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Recombination and the Tel1 and Mec1 checkpoints differentially effect genome rearrangements driven by telomere dysfunction in yeast

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In telomerase-deficient *Saccharomyces cerevisiae*, telomeres are maintained by recombination. Here we used a *S. cerevisiae* assay for characterizing gross chromosomal rearrangements (GCRs) to analyze genome instability in post-senescent telomerase-deficient cells. Telomerase-deficient *tlc1* and *est2* mutants did not have increased GCR rates, but their telomeres could be joined to other DNAs resulting in chromosome fusions. Inactivation of Tel1 or either the Rad51 or Rad59 recombination pathways in telomerase-deficient cells increased the GCR rate, even though telomeres were maintained. The GCRs were translocations and chromosome fusions formed by nonhomologous end joining. We observed chromosome fusions only in mutant strains expressing Rad51 and Rad55 or when Tel1 was inactivated. In contrast, inactivation of Mec1 resulted in more inversion translocations such as the isochromosomes seen in human tumors. These inversion translocations seemed to be formed by recombination after replication of broken chromosomes.

Telomeres function in replication and maintenance of chromosome ends, to prevent DNA ends from being inappropriately joined to each other and to prevent chromosome ends from activating checkpoints[1,2]. Telomeres are maintained by telomerase, which consists of the Est2 catalytic subunit, the Tlc1 RNA and other subunits[2]. Telomere maintenance also requires other proteins. These include the Tel1 protein kinase that functions in telomere protection and length regulation and proteins such as Cdc13 and Ku that target telomerase to telomeres and protect telomeres from degradation[2]. Proteins such as Pif1 help regulate telomere length[3] and prevent telomerase from adding telomeres to broken DNAs[3,4]. In telomerase-deficient *S. cerevisiae* cells telomeres are maintained by recombination[5,6]. Most mammalian cells lack telomerase[7] and have a limited lifespan. Immortalization and cancer progression require increased telomere maintenance capacity, either through upregulation of telomerase activity[7] or through the alternative lengthening of telomere pathway[8].

Table 1 Effect of checkpoint, DNA repair and telomerase defects on the rate of accumulation of GCRs

	Wild-type		*tlc1Δ*		*lig4Δ tlc1Δ*		*est2Δ*	
Relevant genotype	Mutation rate[a]	Strain number	Mutation rate[a]	Strain number	Mutation rate[a]	Strain number	Mutation rate[a]	Strain number
Wild-type	3.5 (1)[b]	3615	3.3 (0.95)	5233	25 (6)	5237	3.1 (0.9)	5234
tel1Δ	2.0 (0.6)[c]	3731	398 (114)	5236	31 (9)	5239	460 (131)	5241
mec1Δ sml1Δ	680 (194)[c]	3735	518 (148)	5246	500 (143)	5247	ND	
rad51Δ	35 (10)[b]	3636	710 (203)	5214	12 (3)	5222	478 (136)	5225
rad55Δ	19 (5)	5203	501 (143)	5215	27 (7)	5223	ND	
rad54Δ	19 (5)[d]	4473	477 (136)	5216	ND		ND	
rad59Δ	75 (21)[d]	4423	391 (112)	5217	9.0 (2)	5224	565 (161)	5226
rdh54Δ	10 (3)[d]	4425	400 (114)	5218	ND		ND	
tel1Δ rad51Δ	87 (25)	5211	433 (124)	5219	ND		ND	
tel1Δ rad55Δ	87 (25)	5212	661 (188)	5220	ND		ND	
tel1Δ rad59Δ	80 (23)	5213	507 (145)	5221	ND		ND	
mec1Δ sml1Δ rad51Δ	4,450 (1271)	5303	256 (73)	5305	ND		ND	
mec1Δ sml1Δ rad59Δ	3,060 (874)	5304	247 (70)	5306	ND		ND	

[a]Can[r] 5-FOA[r] (10^{-10}). Numbers in parentheses indicate the relative increase of the GCR rate relative to wild-type. [b]Data from ref. 10. [c]Data from ref. 11. [d]Data from ref. 4.

RDKY5233 is a *tlc1Δ* type II strain. Additional relevant GCR rates include the *tlc1Δ* type I strain, RDKY5232 (3.1 10^{-10} (0.9)); *lig4Δ* strain, RDKY3641 (1.6 10^{-9} (9); ref. 10); *tel1Δ lig4Δ* strain, RDKY5238 (4.2 10^{-9} (12)); and *tel1Δ lig4Δ est2Δ* strain, RDKY5240 (3.5 10^{-9} (10)). ND, not determined.

