Telomerase-Independent Proliferation Is Influenced by Cell Type in *Saccharomyces cerevisiae*

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Manuscript received September 15, 2002
Accepted for publication March 26, 2003

ABSTRACT

Yeast strains harboring mutations in genes required for telomerase function (*TLC1* and the *EST* genes) exhibit progressive shortening of telomeric DNA and replicative senescence. A minority of cells withstands loss of telomerase through *RAD52*-dependent amplification of telomeric and subtelomeric sequences; such survivors are now capable of long-term propagation with telomeres maintained by recombination rather than by telomerase. Here we report that simultaneous expression in haploid cells of both *MAT***a** and *MAT*α information suppresses the senescence of telomerase-deficient mutants, with suppression occurring via the *RAD52*-dependent survivor pathway(s). Such suppression can be mimicked by deletion of *SIR1–SIR4*, genes that function in transcriptional silencing of several loci including the silent mating-type loci. Furthermore, telomerase-defective diploid strains that express only *MAT***a** or *MAT*α information senesce at a faster rate than telomerase-defective diploids that are heterozygous at the *MAT* locus. This suggests that the *RAD52*-dependent pathway(s) for telomere maintenance respond to changes in the levels of recombination, a process regulated in part by the hierarchy of gene control that includes *MAT* regulation. We propose that cell-type-specific regulation of recombination at human telomeres may similarly contribute to the tissue-specific patterns of disease found in telomerase-deficient tumors.

TELOMERES, the physical ends of chromosomes, are composed of unusual chromatin and are required to prevent such catastrophic cellular events as chromosome loss, degradation, and end-to-end fusions. In most organisms, telomeres are composed of tandemly arrayed short sequence repeats flanked on the centromere-proximal side by middle-repetitive sequence elements (reviewed in Louis 1995). In the yeast *Saccharomyces cerevisiae*, telomeric DNA is composed of $TG_{1–3}$ repeats totaling ~300–500 bp (Shampay *et al.* 1984). These repeats are often abutted by one to four copies of subtelomeric sequences called Y′ elements, each separated by 50–130 bp of $TG_{1–3}$ repeats (Walmsley *et al.* 1984), and by a mosaic of more centromere-proximal subtelomeric sequences, collectively referred to as X elements (Flint *et al.* 1997; Pryde *et al.* 1997). The functions of Y′ and X repeats are unknown, but their presence indicates that recombination has occurred at telomeres (Louis and Haber 1990; Louis *et al.* 1994).

Maintenance of telomeres normally requires the enzyme telomerase, a reverse transcriptase complex containing an RNA molecule that serves as an internal template for the synthesis of new telomeric DNA repeats (reviewed in Nugent and Lundblad 1998). In *S. cerevisiae*, the RNA and catalytic protein components of the core enzyme are encoded by *TLC1* (*te*lomerase *c*omponent) and *EST2* (*e*ver *s*horter *t*elomeres), respectively (Singer and Gottschling 1994; Counter *et al.* 1997; Lingner *et al.* 1997). Three additional yeast genes, *EST1*, *EST3*, and *CDC13* (*EST4*), can be mutated to yield phenotypes identical to those of *tlc1* and *est2* mutants and function in the same pathway as components of telomerase (Lundblad and Szostak 1989; Lendvay *et al.* 1996; Nugent *et al.* 1996; Morris and Lundblad 1997). Of these, Est1p and Est3p are components of the telomerase holoenzyme whereas Cdc13p serves both to protect the telomere from nuclease activity and to recruit telomerase to the telomere (Evans and Lundblad 1999; Hughes *et al.* 2000; Pennock *et al.* 2001).

Disruption of telomerase function in yeast is not immediately detrimental because each telomere loses only a few base pairs of DNA upon each cell division (Lundblad and Szostak 1989; Singer and Gottschling 1994; Lendvay *et al.* 1996). However, progressive shortening of telomeres in *tlc1* and *est* mutants correlates with an eventual increase in cell death, often referred to as replicative senescence. It is noteworthy that after extended growth, a small proportion of *tlc1*, *est2*, *est1*, *est3*, or *cdc13-2* mutants escape senescence (Lundblad and Blackburn 1993; Singer and Gottschling 1994; Lendvay *et al.* 1996). These "survivors" can be classified into two categories that are distinguished by general

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differences in the rearrangements that occur at chromosome ends (reviewed in Lundblad 2002). The more common class, sometimes referred to as type I survivors, has rearranged chromosomal termini that contain massive amplifications of the subtelomeric sequence element Y′ capped with very short tracts of $TG_{1–3}$ repeats. The growth rate and viability of these survivors fluctuates dramatically and is coupled with the reappearance of rare senescent subpopulations. In the second class, referred to as type II survivors, little Y′ amplification is seen. Instead, chromosomes have extremely long terminal $TG_{1–3}$ repeats and exhibit long-term viability and healthy growth rates. Upon sustained outgrowth, type I survivors frequently transform to type II survivors, but the reverse is not observed (Teng and Zakian 1999). Notably, however, in the absence of *RAD52*, neither type of survivor can form, indicating that maintenance of telomeres in the absence of telomerase requires homologous recombination (Lundblad and Blackburn 1993; Singer and Gottschling 1994; Lendvay *et al.* 1996).

Recent studies have further emphasized the role of recombination in extending cell survival in the absence of telomerase. For example, a partial loss of telomerase, resulting in stably short telomeres but no obvious senescence phenotype, can still confer a growth disadvantage (Morris and Lundblad 1997). Such shortened telomeres can also be highly recombinogenic, even prior to becoming critically short. In *Kluyveromyces lactis*, recombination-mediated exchanges among subtelomeric repeats are increased by up to 200-fold in cells in which telomerase is still partially active (McEachern and Iyer 2001). Alterations that increase the frequency of genetic exchanges at chromosomal termini can also influence telomerase-independent survival. Defects in mismatch repair, which relieve the normal inhibition of recombination between homeologous DNA sequences, enhance the growth of strains of either *K. lactis* or *S. cerevisiae* that also lack telomerase (Rizki and Lundblad 2001). In fact, a mismatch repair defect can influence growth during the early stages following loss of telomerase, well before telomeres become critically short and the strain displays a noticeable barrier to proliferation. These studies collectively suggest that at least one pathway for conversion to a telomerase-independent survivor may depend on multiple rounds of recombination and that recombination contributes to telomere maintenance even during the early stages of growth of a telomerase-minus strain (Lundblad and Blackburn 1993). The enhanced recombinogenic nature of telomeres that have undergone only intermediate shortening may be a reflection of a partial loss of telomeric end protection, which increases the risk that chromosomal termini will be exposed to the types of DNA repair activities that normally act on double-strand breaks (McEachern and Blackburn 1996; McEachern and Iyer 2001).

