium length, suggesting a telomere length regulation defect as opposed to failure to replicate telomeres. In addition, a defect in telomere replication does not readily account for the large-cell-with-small-bleb phenotype of *tel2*Δ cells. We suggest that Tel2p is involved in assembling the chromatin structure of the telomere and adjacent DNA. In this respect, Tel2p would resemble the known telomere binding protein Rap1p, an essential gene product which has roles in silencing, transcriptional activation, telomere maintenance, and nuclear organization (7, 23, 53).

The yeast telomeric region has an unusual chromatin structure. Yeast terminal $C_{1-3}A$ repeats are in a nonnucleosomal chromatin structure called the telosome (64), of which Rap1p is a major component (14, 65). The DNA adjacent to the $C_{1-3}A$ repeats is organized into nucleosomes (64), but unlike nucleosomes in most other regions of the genome, the histones in these nucleosomes are hypoacetylated (5). Genes placed in this region are subject to TPE (18) and are protected from methylation by *dam* methylase (17). All of these effects are considered to be due to the special chromatin structure of the telomeric region (3, 5, 16, 20, 48, 56). We hypothesize that Tel2p may be required for chromatin assembly at telomeres and elsewhere in the genome. In this hypothesis, the *tel2-1* cells form an altered telosome which in turn alters telomere length and TPE. A requirement of this hypothesis is that some genes affecting cell morphology are particularly sensitive to changes in Tel2p levels. In this model, as *tel2*Δ cells run out of Tel2p, the transcriptional regulation of these genes is altered, resulting in the knobbed-cell phenotype. The high proportion of rare amino acid codons in the *TEL2* ORF and the observation that the *GAL1-TEL2* fusions that complement *tel2*Δ on galactose also complement on glucose (data not shown) suggest that only low levels of Tel2p are required to carry out its function. Thus, the role of Tel2p in chromatin could be an enzymatic one as opposed to a structural one. Under this hypothesis, *TEL2* resembles *Drosophila Suvar* genes, some of which encode proteins that alter chromatin structure and are essential (for a review, see reference 42).

Analysis of the *tel1-1* and *tel2-1* cells and sequencing of the *TEL2* gene have revealed several common features among mutations affecting telomere length. First, cells with short telomeres due to either the *tel1-1*, *tel2-1* (Fig. 2), or *est1-1* mutation (31, 44a, this work) were impaired for growth at high temperatures. This weak Ts^- phenotype was not observed in these three strains when their telomeres were long (see Results) (44a). This weak Ts^- phenotype may be the result of an inherent temperature lability in essential telomere DNA-protein interactions that is only apparent when telomeres are short. The short-telomere phenotype associated with the *tel1-1* and *tel2-1* mutations is consistent with the hypothesis that *TEL1* and *TEL2* are regulators of telomere length. The *tel1-1* and *tel2-1* mutations may change the range of allowable telomere lengths in cells. The *TEL2* sequence reveals the presence of a short ORF that overlaps the *TEL2* ATG, a structural feature shared with the *EST1* gene (31) but with few other yeast genes (13, 22). The presence of these upstream ORFs, besides suggesting a translational control mechanism similar to that of *GCN4* (22), raises the possibility that the expression of several genes affecting telomere length is coordinately regulated.

Like *tel2-1* cells, *tel1-1* cells did not respond to perturbations that caused telomere lengthening (Fig. 1). The *TEL1* gene has

recently been cloned and encodes a protein with similarity to known phosphoinositide 3-kinases. *TEL1* has the strongest sequence similarity of all known proteins to the ataxia telangiectasia gene product and is also similar to the *S. cerevisiae MEC1* and *Schizosaccharomyces pombe rad3* cell cycle checkpoint genes (19, 35, 51). These data suggest that signal transduction may play a role in regulating or monitoring telomere length. The sequence of the *TEL2* gene is not similar to that of any known gene coding for a product involved in signal transduction or cell cycle checkpoints; however, *tel1-1 tel2-1* double mutants have telomeres the same length as those of *tel1-1* single mutants, suggesting that these two genes may act in the same pathway (32).

Telomere length appears to be regulated in *S. cerevisiae*. When the amount of $C_{1-3}A$ sequences in wild-type yeast cells is increased ~30-fold by using high-copy-number plasmids, chromosomal telomeres reach a new equilibrium length instead of increasing in length without bounds (46) (see the introduction). When linear plasmids are formed in yeasts by using short or long stretches of $C_{1-3}A$, the final length of $C_{1-3}A$ sequence on the plasmid termini is similar in length to that of the chromosomal telomeres of the host strain (37, 41, 46, 59, 61). This result is obtained even when the input plasmid starts with 250 to 350 bp of $C_{1-3}A$ sequence (46, 61). The terminally repeated DNA sequences, and proteins that bind to them, appear to be required for the telomere replication and protection functions in yeasts (14, 38, 41, 49). If telomere DNA length were to become too short, telomere function might be lost. In *Tetrahymena* and *Trypanosoma* spp., in which telomere length can continuously increase during mitotic growth, the subset of cells with average telomere length takes over the population (4, 26). These considerations suggest that there is a growth advantage to maintaining telomere length within a certain range. Thus, the role of telomere length regulation in ciliates and yeasts could be to maintain a telomere length sufficient to accomplish telomere functions without imposing a burden on cell growth. The results presented here suggest that *TEL2* is an essential gene whose product is part of the yeast system that maintains telomere length within a given range, perhaps by altering chromatin structure at the telomere and elsewhere.

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