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Table 2 Translocation, deletion and chromosome fusion breakpoints detected in *tlc1* mutants

Relevant genotype	Translocation or deletion NH	Translocation or deletion MH	Chromosome fusion NH	Chromosome fusion MH	Chromosome fusion CA	N
*tel1*Δ[a]	0	3,3[b]	0	0	0	6
*tlc1*Δ type I	0	1,1[b]	2	5	2	11
*tlc1*Δ type II	2[b]	3	1	0	5	11
*tel1*Δ *tlc1*Δ	4	1	2	2	1	10
*tel1*Δ *lig4*Δ *tlc1*Δ	0	5	1	2	1	9
*mec1*Δ *sml1*Δ *tlc1*Δ	3	5	0	1	0	9
mec1Δ sml1Δ lig4Δ tlc1Δ	0	9	0	1	0	10
*rad51*Δ *tlc1*Δ	6	5,2[b],7[c]	0	1	0	21
*rad55*Δ *tlc1*Δ	1,1[b],2[c]	1,5[b]	0	0	0	10
*rad54*Δ *tlc1*Δ	2[b],1[c]	0	2	2	5	12
*rad59*Δ *tlc1*Δ	0	4,3[b],1[c]	1	3	1	13
*rdh54*Δ *tlc1*Δ	0	5,2[b],1[c]	1	1	3	13
*tel1*Δ *rad51*Δ *tlc1*Δ	1[b],2[c]	1,3[b],1[c]	0	2	0	10
*tel1*Δ *rad59*Δ *tlc1*Δ	0	4,1[c]	0	4	1	10
*mec1*Δ *sml1*Δ *rad51*Δ *tlc1*Δ	0	10	2	0	0	12
*mec1*Δ *sml1*Δ *rad59*Δ *tlc1*Δ	0	10	0	1	1	12

[a]Data from ref. 11. [b]Translocations that occurred at a transposon sequence. [c]Rearrangements that were an interstitial deletion of chromosome V.

The numbers indicate the observed number of translocations or chromosome fusions with nonhomology (NH) or microhomology (MH) breakpoint, or telomeric repeat poly($C_{1-3}A/TG_{1-3}$) at the breakpoint of the chromosome fusion (CA). N indicates the total number of breakpoints characterized. Rearrangements in the wild-type strain (RDKY3615) were 14 telomere additions and 3 translocations. Rearrangements in *rad51*Δ (RDKY3636), *rad59*Δ (RDKY4423) and *rad55*Δ (RDKY5203) single mutants were 50% telomere additions and 50% translocations.

When telomerase-deficient cells senesce, survivors emerge in which telomeres are added by recombination[5]. When only the Rad51-dependent (requires Rad52, Rad54, Rdh54, Rad55, Rad57) recombination pathway is active, telomeres are characterized by short TG_{1-3} repeats (type I telomeres), whereas when only the Rad59-dependent (requires Rad52, Rdh54, Rad50, Mre11, Xrs2) recombination pathway is active, telomeres are characterized by long TG_{1-3} repeats (type II telomeres; refs. 6,9; **Supplementary Table 1** online). We examined the effect of *tlc1* and *est2* mutations and telomere type on the accumulation of GCRs using an assay that detects GCRs that target the left arm of chromosome V (refs. 3,10). Pre-senescent *tlc1* and *est2* mutants did not have higher GCR rates[4], and neither did post-senescent *tlc1* survivors having only type I or only type II telomeres (**Table 1**). In type I and type II *tlc1* mutants, the GCRs were a mixture of translocations and chromosome fusions (**Tables 2** and **3**, **Figs. 1** and **2** and **Supplementary Table 2** online). This suggests that although recombination allows telomerase-deficient cells to proliferate, recombination-mediated telomere maintenance in *tlc1* type I and type II survivors cannot completely compete with telomere end-joining reactions. We observed no chromosome fusions in wild-type cells, in which most GCRs are due to *de novo* telomere addition (**Table 2**)[10,11], indicating that telomerase more effectively competes with telomere end-joining reactions.

Deletion of *RAD51*, *RAD55*, *RAD54*, *RAD59* or *RDH54* caused a small increase in the GCR rate[4], and combining these mutations with a *tlc1* mutation caused a synergistic increase in the GCR rate in post-senescent cells (**Table 1** and **Supplementary Table 3** online). We did not examine *rad52* mutations because they are lethal in combination with telomerase defects[5]. The GCR rates in *rad51 tlc1*, *rad55 tlc1*, *rad54 tlc1*, *rad59 tlc1* and *rdh54 tlc1* double mutants were not different ($P > 0.10$). We observed similar results with mutations in *EST2* (**Table 1**). This suggests that when maintenance of telomeres is partially compromised by inactivation of only one recombination pathway, increased GCRs result even though telomeres are still maintained by recombination.