DNA repair and recombination activities are also influenced by changes in cell identity or mating type (Lovett and Mortimer 1987; Heude and Fabre 1993; Schild 1995; Yan *et al.* 1995; Frank-Vaillant and Marcand 2001; Kegel *et al.* 2001; Ooi *et al.* 2001; Valencia *et al.* 2001; Morgan *et al.* 2002). In *S. cerevisiae*, a cell's identity is established by the mating-type information at the *MAT* locus that encodes transcription factors affecting the expression of a variety of haploid and diploid specific genes. Haploid cells express either *MAT***a** or *MAT*α information, whereas diploids have and express both types of information simultaneously. Additional copies of the mating-type information are at *HML* and *HMR*, and these loci are ordinarily transcriptionally silenced through the action of a variety of *cis*-acting sequences and *trans*-acting factors (reviewed in Freeman-Cook *et al.* 2000). This latter category includes Sir2–4p, along with Sir1p. The *SIR2–4* (*s*ilent *i*nformation *r*egulator) genes encode components of telomeric chromatin and are required to maintain both transcriptional silencing and wild-type length of the telomeres (Aparicio *et al.* 1991; Palladino *et al.* 1993; Hecht *et al.* 1996; Gotta *et al.* 1997; Strahl-Bolsinger *et al.* 1997). In contrast, the function of *SIR1* appears most significant at the silent mating-type loci, although a modest role has also been noted for it within the subtelomeric repeats (Fourel *et al.* 1999; Pryde and Louis 1999). In the absence of *SIR* gene functions, a haploid cell gains *MAT***a**/*MAT*α characteristics and, consequently, it is nonmating and transcription of haploid specific genes is repressed.

Here we present evidence that cell mating-type identity influences the ability of cells to survive in the absence of telomerase. Specifically, simultaneous expression of *MAT***a** and *MAT*α information in a haploid cell suppresses the senescence phenotype of telomerase mutants. This suppression is also induced by deletion of *SIR1–4*, but only when such deletions are accompanied by a state of *MAT* heterozygosity. Suppression of senescence is dependent on *RAD52*, indicating that coexpression of *MAT***a** and *MAT*α enhances the formation of telomerase-independent survivors. This effect is also observed in telomerase-defective diploid strains, where the severity of the telomere replication defect exhibits a clear correlation with the *MAT***a**/*MAT*α program of gene expression. Together, our findings suggest that changes in cell identity that lead to alterations in gene expression enhance the efficiency of recombination-dependent telomere maintenance pathways in strains that lack telomerase.

MATERIALS AND METHODS

Yeast strains and media: The strains used in this study are listed in Table 1. Since yeast harboring mutations in the *TLC1* or *EST* genes undergo senescence, *tlc1* and *est1* mutants were covered by a plasmid copy of the appropriate gene prior to

TABLE 1

Strains used in this study

Strain[a]	Genotype
LPY2691	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hml*Δ*::TRP1*
LPY3085	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 tlc1*-Δ*::LEU2 sir4*Δ*::HIS3 rho*° pSD120
LPY3105	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 tlc1*-Δ*::LEU2 sir3*Δ*::TRP1 rho*° pSD120
LPY3107	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rho*° pSD120
LPY3109	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 sir3*Δ*::TRP1 rho*° pSD120
LPY3111	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 sir4*Δ*::HIS3 rho*° pSD120
LPY3143	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 est1*-Δ*1::HIS3 rho*° pVL308
LPY3146	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 est1*-Δ*1::HIS3 sir3*Δ*::TRP1 rho*° pVL308
LPY3147	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 lys2 trp1-1 ura3-1 est1*-Δ*1::HIS3 sir4*Δ*::HIS3 rho*° pVL308
LPY3149	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 est1*-Δ*1::HIS3 rho*° pVL308
LPY3409	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rho*° pLP923
LPY3410	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 est1*-Δ*1::HIS3 rho*° pLP923
LPY3411	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 sir3*Δ*::TRP1 rho*° pLP923
LPY3412	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad52::LEU2 rho*° pLP923
LPY3413	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 est1*-Δ*1::HIS3 sir3*Δ*::TRP1 rho*° pLP923
LPY3414	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 est1*-Δ*1::HIS3 rad52*Δ*::LEU2 rho*° pLP923
LPY3415	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad52::LEU2 sir3*Δ*::TRP1 rho*° pLP923
LPY3416	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 est1*-Δ*1::HIS3 rad52::LEU2 sir3*Δ*::TRP1 rho*° pLP923
LPY3473	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 sir2*Δ*::TRP1 rho*° pVL308
LPY3474	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 tlc1*-Δ*::LEU2 sir2*Δ*::TRP1 rho*° pSD120
LPY3477	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 est1*-Δ*::HIS3 sir2*Δ*::TRP1 rho*° pVL308
LPY3478	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 sir1*Δ*::TRP1 rho*° pSD120
LPY3479	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1tlc1*-Δ*::LEU2 sir1*Δ*::TRP1 rho*° pSD120
LPY3480	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 est1*-Δ*1::HIS3 sir1*Δ*::TRP1 rho*° pVL308
LPY4419	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 tlc1*-Δ*::LEU2 rho*° pSD120 pLP1185
LPY4421	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 tlc1*-Δ*::LEU2 rho*° pSD120 pRS314
LPY4422	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rho*° pSD120 pLP1185
LPY4424	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rho*° pSD120 pRS314
LPY4425	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 est1*-Δ*1::HIS3 rho*° pVL308 pLP1185
LPY4427	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 est1*-Δ*1::HIS3 rho*° pVL308 pRS314
LPY4737	*MAT***a***/MAT*α *ade2-1/ade2-1 can1-100/can1-100 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1 est1*-Δ*1::HIS3/est1*-Δ*1::HIS3 rho*° pVL308
LPY4745	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hml*Δ*::TRP1 est1*-Δ*1::HIS3*
LPY4749	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hml*Δ*::TRP1 est1*-Δ*1::HIS3 sir3*Δ*::TRP1*
LPY4884	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hml*Δ*::TRP1 sir3*Δ*::TRP1*
LPY4994	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hml*Δ*::TRP1 est1*-Δ*1::HIS3 sir1*Δ*::TRP1*
LPY4999	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hml*Δ*::TRP1 est1*-Δ*1::HIS3 sir2*Δ*::TRP1*
LPY5020	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 est1*-Δ*1::HIS3 rho*° pLP923 pRS324
LPY5022	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 est1*-Δ*1::HIS3 rho*° pLP923 pLP1185
LPY5024	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad52::LEU2 rho*° pLP923 pRS324
LPY5026	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad52::LEU2 rho*° pLP923 pLP1185
LPY5028	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 est1*-Δ*1::HIS3 rad52*Δ*::LEU2 rho*° pLP923 pRS324
LPY5030	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 est1*-Δ*1::HIS3 rad52*Δ*::LEU2 rho*° pLP923 pLP1185
LPY7501	*MAT***a***/MAT*α *ade2-1/ade2-1 can1-100/can1-100 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1 rho*° pVL308
LPY7507	*MAT***a***/MAT***a** *ade2-1/ade2-1 can1-100/can1-100 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1 rho*° pVL308
LPY7568	*MAT*α*/MAT*α *ade2-1/ade2-1 can1-100/can1-100 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1 est1*-Δ*1::HIS3/est1*-Δ*1::HIS3 rho*° pVL308
LPY7571	*MAT***a***/MAT***a** *ade2-1/ade2-1 can1-100/can1-100 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1 est1*-Δ*1::HIS3/est1*-Δ*1::HIS3 rho*° pVL308
TVL249	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 tlc1*-Δ*::LEU2 rho*° pSD120

[a] To simplify nomenclature, the strains cited in Figures 1–7 are referred to by the same identification number as the strains presented in Table 1. It should be noted, however, that for experiments in Figures 1–7, strains have been cured of pSD120, pVL308, or pLP923 immediately prior to the start of each assay. Except for LPY2691 (gift of E. Stone), all strains listed were constructed and characterized in the course of this study.