Table 3 Translocation-deletion and chromosome fusion rates

Relevant genotypes	Translocation-deletion rate[a]		Chromosome fusion rate[a]
Wild-type[b]	0.6		<0.6
*tlc1*Δ (type I)	0.6		2.5
*tlc1*Δ (type II)	1.3	(0.3)	2
*tel1*Δ *tlc1*Δ	199		199
*tel1*Δ *lig4*Δ *tlc1*Δ	17	(7)	14
*mec1*Δ *sml1*Δ *tlc1*Δ	460	(115)	58
*mec1*Δ *sml1*Δ *lig4*Δ *tlc1*Δ	450	(250)	50
*rad51*Δ *tlc1*Δ	676		34
*rad55*Δ *tlc1*Δ	501		<50
*rad54*Δ *tlc1*Δ	119		358
*rad59*Δ *tlc1*Δ	241	(30)	150
*rdh54*Δ *tlc1*Δ	246	(31)	154
tel1Δ rad51Δ tlc1Δ	347		86
*tel1*Δ *rad59*Δ *tlc1*Δ	253		253
*mec1*Δ *sml1*Δ *rad51*Δ *tlc1*Δ	213	(<21)	43
*mec1*Δ *sml1*Δ *rad59*Δ *tlc1*Δ	205	(20)	42

[a]Values are 10^{-10}. [b]Data from ref. 10.

The rate for each class of rearrangements in a given mutant strain was determined by multiplying the fraction of total events that each class represents by the GCR rate of the mutant strain. < indicates cases where no rearrangements were observed and therefore the rate was less than that if one rearrangement was observed. The translocations were determined to be monocentric or dicentric based on the orientation of the DNA sequences at the breakpoint. The number in parentheses is the rate of dicentric inversion translocations (see **Fig. 3**).

We determined the breakpoint sequences of GCRs from each strain (**Figs. 1** and **2** and **Table 2**) and the rate of formation of each type of GCR (**Table 3**). In the double mutants, each class of rearrangement, except as discussed below, was formed at a higher rate than in either wild-type or *tlc1* type I and type II strains ($P < 0.0007$; **Table 3**). We observed no telomere addition GCRs because their formation requires telomerase[4]. The GCRs in *rad54 tlc1*, *rad59 tlc1* and *rdh54 tlc1* double mutants were translocations, deletions and chromosome fusions. In contrast, *rad51 tlc1* and *rad55 tlc1* resulted in an increased translocation and deletion rate with little or no effect on the chromosome fusion rate (**Table 3**). We observed no chromosome fusions in the *rad55 tlc1* mutant, and the chromosome fusion rate in the *rad51 tlc1* mutant was lower than those in *rad54 tlc1*, *rad59 tlc1* and *rdh54 tlc1* double mutants ($P = 0.003$, $P = 0.0003$ and $P = 0.03$, respectively). The breakpoints were predominantly (except in the *rad54 tlc1* mutant) at regions of microhomology,

Translocations and deletions

mec1Δ sml1Δ lig4Δ tlc1Δ (inversion translocation, dicentric)
CCGTATCTTAACCTTCTACATTGGC:TCTCTATTATTCATTGGACTTTTAG chr V 32465
CAATATAACGGGACGTAACATTGGC:CCCCTTTTATATTCATCGCGCTCTT chr V 42063

rad59Δ tlc1Δ (translocation, monocentric)
CTTTTTGTCTTTGCAACCAGGTTCT:GATAGTGAATTTTTCGAATTGGGCC chr V 40337
AAGGAACAGGCATATTTATTATTCT:ACACCATTCGTCAAGTAAATTGATT chr XIII 718107

rad51Δ tlc1Δ (interstitial deletion)
CGACGGGAGATACCGGTATAACTGC:TCAAATGAATGCCAAGAGATACACA chr V 39293
GTGGTGCTCCTGCATCAGACGGTGC:ATGAAAACAGCTAAATGGGAACGGT chr V 19753

Chromosome fusions

tlc1Δ type I (chromosome fusion in Y′ element)
ATAAATAAAAAATTTTGCCAAGACT:TTTTTAAACTGCACCCGACAGATCA chr V 42341
CATGCTCGTCCACAGAGGGAATAAT:GGGTTCTTCATTCGTAATAGATCGA chr IX 5257

tel1Δ tlc1Δ (chromosome fusion in small subtelomeric repeats)
AATCGAAAGTTTATTTCAGAGTTCT:TCAGACTTCTTAACTCCTGTAAAAA chr V 33515
CCTAACACTACCCTAACACTACCCT:AACCCTATTCTAATCCAACCCTGAT chr X 745327

tel1Δ rad59Δ tlc1Δ (chromosome fusion in Y′ element)
AAAGTTCCATAAGAAGGGCCGCAAG:GGCCAAGACAAGGAGTCTCCGGAAT chr V 41732
TACAACTTCTTCCGCTCTCGAAAAG:ACCAATATAATAGAAAGTTATAAAT chr VI 4316

rad54Δ tlc1Δ (chromosome fusion in $C_{1-3}A/TG_{1-3}$ repeats)
TAAGATTGACAGTCACTAGCCATTA:GTGGATCAGTCAAAAAATTCCTAATTAATGAAGA chr V 35392
Poly($C_{1-3}A$):CCACACCCACACCCACCAAACACATACCCTAACA chr VII 10

although we observed some nonhomology breakpoints (**Table 2** and **Fig. 1**). In most cases, the orientation of the breakpoint partners based on analysis of the breakpoint sequences was consistent with the structure of a monocentric translocation chromosome (**Fig. 2**). These results indicate that GCRs are formed by joining of broken DNAs with each other or with chromosome ends and that homologous recombination proteins may function at chromosome ends to prevent or promote the participation of telomeres in chromosome fusions.

When combined with *tlc1* or *est2* mutations, a *tel1* mutation causes a synergistic increase in the GCR rate and high frequencies of translocations and spontaneous[4] and double-strand break (DSB)-directed[12] chromosome fusions (**Tables 1** and **3**). A *tel1* mutation had no effect on GCR rates in combination with mutations in *RAD51, RAD55* or *RAD59*, and the GCR rates of *tel1 rad51 tlc1*, *tel1 rad55 tlc1* and *tel1 rad59 tlc1* triple mutants were not different from the those of *rad51 tlc1*, *rad55 tlc1* and *rad59 tlc1* double mutants ($P > 0.13$; **Table 1**). The rates of translocations and chromosome fusions were similar in *rad59 tlc1*, *tel1 tlc1* and *tel1 rad59 tlc1* strains. The *tel1 rad51 tlc1* strain accumulated mostly translocations, but chromosome fusions were formed at a higher rate than in the *rad51 tlc1* strain ($P = 0.02$; **Table 3**). Because mutations in *TEL1* are not known to cause defects in the Rad51 or Rad59 recombination pathways, this suggests that defects in a Tel1-dependent checkpoint function[3,11] or telomere-capping function[4,12,13] and expression of Rad51 (and Rad55) could both disrupt the protective function that prevents telomeres from being joined to broken DNAs.

Because nonhomologous end joining (NHEJ) proteins Ku70, Ku80 and ligase 4 promote translocations[3,4], telomere-telomere fusions[14–18] and fusion of telomeres to an induced DSB[12], we examined the role of ligase 4 in chromosome fusions. When we introduced a *lig4* mutation into the *rad51 tlc1*, *rad55 tlc1*, *rad59 tlc1* and *tel1 tlc1* double mutants, the GCR rate was reduced to almost wild-type rates and was indistinguishable from that of the *lig4 tlc1* double mutant ($P > 0.14$; **Table 1**). We obtained similar results with an *est2* mutation. The GCR rate and the rates of chromosome fusions and translocations were greatly reduced when we introduced a *lig4* mutation into the *tel1 tlc1* mutant

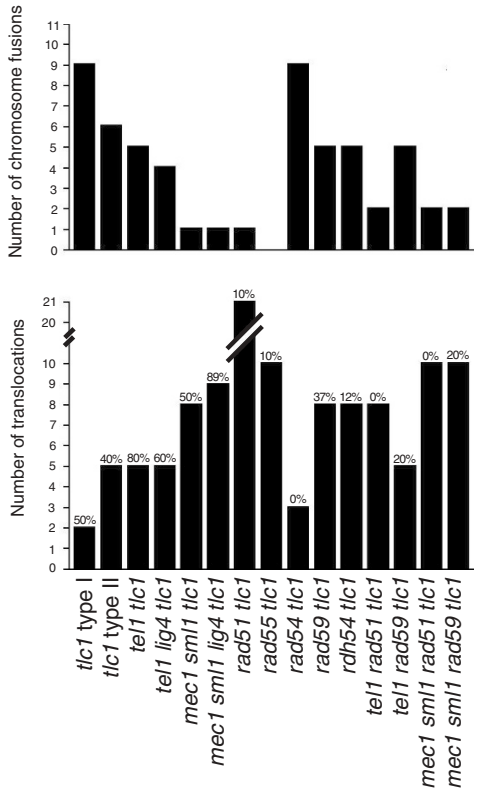

Figure 1 Representative examples of translocations and chromosome fusions. The DNA sequence of all rearrangement breakpoints characterized is available in **Supplementary Tables 1–3** online. The underlined portion of the upper DNA sequence is the sequence of chromosome V followed by the standard SGD nucleotide coordinates for the last recognizable nucleotide of chromosome V at the breakpoint. The underlined portion of the lower sequence is the DNA sequence found at the breakpoint followed by the standard SGD nucleotide coordinates for the first nucleotide for the corresponding indicated chromosome. The nucleotides in bold are identical.

Figure 2 Genetic requirements for translocations and chromosome fusions. Number of events observed in the indicated genetic backgrounds is shown. Bars represent the number of chromosome fusions (upper panel) and translocations (lower panel) observed. The number above each bar in the translocation panel indicates the percentage of the observed translocations that were dicentric, based on the orientation of the DNA sequence at the breakpoint. In this study, all dicentric translocations were nonreciprocal and were associated with the presence of an apparently intact copy of the non–chromosome V target chromosome. This would limit any lethality resulting from subsequent rounds of breakage of dicentric chromosomes. Strains with apparent dicentric chromosomes or chromosome fusions did not show any obvious growth defects.

(**Tables 1** and **3**). This indicates that GCRs resulting from combining Tel1 defects or recombination defects with telomerase defects are primarily formed by NHEJ of broken DNAs with each other or with chromosome ends.