TABLE 2

Plasmids used in this study

Plasmid	Description	Source[a]
pLP923	*SIR3 EST1 URA3 CEN*	
pLP1185	*MAT***a** *TRP1 CEN*	
pRS314	*TRP1 CEN*	Sikorski and Hieter (1989)
pSD120	*TLC1 URA3 CEN*	S. Diede and D. Gottschling
pSM20	*rad52::LEU2*	Schild *et al.* (1983)
pVL154	*est1-*Δ*1::HIS3*	Lundblad and Szostak (1989)
pVL308	*EST1 URA3 CEN*	

[a] Except where noted, plasmids listed were constructed and characterized in the course of this study.

performing experiments. All strains are in the W303 background. The *sir3*Δ*::TRP1* (Stone *et al.* 1991), *sir4*Δ*::HIS3* (Marshall *et al.* 1987), *tlc1-*Δ*::LEU2* (Lendvay *et al.* 1996), *est1-*Δ*1::HIS3* (Lundblad and Szostak 1989), *rad52::LEU2* (Schild *et al.* 1983), and *hml*Δ*::TRP1* alleles were introduced into these strains through crosses or standard disruption techniques (Rothstein 1983; Baudin *et al.* 1993). Both *sir1*Δ*::TRP1* and *sir2*Δ*::TRP1* are null alleles (gifts of J. Rine) that were generated by completely deleting the open reading frames of *SIR1* or *SIR2*, respectively, replacing them with *TRP1*, and introducing them into strains through crosses. LPY4737, a diploid *MAT***a**/*MAT*α *est1/est1* mutant strain, was generated by crossing LPY3143 with LPY3149. Subsequently, LPY4737 was subjected to low doses of UV irradiation to stimulate recombination at *MAT*. This treatment resulted in the production of *est1/est1* diploid mutants that were homozygous for either *MAT*α (LPY7568) or *MAT***a** (LPY7571) as confirmed by mating assays and molecular amplification using *MAT***a**- and *MAT*α-specific primers. The control strain LPY7507 was likewise constructed and confirmed.

We observed that strains in the W303 background spontaneously produce *pet* mutants at a high frequency, a situation complicating analysis of the mutant strains. To circumvent this problem, all of the strains used in this study, except LPY2691, LPY4745, LPY4749, LPY4884, LPY4994, and LPY4999, were made *rho*° as described (Fox *et al.* 1991), and results in otherwise isogenic *rho*° and *rho*$^+$ strains were comparable.

YPD, YPG, YPAD, supplemented synthetic medium lacking specific nutrients used to maintain plasmid selection, and liquid sporulation media were prepared as described (Adams *et al.* 1998). 5-Fluoroorotic acid (5-FOA; Toronto Research Chemicals) was added to supplemented synthetic medium at a concentration of 0.1% (Boeke *et al.* 1987).

DNA manipulations: The plasmids used in this study are listed in Table 2. pSD120 (a gift of S. Diede and D. Gottschling) was constructed by cloning the ~2.6-kb *Hpa*II fragment of *TLC1* into pRS316. pVL308 was made by cloning the ~2.6-kb *Bam*HI/*Sal*I fragment of *EST1* into YCplac33 opened with *Bam*HI and *Sal*I. pLP923 was created by inserting the ~4.5-kb *Sal*I fragment of *SIR3* into pVL308 opened with *Sal*I. pLP1185 was constructed by inserting an ~4-kb *Pst*I/*Cla*I fragment of *MAT***a** into pRS314 opened with *Pst*I and *Cla*I. Yeast transformations were performed as described (Schiestl and Gietz 1989). Disruption plasmids pVL154 and pSM20 were used as described (Schild *et al.* 1983; Lundblad and Szostak 1989), and disruptions were confirmed by molecular amplification and/or by genomic DNA blotting.

Senescence assays: The growth phenotypes of haploid and diploid mutant and control strains were assayed for senescence using a protocol previously established for analysis of *est* mutant strains (Lendvay *et al.* 1996). In the experiments presented here, *tlc1* and *est1* haploid single-mutant strains or *est1/est1* diploid mutants were generated by a plasmid shuffle in which spontaneous loss of covering plasmids with wild-type genes was selected by plating on 5-FOA (Boeke *et al.* 1987). This plasmid shuffle results in some variability in the onset and severity of the senescence phenotype of *tlc1* and *est1* mutants (presumably a reflection of variability in the time at which the covering plasmid was lost). To address this variability, large numbers of isolates of each mutant and mutant combination were analyzed.

Fresh isolates of each strain were streaked onto synthetic complete medium containing 5-FOA and incubated for ~96 hr at 30° to select for the loss of plasmids containing the relevant wild-type gene (pSD120, pVL308, or pLP923). In a typical experiment, 4–12 single, small colonies (<1 mm in diameter) of each strain were streaked from 5-FOA onto YPAD, incubated for ~96 hr at 30°, examined for growth phenotypes, and photographed (representing ~25 generations). Single colonies from the first passage were restreaked and analyzed similarly (~50 generations). This process was repeated a third time (~75 generations). In experiments conducted with haploid strains for the majority (~75%) of *tlc1* and *est1* mutant isolates examined, the most extreme senescence was observed at ~75 generations after which time *RAD52*-dependent survivors overwhelmed the population. All *tlc1 sir* and *est1 sir* double-mutant isolates behaved comparably, and senescence among double mutants was only infrequently observed over the course of an experiment. Examples of typical experiments are presented in the results.

For strains harboring pRS314 or pLP1185 in addition to pSD120, pVL308, or pLP923, senescence assays were performed as described above except that the medium used lacked tryptophan.

Telomeric DNA analysis: In parallel with the senescence experiments described above, individual colonies grown for ~25 generations were inoculated into 8 ml of YPAD and grown at 30° for ~48 hr. Genomic DNA was prepared (Hoffman and Winston 1987), digested with *Xho*I, separated on a 0.7% agarose gel, transferred to nylon membrane, and probed with poly d(GT/CA) as described (Lundblad and Szostak 1989). The relative amplification of Y′ elements in *tlc1* and *tlc1 sir* mutants was quantified by PhosphorImager analysis and the "Imagequant" software package (Molecular Dynamics, Sunnyvale, CA) by comparing the intensity of the signal corresponding to both Y′ elements relative to a nontelomeric 4-kb band also detected by the poly d(GT/CA) probe. The data presented in Figure 3 represent multiple independent experiments. Genomic DNA from a total of 27 *tlc1* or *est1* single-mutant isolates and 44 *sir tlc1* or *sir est1* double-mutant isolates at early cultivation times was examined by Southern blotting. From these analyses, indication of Y′ amplification was observed in only eight *tlc1/est1* mutants, but was clearly apparent in 38 of the *sir tlc1/sir est1* double-mutant isolates.

RESULTS

Coexpression of *MAT*a and *MAT*α suppresses senescence of haploid telomerase mutants: *S. cerevisiae* containing a mutation either in *TLC1* or in any of the *EST* genes exhibit progressive shortening of telomeric TG_{1-3} DNA and an accompanying senescence phenotype (Lundblad and Szostak 1989; Singer and Gottsch-

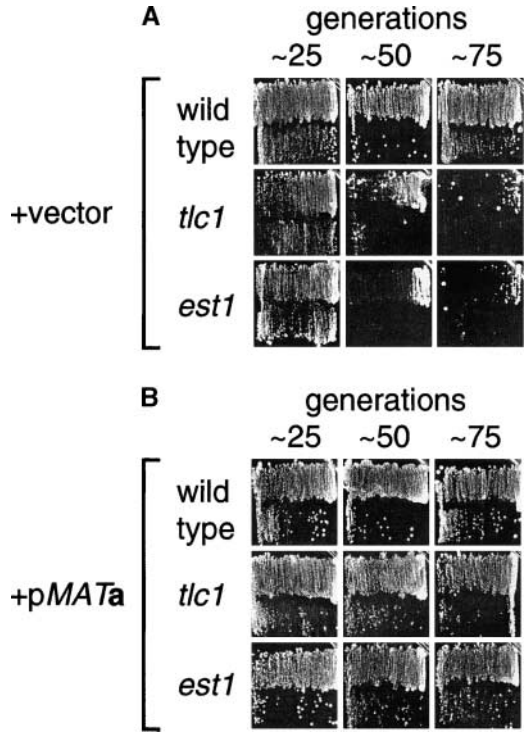

FIGURE 1.—The senescence of haploid telomerase mutants is suppressed by the coexpression of *MAT***a** and *MAT*α. Viability of (A) wild-type (LPY4424), *tlc1* (LPY4421), and *est1* (LPY4427) strains harboring a vector-control plasmid and (B) wild-type (LPY4422), *tlc1* (LPY4419), and *est1* (LPY4425) strains harboring plasmid-borne *MAT***a** was assayed by successive streak-outs. Each streak-out represents ~25 generations of growth.

LING 1994; LENDVAY *et al.* 1996). At its *MAT* locus, a haploid cell ordinarily expresses either "**a**" or "α" information, but not both simultaneously. In fact, the coexpression of *MAT***a** and *MAT*α information leads to the production of an **a**1/α2 heterodimer that blocks the transcription of a variety of haploid-specific genes. To test whether a cell's identity influences its response to loss of telomerase, we introduced plasmids containing *MAT***a** (p*MAT***a**) or a vector control (vector) into a *MAT*α *tlc1* mutant or *MAT*α wild-type strains. Senescence was monitored by the successive restreaking of multiple isolates of each strain, with each streak-out representing ~25 generations of growth (see MATERIALS AND METHODS). As expected, a *MAT*α *tlc1* mutant harboring a vector-control plasmid exhibited senescence, showing a moderate growth defect at ~50 generations and a severe loss of viability by ~75 generations (Figure 1A). Strikingly, however, a *MAT*α *tlc1* mutant harboring p*MAT***a** did not exhibit notable levels of inviability by this colony assay (Figure 1B), suggesting that changing a haploid cell's identity from α to **a**/α results in the suppression of *tlc1* senescence. In control experiments, neither p*MAT***a** nor vector plasmids affected the growth of a *MAT*α wild-type strain (Figure 1, A and B).

To test the specificity of the suppression, p*MAT***a** or vector-control plasmids were introduced into a *MAT*α *est1* mutant. In the presence of the vector-control plasmid, *MAT*α *est1* senesced (Figure 1A), whereas in the presence of p*MAT***a**, suppression was again observed (Figure 1B). Thus, suppression of senescence that results from the coexpression of *MAT***a** and *MAT*α is not restricted to loss of function of the RNA component of telomerase. This finding supports previous epistasis analysis demonstrating that *tlc1* and *est1*, along with *est2*, *est3*, and *cdc13-2* mutants, share identical phenotypes, consistent with their involvement in the same process of telomere maintenance and replication (LENDVAY *et al.* 1996; NUGENT *et al.* 1996). We predict that coexpression of *MAT***a** and *MAT*α would have comparable effects on *est2*, *est3*, and *cdc13-2* mutants, although these double-mutant combinations have not been tested.

Mutations in *SIR* genes suppress the senescence of telomerase mutants: The silent mating-type loci are transcriptionally silenced by a combination of *cis*-acting elements and *trans*-acting factors including the Sir1–4 proteins (FREEMAN-COOK *et al.* 2000). Loss of *SIR* function leads to the expression of the mating-type information at *HML* and *HMR*, resulting in a nonmating, **a**/α haploid. On the basis of our observation that simultaneous expression of *MAT***a** and *MAT*α suppresses the senescence of haploid telomerase mutants, we hypothesized that loss of *SIR* function would similarly suppress senescence. To address this hypothesis, we evaluated genetic interactions between *sir* and *tlc1*/*est* mutants.

As expected, wild-type, *sir1*, *sir2*, *sir3*, and *sir4* strains did not display a decline in viability following extensive propagation (Figure 2A). To examine if the absence of *SIR* function suppresses the senescence of telomerase mutants, we introduced *sir1*, *sir2*, *sir3*, or *sir4* mutations into *tlc1* mutant strains. By this colony assay, all *tlc1 sir* double mutants failed to senesce over the course of ~75 generations, indicating that the loss of *SIR1*, *SIR2*, *SIR3*, or *SIR4* function suppressed *tlc1* senescence (Figure 2B). To test whether the *sir*-mediated suppression of senescence was specific for *tlc1*, we similarly introduced *sir* mutations into an *est1* strain. All *sir* mutations suppressed *est1* senescence in a manner comparable to their suppression of senescence in a *tlc1* mutant (compare B and C in Figure 2). Suppression of senescence was also observed in *sir3 est2* and *sir4 est2* mutant strains (data not shown). Thus, by the criterion that *sir* suppression of senescence was not specific for a single telomerase-defective strain, *sir* suppression appeared comparable to that of the coexpression of *MAT***a** and *MAT*α.

On the basis of growth rates and variability in colony size and morphology, a subset of *tlc1 sir*, *est1 sir*, and *est2 sir* double-mutant isolates could be distinguished from wild-type strains (data not shown). In fact, *est1 sir* double-mutant isolates analyzed by restreaking for up to ~250 generations fluctuated through periods of senescence and periods of viability much like *est1* single-

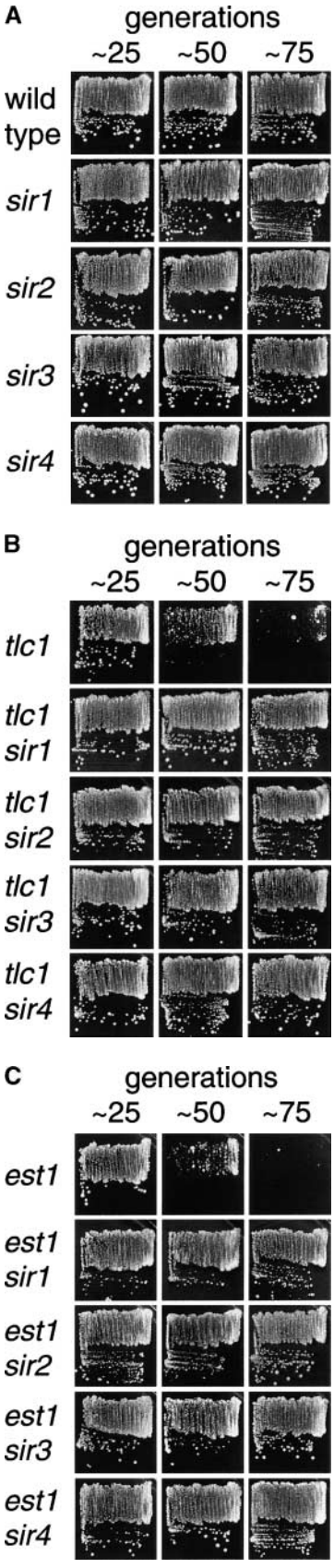

FIGURE 2.—The senescence of telomerase mutants is suppressed by *sir1*, *sir2*, *sir3*, and *sir4* mutations. Viability of (A) wild-type (LPY3107), *sir1* (LPY3478), *sir2* (LPY3473), *sir3* (LPY3109), and *sir4* (LPY3111) control strains; (B) *tlc1* (TVL249), *tlc1 sir1* (LPY3479), *tlc1 sir2* (LPY3474), *tlc1 sir3* (LPY3105), and *tlc1 sir4* (LPY3085) mutant strains; and (C) *est1* (LPY3143), *est1 sir1* (LPY3480), *est1 sir2* (LPY3477), *est1 sir3* (LPY3146), and *est1 sir4* (LPY3147) mutant strains was assayed by successive streak-outs. Each streak-out represents ~25 generations of growth.

mutant type I survivors (LUNDBLAD and BLACKBURN 1993). This suggests that suppression occurs by enhancement of the pathway(s) that mediates the formation of survivors in *tlc1* and *est* single-mutant strains (see also below).