Combining a *mec1* mutation with *tlc1* or *est2* mutations did not change the GCR rate relative to that of a *mec1* single mutant (**Table 1**). But the GCR spectrum changed from all telomere additions in a *mec1* mutant[11] to chromosome fusions, translocations and inversion translocations, a translocation structure we have not previously observed[4,10,11], in the *mec1 tlc1* double mutant (**Fig. 1** and **Table 3**). Analysis of GCR breakpoints in *mec1 tlc1* double mutants detected several (two of nine GCRs) inversion translocations at a region of microhomology; we observed a higher proportion (five of ten GCRs) of these events in a *mec1 lig4 tlc1* mutant (**Tables 2** and **3** and **Fig. 1**). We also observed inversion translocations at low rates in *tlc1* type II, *tel1 tlc1 lig4*, *rad59 tlc1* and *rdh54 tlc1* strains. The GCR rates in the *mec1 rad51 tlc1* and *mec1 rad59 tlc1* mutants was roughly two times lower than that in the *mec1 tlc1* mutant (**Table 1**), although this difference was of borderline significance ($P < 0.09$). The reduced GCR rate in *mec1 rad51 tlc1* and *mec1 rad59 tlc1* mutants was mainly the result of a large reduction in inversion translocations; other translocations and chromosome fusions were moderately reduced (**Fig. 2** and **Table 3**). This suggests that inversion translocations are formed by Rad51- or Rad59-dependent recombination. Such translocations are similar to the isochromosomes seen in human tumors.

A model describing how defects in telomere maintenance result in increased GCRs is presented in **Figure 3**. In the absence of telomerase, the telomeres added by recombination can participate in NHEJ at low rates, because telomere maintenance functions do not completely compete with NHEJ, but this does not increase the GCR rate. In the absence of telomerase and either the Rad51 or Rad59 recombination pathways or Tel1, there are more broken chromosomes that escape repair owing to overburdening of the existing recombination capacity by telomere maintenance, less telomere protection or both, resulting in increased GCR rates even though there is sufficient recombination to maintain telomeres. There are three potential sources of broken chromosomes: replication errors that escape repair[3,11], dicentric chromosomes due to telomere-telomere fusion followed by breakage of the dicentric chromosomes[1,2,15,19] and degradation of chromosome ends by exonucleases such as Exo1 (ref. 20). The observation of chromosome fusion junctions at subtelomeric regions suggests that chromosome ends are degraded, but this is probably not a major source of 'broken' chromosomes, because this predicts that 100% of translocation breakpoints would be in a dicentric orientation, which is not observed. The subsequent rearrangements are predominantly due to NHEJ; joining of broken DNAs to each other yields translocations and joining of broken DNAs to unprotected telomeres yields chromosome fusions. Finally, in the absence of Mec1-dependent DNA damage checkpoints[11,21,22], broken chromosomes seem to replicate and then fuse by Rad51- or Rad59-dependent recombination to yield dicentric inversion translocations. This is in contrast to telomerase-defective mammalian cells, in which chromosome fusions and translocations in cells that escape cell cycle arrest and apoptosis because of a p53 mutation result from a ligase 4–dependent NHEJ reaction[17,23].

The data presented here illustrate the complex nature of the mechanisms that suppress and promote genome rearrangements. When we combined a telomerase defect with mutations in *TEL1*, *RAD59*, *RAD54* or *RDH54*, we observed high levels of chromosome fusions, whereas when we combined telomerase defects with mutations in *RAD51* or *RAD55*, we observed significantly lower chromosome fusion rates. This suggests not only that recombination suppresses chromosome fusions by maintaining telomeres and repairing broken chromosomes but also that assembly of Rad51 on chromosome ends in the presence of Rad55-Rad57 during recombination may disrupt end-protection, facilitating NHEJ when telomere maintenance is partially compromised. In contrast, proteins such as Tel1 seem to help maintain chromosome ends by mediating checkpoint activation[3,11] and telomere capping[4,12], precluding participation of telomeres in NHEJ. The interaction of a *mec1* mutation with telomerase defects uses a different mechanism. Checkpoint defects[3] allow broken DNAs to replicate, which allows