Loss of *SIR* function promotes the formation of a telomerase-independent telomere maintenance pathway: In seeking to identify the mechanism by which *sir* mutations suppress *tlc1* and *est* senescence, we hypothesized at least two possibilities. Suppression might occur such that characteristic shortening of telomeric TG_{1-3} DNA in telomerase-defective strains was prevented. Alternatively, the absence of *SIR* might facilitate recombination at telomeres, resulting in an increased frequency of appearance of telomerase-independent survivors. To test these possibilities, we examined telomeric DNA from multiple isolates of wild-type, *sir3*, *sir4*, *tlc1*, *tlc1 sir3*, and *tlc1 sir4* strains. The strains were grown in parallel for ~25 generations, a time at which amplification of either subtelomeric or telomeric sequences is not normally observed in survivors (LUNDBLAD and BLACKBURN 1993; V. LUNDBLAD, unpublished data). As described previously (SINGER and GOTTSCHLING 1994), telomeric DNA in *tlc1* mutants was ~150 bp shorter than telomeric DNA prepared from wild-type strains after 25 generations (Figure 3). Likewise in agreement with previous results (PALLADINO *et al.* 1993), *sir3* and *sir4* single mutants had telomeric DNA repeats ~50 and ~100 bp shorter than those found in wild type. In *tlc1 sir3* and *tlc1 sir4* double mutants, telomeric TG_{1-3} DNA was shortened to an extent similar to that observed for *tlc1* single mutants. These data thus demonstrated that *sir3* and *sir4* did not block the telomeric shortening of *tlc1* mutants.

Although the length of the telomeric TG_{1-3} DNA did not differ, examination of the subtelomeric DNA structure in *tlc1 sir3* and *tlc1 sir4* mutants indicated that the telomerase-independent pathway occurred earlier than it did in *tlc1* single-mutant strains. In *S. cerevisiae*, the appearance of this telomerase-independent telomere maintenance pathway is heralded not only by a wild-type-like growth phenotype but also by changes in telomeric DNA composition that are visualized easily on genomic blots (LUNDBLAD and BLACKBURN 1993; TENG and ZAKIAN 1999). One change characteristic of this pathway is an increase in the copy number of two size classes of the Y′ subtelomeric repeats (LUNDBLAD and BLACKBURN 1993; TENG and ZAKIAN 1999). These ele-

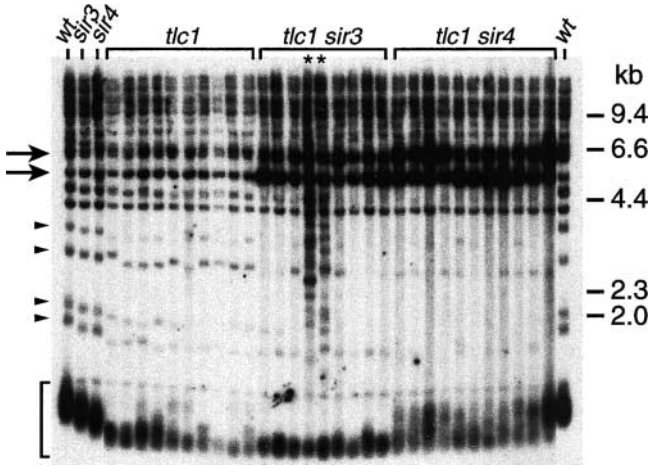

FIGURE 3.—Telomeric rearrangements occur early in *tlc1 sir3* and *tlc1 sir4* mutants. A Southern blot of genomic DNA prepared from isolates of wild-type (LPY3107), *sir3* (LPY3109), *sir4* (LPY3111), *tlc1* (TVL249), *tlc1 sir3* (LPY3105), and *tlc1 sir4* (LPY3085) grown in the absence of *TLC1* for ~25 generations was probed with a telomere-specific probe [poly d(GT/CA)]. Telomeric TG_{1-3} DNA from Y′-containing telomeres is bracketed. The two major classes of Y′ elements are marked by arrows, and examples of non-Y′-containing telomeres are indicated by arrowheads. In addition, lanes marked by asterisks correspond to isolates that have an appearance characteristic of type II survivors. Note that the telomeric TG_{1-3} DNA from a *tlc1 sir4* double mutant is heterogeneous in length; although we do not understand the basis for this phenomenon, it is apparently genotype independent (C. NUGENT and V. LUNDBLAD, unpublished results; see also Figure 1A in NUGENT *et al.* 1996 for further examples).

ments are not amplified early in the outgrowth of *tlc1* or *est* mutant strains, but survivors generated after >100 generations of growth of a telomerase-defective strain exhibit increases in Y′ copy number ranging from 10- to 200-fold, in parallel with amplification of TG_{1-3} DNA (LUNDBLAD and BLACKBURN 1993; LENDVAY *et al.* 1996). As shown in Figure 3, amplification of Y′ elements was observed even earlier in the outgrowth of *tlc1 sir3* and *tlc1 sir4* mutant strains relative to *tlc1*, *sir3*, *sir4*, and wild-type strains. Quantitation showed that Y′ copy number was only marginally increased in *tlc1* strains (by a factor of 1.9-fold, relative to wild type) at this early time point. In contrast, Y′ copy number was increased by 4.3- and 6.5-fold in *tlc1 sir3* and *tlc1 sir4* strains, respectively, relative to wild-type and *sir* control strains (see MATERIALS AND METHODS).

A second change in telomeric structure that is diagnostic of the Y′ amplification survivor pathway is the acquisition of Y′ elements by telomeres that did not previously have this subtelomeric repeat. This rearrangement in telomeric structure can be detected by monitoring individual telomeric restriction fragments that lack Y′ elements (indicated by arrowheads in Figure 3); such bands diminish or disappear in survivors (LUNDBLAD and BLACKBURN 1993). Consistent with the observed increase in Y′ copy number, these bands were absent or weaker in intensity in many of the *tlc1 sir3* and *tlc1 sir4* double mutants (Figure 3). Analysis of telomeric DNA prepared from *est1*, *est1 sir3*, and *est1 sir4* mutants prepared after ~25 generations of growth revealed similar results: Y′ elements in *est1 sir3* and *est1 sir4* double mutants were amplified and rearranged relative to *est1* single-mutant isolates and control strains (data not shown). Although the predominant genomic change detected in *tlc1 sir3* and *tlc1 sir4* double-mutant strains was amplification and dispersal of Y′ elements, we also observed changes characteristic of the appearance of type II survivors (see lanes marked with asterisks, Figure 3).

Thus, telomeric rearrangements occurred earlier in *tlc1 sir* or *est1 sir* double mutants than in *tlc1* or *est1* single mutants. These results suggest that suppression of *tlc1* and *est* senescence in *sir3* and *sir4* mutants occurs through the same processes by which survivors ordinarily form. Previous work demonstrated that this telomerase-independent pathway is mediated via recombination, as elimination of *RAD52* function blocks both the formation (LUNDBLAD and BLACKBURN 1993; LENDVAY *et al.* 1996) and maintenance (TENG and ZAKIAN 1999) of survivors. We therefore tested whether the suppression of *est1* senescence by a *sir3* mutation is similarly dependent upon *RAD52*. Figure 4 shows that the absence of *RAD52* function had no effect on the growth of a *sir3* mutant strain, and as expected, the senescence phenotype exhibited by the *est1* mutant at ~50 generations was suppressed in an *est1 sir3* double mutant. However, both the *est1 rad52* double mutant and the *est1 sir3 rad52* triple mutant were inviable by ~50 generations, and the growth patterns of these two strains were indistinguishable. Similarly, *est1 sir1 rad52*, *est1 sir2 rad52*, and *est1 sir4 rad52* triple mutants exhibited the same rapid senescence phenotype of an *est1 rad52* mutant (data not shown). Therefore, the suppression of senescence observed in *est sir* mutant strains was recombination mediated. This observation, in combination with the telomeric rearrangements observed in *tlc1 sir* and *est sir* strains, strongly suggests that *sir* suppression utilizes the same *RAD52*-dependent mechanisms originally defined for survivor formation (LUNDBLAD and BLACKBURN 1993).

Suppression of senescence by coexpression of *MAT*a and *MAT*α occurs through a *RAD52*-dependent mechanism: To determine whether the suppression of senescence observed by coexpression of *MAT***a** and *MAT*α occurred by stimulating the survivor pathway(s), we asked whether suppression was similarly dependent on *RAD52*. We introduced either a vector control or p*MAT***a** into *est1*, *rad52*, or *est1 rad52* mutants. As shown in Figure 5A, the vector control did not block the senescence of *est1* or *est1 rad52* mutants and had no effect on the growth of a *rad52* mutant. Figure 5B reveals that p*MAT***a**, as observed previously (see Figure 1B), suppressed *est1* senescence and had no effect on a *rad52* single mutant.

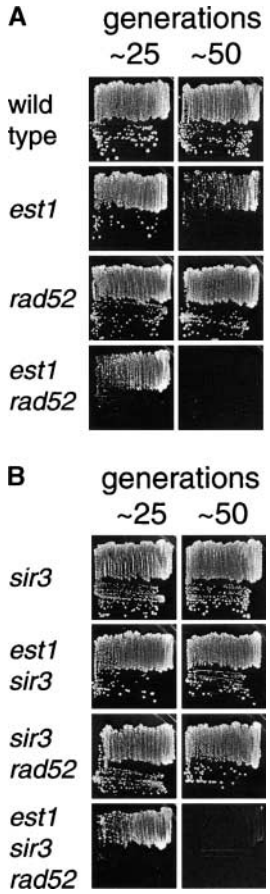

FIGURE 4.—*sir*-mediated suppression of senescence is *RAD52* dependent. Viability of (A) wild-type (LPY3409), *est1* (LPY3410), *rad52* (LPY3412), and *est1 rad52* (LPY3414) control strains and (B) *sir3* (LPY3411), *est1 sir3* (LPY3413), *sir3 rad52* (LPY3415), and *est1 sir3 rad52* (LPY3416) mutant strains was assayed by successive streak-outs. To ensure that telomere lengths were comparable in both *SIR3* wild-type and *sir3* mutant strains at the start of this experiment, all strains initially harbored a *URA3*-marked plasmid bearing both *EST1* and *SIR3* (pLP923), which was lost upon growth on 5-FOA prior to the first streak-out depicted here.

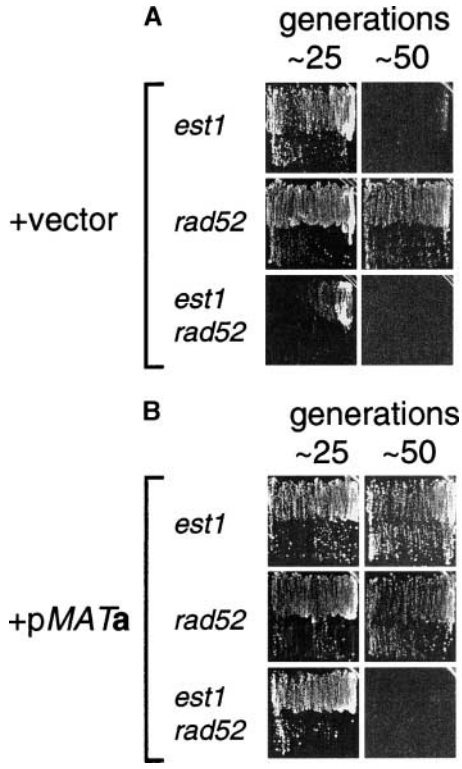

FIGURE 5.—Cell-type-specific suppression of *est1* senescence is *RAD52* dependent. Viability of (A) *est1* (LPY5020), *rad52* (LPY5024), and *est1 rad52* (LPY5028) strains harboring a vector-control plasmid and (B) *est1* (LPY5022), *rad52* (LPY5026), and *est1 rad52* (LPY5030) strains harboring plasmid-borne *MAT***a** was assayed by successive streak-outs. We note that *est1* and *est1 rad52* mutants harboring the vector-control plasmid senesced especially rapidly in this experiment.

Importantly, however, in the absence of *RAD52*, p*MAT***a** was unable to suppress the senescence of an *est1* mutant. From these data, we conclude that suppression by coexpression of *MAT***a** and *MAT*α requires *RAD52*-dependent homologous recombination and therefore is due to enhanced survivor formation.

Coexpression of *MAT*a and *MAT*α information is required for the *sir* mutant-mediated suppression of senescence: The suppression of senescence observed in telomerase-defective strains by *sir* mutations bears remarkable similarity to that of the suppression of *tlc1* and *est1* mutants by the coexpression of *MAT***a** and *MAT*α. Notably, the suppression phenotypes are indistinguishable from one another and are likewise *RAD52* dependent. However, given that Sir2–4p not only are required for silencing at the silent mating-type loci but also are components of telomeric chromatin (reviewed in THAM and ZAKIAN 2002), it was formally possible that although *MAT***a** and *MAT*α coexpression is sufficient for suppression of telomerase-defective mutants, loss of *SIR* could directly promote survivor formation in telomerase-defective strains. To test this possibility, *sir3* was combined with *est1* in a genetic background in which the silent mating-type locus *HML* was deleted (*hml*), resulting in *sir3 est1* cells that expressed only *MAT***a** information. If *sir* mutations had effects on suppression unrelated to the simultaneous expression of both forms of mating-type information, suppression of senescence in telomerase-defective strains would still be observed. On the other hand, if *sir* suppression required coexpression of *MAT***a** and *MAT*α, then *sir est1* strains would be predicted to senesce. The *hml* single and *hml sir3* double mutants were mating competent, as expected, and did not display any loss in viability over the course of a senescence assay (Figure 6A and data not shown). The *sir3* mutation, however, was no longer capable of suppressing *est1* senescence in an *hml* background. These strains displayed a marked loss in viability by 50–75 generations (Figure 6A). Moreover, *sir1* and *sir2* mutations also failed to suppress the senescence of an *hml*