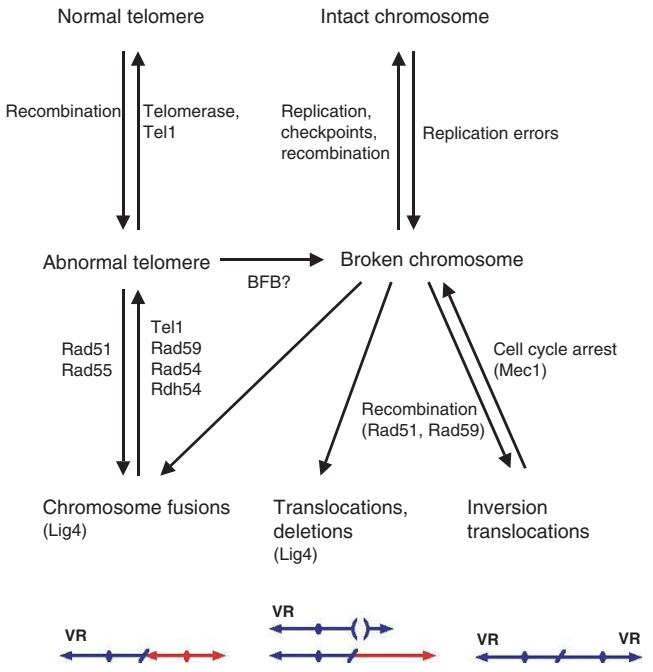

Figure 3 Model for the formation of GCRs in telomerase-deficient cells. In the absence of telomerase, the telomeres added by recombination[5,6] do not prevent the joining of telomeres to broken DNAs at low rates. In the absence of either of the Rad51 or Rad59 recombination pathways or Tel1, there are either increased levels of broken chromosomes that escape repair, decreased telomere protection, or both such that increased GCRs occur. However, the telomeres may also fail to protect chromosomes from telomere-telomere fusions leading to breakage-fusion-bridge cycles (BFB)[1,2,15,19] or exonuclease digestion[20]. The broken chromosomes are then joined to each other or to telomeres by NHEJ to yield GCRs. Chromosome fusions appear to be suppressed by telomere capping proteins Tel1, Rad59, Rad54 and Rdh54 whereas the recombination proteins Rad51 and Rad55-Rad57 may promote chromosome fusions by possibly disrupting telomere capping. In the absence of Mec1, inversion translocations are formed at high rates. This translocation structure is consistent with a mechanism in which broken chromosomes replicate and then fuse in inverted orientation at the broken ends by Rad51- or Rad59-dependent recombination. *mec1* mutants also have a defect in Rad55 phosphorylation and reduced homologous recombination[21,30] either of which might alter the balance between the types of recombination events available to broken chromosomes. Blue indicates chromosome V, red indicates any other chromosome. The arrowheads indicate telomeres (or sub-telomeric regions), the filled circles indicate centromeres and VR indicates the right arm of chromosome V.

other rearrangement mechanisms to lead to inversion translocations (under these conditions, there is no further increase in damaged chromosomes, but rather the damaged chromosomes are channeled into a different outcome). These and other studies describing *de novo* telomere addition GCRs[4,10,24], telomere-telomere[14,15,17,19,23,25] and fusions of telomeres to spontaneous[4] and induced DSBs[12,13] (and potentially unstable dicentric chromosomes as well as circular chromosomes[14]) illustrate the diversity of ways in which telomere or telomerase dysfunction results in genome rearrangements. This raises the possibility that suppression of telomerase activity observed in human genetic diseases[26–28] and the increased telomere maintenance capacity seen in cancer cells due to upregulation of telomerase[7] or activation of the alternative lengthening of telomere pathway[8] could each contribute to genome instability.

METHODS

Yeast strains. We disrupted genes of interest in the isogenic strains RDKY3615 (*MATa, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2ΔBgl, hom3-10, ade2Δ1, ade8, hxt13:URA3*) and RDKY5027 (*MATα, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2ΔBgl, hom3-10, ade2Δ1, ade8, hxt13:URA3*) using standard PCR-based methods. We crossed these strains to generate the series of isogenic MATa strains that we used in the studies described here. We selected the tlc1 or est2 mutant derivatives and subcultured them in liquid media or restreaked them on plates until post-senescence survivors emerged. We then genotyped the survivors by PCR and determined the survivor type by Southern blotting of XhoI-digested genomic DNA with a poly(C_{1-3}A /TG_{1-3}) probe. All the post-senescence strains used contained type II telomeres, except for *rad59 tlc1*, *rad59 lig4 tlc1*, *rad59 tel1 est2*, *rad59 tlc1*, *mre11 tlc1*, *mre11 lig4 tlc1* and *mre11 est2* strains, which contained type I telomeres. We generated post-senescent *tlc1* single mutants both by restreaking on rich media and by liquid subculture to generate type I and type II survivors, respectively[6]. For a given mutant, survivors obtained independently had similar GCR rates.