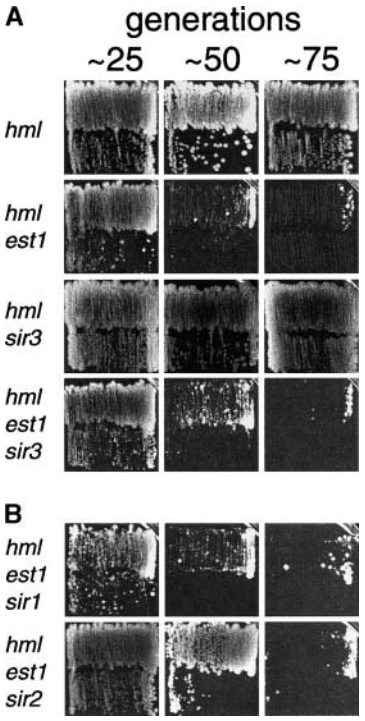

FIGURE 6.—Suppression of senescence requires coexpression of *MAT***a** and *MAT*α information. (A) Viability of *MAT***a** *hml* (LPY2691), *MAT***a** *hml est1* (LPY4745), *MAT***a** *hml sir3* (LPY4884), and *MAT***a** *hml est1 sir3* (LPY4749) and (B) *MAT***a** *hml est1 sir1* (LPY4994) and *MAT***a** *hml est1 sir2* (LPY4999) strains were assayed by successive streak-outs.

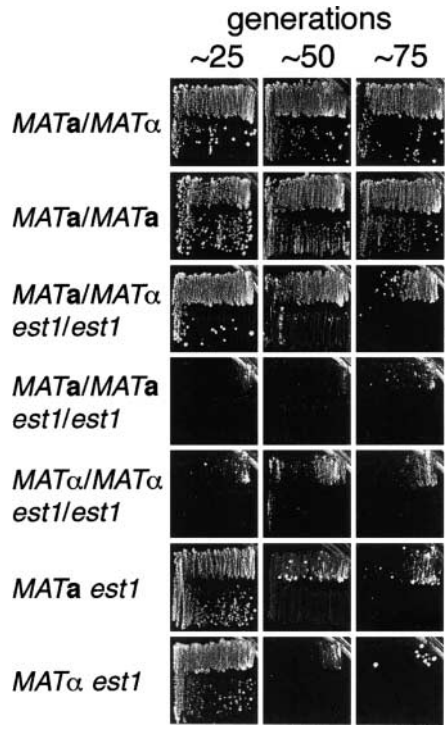

FIGURE 7.—Senescence of diploid telomerase mutants is influenced by *MAT*. Viability of diploid control strains *MAT***a**/*MAT*α (LPY7501) and *MAT***a**/*MAT***a** (LPY7507) and diploid mutant strains *MAT***a**/*MAT*α *est1/est1* (LPY4737), *MAT***a**/*MAT***a** *est1/est1* (LPY7571), and *MAT*α/*MAT*α *est1/est1* (LPY7568) was assayed by successive streak-outs and compared to haploid mutant controls *MAT***a** *est1* (LPY3149) and *MAT*α *est1* (LPY3143). Each streak-out represents ~25 generations of growth.

est1 strain (Figure 6B), indicating that coexpression of *MAT***a** and *MAT*α is a requirement of *sir* suppression of senescence.

Senescence of diploid telomerase mutants is also influenced by *MAT* expression: The results above demonstrated that in haploid cells, *MAT* heterozygosity can modulate the onset of a recombination-dependent mechanism for telomere maintenance. This finding suggests that in diploid strains coexpression of both *MAT* loci, when compared to strains expressing only one type of mating-type information, should similarly influence survival in the absence of telomerase. To test this prediction, we generated isogenic *est1/est1* diploid mutants that were either heterozygous (*MAT***a**/*MAT*α) or homozygous (*MAT***a**/*MAT***a** or *MAT*α/*MAT*α) at the mating-type locus (see MATERIALS AND METHODS for details of strain construction). Diploid *EST1/EST1* and haploid *est1* strains were included as controls (Figure 7). As expected, diploid strains in which telomerase was intact did not exhibit any signs of senescence regardless of whether they were heterozygous or homozygous for *MAT*. In contrast, a *MAT***a**/*MAT*α *est1/est1* diploid displayed a clear senescence phenotype, although the onset of senescence in this strain was slightly delayed relative to *est1* haploid controls (~75 generations *vs.* ~50 generations). Therefore, in a diploid strain, simply expressing both *MAT***a** and *MAT*α is not sufficient to suppress senescence. By contrast, both *MAT***a**/*MAT***a** *est1/est1* and *MAT*α/*MAT*α *est1/est1* diploid mutants exhibited accelerated senescence relative to either the heterozygous *MAT***a**/*MAT*α *est1/est1* diploid or the haploid *est1* mutant controls. Thus, similarly to the observations described above for haploid telomerase mutants, expression of both *MAT***a** and *MAT*α information also facilitates survival in a diploid strain in the absence of telomerase.

DISCUSSION

Over the past two decades, a mechanistic understanding of how organisms with linear chromosomes solve the "end replication" problem (WATSON 1972) has increased significantly. Components of telomerase, the end-replicating enzyme, have been identified in an enormous range of organisms (reviewed in NUGENT and LUNDBLAD 1998), implying that this mechanism of telomere replication is evolutionarily conserved. Initially, however, the discovery that telomeric DNA is highly repetitive and the observation that conserved, subtelomeric DNA sequences are frequently dispersed among

chromosome ends was interpreted to suggest that recombination might play a central role in telomere replication. This viewpoint has recently gained renewed interest on the basis of the discovery that in *S. cerevisiae*, *K. lactis*, *Schizosaccharomyces pombe*, and certain human tumors and tumor cell lines, telomeres can sometimes be maintained in the absence of telomerase (Lundblad and Blackburn 1993; McEachern and Blackburn 1996; Bryan *et al.* 1997; Nakamura *et al.* 1998).

The process by which telomerase-independent "survivors" arise in the budding yeast has been the subject of numerous studies (reviewed in Lundblad 2002). Insight into the mechanism promoting this process came from the observation that telomerase-independent survivors could not form in the absence of *RAD52*, the gene required for most homologous recombination (Lundblad and Blackburn 1993; McEachern and Blackburn 1996). Not only did loss of *RAD52* function ultimately block the formation of survivors, but also, in this genetic context, the senescence characteristics of a telomerase-defective strain were exaggerated, indicating that recombination contributed to telomere maintenance immediately after the loss of telomerase. In contrast, the viability of telomerase mutants harboring defects in the mismatch-repair pathway was enhanced, implying that a system that safeguards genomic stability interferes with survivor formation (Rizki and Lundblad 2001).

MAT* heterozygosity suppresses senescence in telomerase mutants:** This work demonstrates that the senescence phenotype of telomerase mutants can also be influenced by the status of *MAT* in both haploid and diploid strains. A comparison of a *MAT*α *tlc1* or a *MAT*α *est1* strain to the identical strain harboring a p*MATa** plasmid revealed a striking phenotype: over the course of ~75 generations, the senescence phenotype evident in the telomerase-defective strains was suppressed in those strains containing p*MAT***a** (see Figure 1). Consistent with the change in the status of *MAT* that accompanies mutations in *SIR*, we were able to mimic this phenotype by combining mutations in *SIR1–4* with mutations in *TLC1* or *EST1* (see Figure 2). Suppression is not seen in the absence of *RAD52* function (Figure 4), indicating that suppression requires homologous recombination. Such *RAD52* dependence was also true for experiments with episomal expression of *MAT* (Figure 5). These data strongly suggest that the suppression we observed was due to an early arrival of survivors in a population of cells that would ordinarily be undergoing senescence.