The strains used for the experiments were as follows: RDKY3615 wild-type; RDKY3633 *mre11:HIS3*; RDKY3636 *rad51:HIS3*; RDKY3652 *mre11:KAN lig4:HIS3*; RDKY3731 *tel1:HIS3*; RDKY3735 *mec1:HIS3 sml1:KAN*; RDKY4423 *rad59:TRP1*; RDKY4425 *rdh54:TRP1*; RDKY4473 *rad54:HIS3*; RDKY5203 *rad55::TRP1*; RDKY5211 *tel1:KAN rad51:HIS3*; RDKY5212 *tel1:HIS3 rad55:TRP1*; RDKY5213 *tel1:KAN rad59:TRP1*; RDKY5214 *rad51:HIS3 tlc1:TRP1*; RDKY5215 *rad55:HIS3 tlc1:TRP1*; RDKY5216 *rad54:HIS3 tlc1:TRP1*; RDKY5217 *rad59:TRP1 tlc1:HIS3*; RDKY5218 *rdh54:TRP1 tlc1:HIS3*; RDKY5219 *tel1:KAN rad51:HIS3 tlc1:TRP1*; RDKY5220 *tel1:KAN rad55:HIS3 tlc1:TRP1*; RDKY5221 *tel1:KAN rad59:TRP1 tlc1:HIS3*; RDKY5222 *rad51:TRP1 lig4:KAN tlc1:HIS3*; RDKY5223 *rad55:HIS3 lig4:KAN tlc1:TRP1*; RDKY5224 *rad59:TRP1 lig4:KAN tlc1:HIS3*; RDKY5225 *rad51:HIS3 est2:TRP1*; RDKY5226 *rad59:TRP1 est2:HIS3*; RDKY5227 *mre11:KAN lig4:HIS3 tlc1:TRP1*; RDKY5228 *mre11:KAN tlc1:TRP1*; RDKY5229 *mre11:HIS3 est2:TRP1*; RDKY5232 *tlc1:TRP1* (type I); RDKY5233 *tlc1:TRP1* (type II); RDKY5234 *est2:TRP1*; RDKY5236 *tel1:HIS3 tlc1:TRP1*; RDKY5237 *lig4:HIS3 tlc1:TRP1*; RDKY5238 *tel1:HIS3 lig4:KAN*; RDKY5239 *tel1:HIS3 lig4:KAN tlc1:TRP1*; RDKY5240 *te1:;HIS3 lig4:KAN est2:TRP1*; RDKY5241 *tel1:HIS3 est2:TRP1*; RDKY5246 *mec1:HIS3 sml1:KAN tlc1:TRP1*; RDKY5247 *mec1:HIS3 sml1:KAN lig4:Kan tlc1:TRP1*; RDKY5303 *mec1:TRP1 sml1:KAN rad51:HIS3*; RDKY5304 *mec1:HIS3 sml1:KAN rad59:KAN*; RDKY5305 *mec1:HIS3 sml1:KAN rad51:KAN tlc1:TRP1*; RDKY5306 *mec1:HIS3 sml1:KAN rad59:KAN tlc1:TRP1*.

GCR rates. We determined each GCR rate independently by fluctuation analysis two or more times using either 5 or 15 cultures and report the median value[10,11]. We evaluated statistical significance using the Mann-Whitney test (see URL). We determined the sequences of independent rearrangement breakpoints and the rates of individual breakpoint classes as described[10,11]. We compared the sequences on both sides of each breakpoint with the Saccharomyces Genome Database (SGD) to identify the fused chromosome partners and to determine the predicted orientation of each chromosome relative to the centromere or telomere.

Translocations consist of a broken chromosome V joined to a fragment of another chromosome or, in some cases, another fragment of chromosome V. We scored all chromosome V sequences fused to telomeric repeats poly(C_{1-3}A /TG_{1-3}) and chromosome V sequences fused to subtelomeric sequences oriented towards the centromere of the target chromosome as chromosome fusions. Inversion translocations are dicentric chromosome V–chromosome V translocations in which a centromere-containing fragment of chromosome V is fused to a virtually identical centromere-containing fragment of chromosome V in inverted orientation at a region of microhomology on the left arm of the broken chromosomes V. For translocations, when the target chromosome arm was predicted to contain a centromere, we classified the translocation as dicentric, and when the target chromosome arm was predicted not to contain a centromere, we classified it as monocentric (the chromosome V fragment that results when the left arm of chromosome V is deleted is always predicted to contain a centromere in the assay used here). We did not confirm these classifications using other methods. Translocations were found by PCR to be nonreciprocal, as previously described[10]; the presence of an intact target chromosome suggests that the translocations observed do not cause a disadvantage to the cell.

URL. The programs we used for the Mann-Whitney statistical test are available at http://faculty.vassar.edu/lowry/vshome.html