It was noteworthy that simple loss of *SIR* function was not adequate to suppress senescence (Figure 6). Instead, mutations in *SIR* suppressed senescence only with simultaneous *MAT* heterozygosity (Figure 2), implying that **a**/α coexpression was necessary. Thus, this shows that the effects of mutations in the *SIR* genes are most likely indirect, rather than the consequence of a change in telomeric chromatin due to loss of the Sir complex.

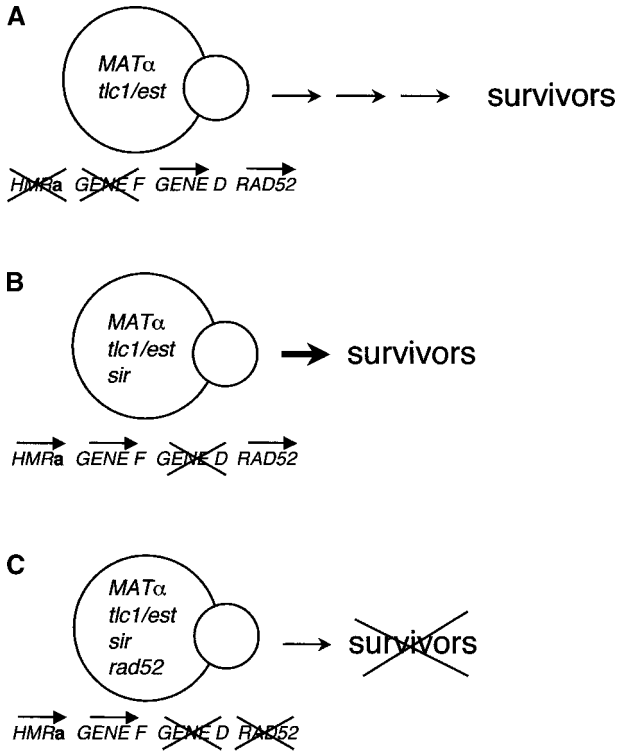

Figure 8.—A model for influence of cell identity on telomerase-independent proliferation. (A) In haploid telomerase mutants (*MAT*α *tlc1*), the silent mating-type loci (here represented by *HMR***a**) are transcriptionally silenced and homologous recombination DNA repair systems are ordinarily intact (indicated by the transcription of *RAD52*). As-yet-unidentified genes that facilitate recombination in diploid cells (*GENE F*) or inhibit recombination in haploid cells (*GENE D*) are, respectively, repressed and transcribed. Telomerase mutants senesce, but over time survivors form. (B) When a *sir* mutation is combined with a telomerase mutation, *HMR***a** is transcribed, resulting in the formation of the **a**1/α2 transcription factor. This change in cell-type transcription may then activate *GENE F* and/or repress *GENE D*. The resulting, new transcriptional program can then promote *RAD52*-dependent recombination and, hence, survivors form earlier than in telomerase single-mutant cells. (C) In the absence of *RAD52*, homologous recombination cannot occur and survivors do not form, regardless of cell mating-type identity.

These observations in haploid strains were also supported by an examination of the senescence phenotypes in telomerase-defective *est1/est1* diploid strains, where a clear correlation between the severity of the telomere replication defect and the *MAT***a**/*MAT*α program of gene expression was observed. Notably, however, senescence was not suppressed in an *est1/est1* diploid expressing both *MAT***a** and *MAT*α information, in contrast to the suppression observed in a haploid *est1* strain expressing both *MAT* loci. This might be attributable to the twofold increase in the number of telomeres that must be processed to sustain viability, which could exceed the capacity of the recombination machinery in a diploid strain.

Recombination and repair phenotypes are associated with *MAT* heterozygosity: Maintenance of silencing at the silent mating-type loci ensures that a haploid cell will be able to conjugate with a cell of its opposite mating type, thereby promoting the exchange of genetic material when the resulting diploid subsequently undergoes meiosis and sporulation. When a haploid cell becomes heterozygous for *MAT*, the resulting **a**1/α2 heterodimer represses the expression of haploid-specific genes and the cell is unable to mate. Microarray techniques have facilitated the identification of genes whose expression is affected by the state of *MAT* (Primig *et al.* 2000; additional microarray data cited in Galitski *et al.* 1999; Kegel *et al.* 2001; Ooi *et al.* 2001; Valencia *et al.* 2001), underscoring the fact that many differences exist between haploid and diploid yeast. The finding that at least one of these genes, *NEJ1*, is involved in nonhomologous end joining (NHEJ) is in accordance with previous observations that haploid and diploid cells rely on different types of recombination as their primary means of repairing double-strand DNA breaks. However, the suppressing effects we observe are clearly distinct from NHEJ mechanisms, since it has previously been reported that *est1* mutants in which NHEJ is disrupted due to a mutation in *LIG4* senesce with the same properties as *est1* mutants alone (Hackett *et al.* 2001). Moreover, *MAT* heterozygosity increases homologous recombination in diploid cells (Lee *et al.* 1999; Clikeman *et al.* 2001), suppresses the X-ray-sensitive phenotype of a *rad52-20* mutant (Schild 1995) and a *rad54* null mutant (Lovett and Mortimer 1987), bypasses the ATP hydrolysis requirement for Rad51p (Morgan *et al.* 2002), and influences the ability of a cell to withstand an unrepaired, double-strand break (Bennett *et al.* 2001). Thus, *MAT* heterozygosity has widespread implications on processes that extend beyond the ability of a cell to mate.

We propose that changes in gene expression that accompany an alteration in cell identity can alter the frequency of recombination events at chromosomal termini as a consequence of either increased expression of genes whose products promote recombination or, alternatively, decreased expression of genes responsible for keeping recombination in check (Figure 8). Future identification of the specific genes responsible for the cell-type-specific effects on telomere maintenance will further advance our understanding of the mechanisms that promote proliferation in the absence of telomerase.

Alternative telomere maintenance in mammalian cells and tumors: Although ~90% of immortal mammalian cell lines and tumors are positive for telomerase activity (Kim *et al.* 1994), indicating that they have escaped crisis by reactivating telomerase, numerous cell lines that are deficient in telomerase activity and yet contain grossly amplified tracts of telomeric repeats have also been identified (Bryan *et al.* 1995, 1997). This phenomenon, known as alternative telomere maintenance (ALT), exhibits similarities to the properties exhibited by survivors recovered from both *S. cerevisiae* and *K. lactis* strains (reviewed in Henson *et al.* 2002; Lundblad 2002), including evidence for recombination between telomeres in ALT cell lines (Dunham *et al.* 2000; Varley *et al.* 2002). Further parallels between ALT in human cells and tumors and survivor formation in yeast may well exist. Our observation that loss of silencing at the silent mating-type loci has indirect consequences in survivor formation in yeast hints that there may be cell-type-specific regulation of recombination at human telomeres. Ultimately, such cell-type differences may contribute to the tissue-specific patterns of disease found in individual telomerase-deficient tumors.

We thank A. Salinger for technical assistance with Figure 3 and E. J. Spedale and S. Jacobson for help with strain construction. S. Diede, D. Gottschling, J. Rine, V. Zakian, K. Friedman, and R. Sternglanz generously provided strains, plasmids, and other reagents. We thank F. Winston and the anonymous reviewers of this and an earlier version of this work for their suggestions and insight. We also thank E. Stone and S. Jacobson for critical comments on the manuscript. J. Lowell was supported in part by a National Institutes of Health Training grant (GM-07135-22). This work was supported by grants from the National Institutes of Health to the labs of L.P. and V.L.

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Communicating editor: F. Winston