Note: Supplementary information is available on the Nature Genetics website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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1. Blackburn, E.H. Telomere states and cell fates. *Nature* **408**, 53–56 (2000).
2. Cervantes, R.B. & Lundblad, V. Mechanisms of chromosome-end protection. *Curr. Opin. Cell Biol.* **14**, 351–356 (2002).
3. Kolodner, R.D., Putnam, C.D. & Myung, K. Maintenance of genome stability in *Saccharomyces cerevisiae. Science* **297**, 552–557 (2002).
4. Myung, K., Chen, C. & Kolodner, R.D. Multiple pathways cooperate in the suppression of genome instability in *Saccharomyces cerevisiae. Nature* **411**, 1073–1076 (2001).
5. Lundblad, V. & Blackburn, E.H. An alternative pathway for yeast telomere maintenance rescues est1- senescence. *Cell* **73**, 347–360 (1993).
6. Teng, S.C. & Zakian, V.A. Telomere-telomere recombination is an efficient bypass pathway for telomere maintenance in *Saccharomyces cerevisiae. Mol. Cell. Biol.* **19**, 8083–8093 (1999).
7. Kim, N.W. *et al.* Specific association of human telomerase activity with immortal cells and cancer. *Science* **266**, 2011–2015 (1994).
8. Bryan, T.M., Englezou, A., Dalla-Pozza, L., Dunham, M.A. & Reddel, R.R. Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines. *Nat. Med.* **3**, 1271–1274 (1997).
9. Chen, Q., Ijpma, A. & Greider, C.W. Two survivor pathways that allow growth in the absence of telomerase are generated by distinct telomere recombination events. *Mol. Cell. Biol.* **21**, 1819–1827 (2001).
10. Chen, C. & Kolodner, R.D. Gross chromosomal rearrangements in *Saccharomyces cerevisiae* replication and recombination defective mutants. *Nat. Genet.* **23**, 81–85 (1999).
11. Myung, K., Datta, A. & Kolodner, R.D. Suppression of spontaneous chromosomal rearrangements by S phase checkpoint functions in *Saccharomyces cerevisiae. Cell* **104**, 397–408 (2001).
12. Chan, S.W. & Blackburn, E.H. Telomerase and ATM/Tel1p protect telomeres from nonhomologous end joining. *Mol. Cell* **11**, 1379–1387 (2003).
13. DuBois, M.L., Haimberger, Z.W., McIntosh, M.W. & Gottschling, D.E.A quantitative assay for telomere protection in *Saccharomyces cerevisiae. Genetics* **161**, 995–1013 (2002).
14. Liti, G. & Louis, E.J. NEJ1 prevents NHEJ-dependent telomere fusions in yeast without telomerase. *Mol. Cell* **11**, 1373–1378 (2003).
15. Mieczkowiski, P., Mieczkowska, J., Dominska, M. & Petes, T. D. Genetic regulation of telomere-telomere fusions in the yeast *Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA* **100**, 10854–10859 (2003).
16. Ferreira, M.G. & Cooper, J.P. The fission yeast Taz1 protein protects chromosomes from Ku-dependent end-to-end fusions. *Mol. Cell* **7**, 55–63 (2001).
17. Smogorzewska, A., Karlseder, J., Holtgreve-Grez, H., Jauch, A. & de Lange, T. DNA

ligase IV-dependent NHEJ of deprotected mammalian telomeres in G1 and G2. *Curr. Biol.* **12**, 1635–1644 (2002).
18. Espejel, S. *et al.* Mammalian Ku86 mediates chromosomal fusions and apoptosis caused by critically short telomeres. *EMBO J.* **21**, 2207–2219 (2002).
19. Hackett, J.A., Feldser, D.M. & Greider, C.W. Telomere dysfunction increases mutation rate and genomic instability. *Cell* **106**, 275–286 (2001).
20. Maringele, L. & Lydall, D. EXO1-dependent single-stranded DNA at telomeres activates subsets of DNA damage and spindle checkpoint pathways in budding yeast yku70Delta mutants. *Genes Dev.* **16**, 1919–1933 (2002).
21. Weinert, T.A., Kiser, G.L. & Hartwell, L.H. Mitotic checkpoint genes in budding yeast and the dependence of mitosis on DNA replication and repair. *Genes Dev.* **8**, 652–665 (1994).
22. Cha, R.S. & Kleckner, N. ATR homolog Mec1 promotes fork progression, thus averting breaks in replication slow zones. *Science* **297**, 602–606 (2002).
23. Artandi, S.E. *et al.* Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. *Nature* **406**, 641–645 (2000).
24. Fouladi, B., Sabatier, L., Miller, D., Pottier, G. & Murnane, J.P. The relationship between spontaneous telomere loss and chromosome instability in a human tumor cell line. *Neoplasia* **2**, 540–554 (2000).
25. Hande, M.P., Samper, E., Lansdorp, P. & Blasco, M.A. Telomere length dynamics and chromosomal instability in cells derived from telomerase null mice. *J. Cell Biol.* **144**, 589–601 (1999).
26. Vulliamy, T. *et al.* The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita. *Nature* **413**, 432–435 (2001).
27. Mitchell, J.R., Wood, E. & Collins, K.A telomerase component is defective in the human disease dyskeratosis congenita. *Nature* **402**, 551–555 (1999).
28. Zhang, A. *et al.* Deletion of the telomerase reverse transcriptase gene and haploinsufficiency of telomere maintenance in Cri du chat syndrome. *Am. J. Hum. Genet.* **72**, 940–948 (2003).
29. Symington, L.S. Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. *Microbiol. Mol. Biol. Rev.* **66**, 630–670 (2002).
30. Bashkirov, V.I., King, J.S., Bashkirova, E.V., Schmuckli-Maurer, J. & Heyer, W.D. DNA repair protein Rad55 is a terminal substrate of the DNA damage checkpoints. *Mol. Cell. Biol.* **20**, 4393–4404 (2000).