**EXO1 Contributes to Telomere Maintenance in Both Telomerase-Proficient and Telomerase-Deficient Saccharomyces cerevisiae**

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**ABSTRACT**

Previous work in budding yeast has indicated that telomeres are protected, at least in part, from the action of Exo1, which degrades the G-rich strand of partially uncapped telomeres. To explore this further, we examined the consequences of Exo1-mediated activity in strains that lacked Ku, telomerase, or both. Loss of Exo1 partially rescued the telomere length defect in a yku80Δ strain, demonstrating that exonuclease action can directly contribute to telomere shortening. The rapid loss of inviability displayed by a yku80Δ est2Δ strain was also partially alleviated by an exo1Δ mutation, further supporting the proposal that Exo1 is one target of the activities that normally protect wild-type telomeres. Conversely, however, Exo1 activity was also capable of enhancing telomere function and consequently cell proliferation, by contributing to a telomerase-independent pathway for telomere maintenance. The recovery of recombination-dependent survivors that arose in a yku80Δ est2Δ strain was partially dependent on Exo1 activity. Furthermore, the types of recombination events that facilitate telomerase-independent survival were influenced by Exo1 activity, in both est2Δ and yku80Δ est2Δ strains. These data demonstrate that Exo1 can make either positive or negative contributions to telomere function and cell viability, depending on whether telomerase or recombination is utilized to maintain telomere function.

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**T**he proteins that associate with telomeric DNA, the short G-rich repetitive sequences present at the ends of linear chromosomes, serve two essential roles (reviewed in McEachern et al. 2000). First, they mediate replication of telomeric DNA, thereby preventing the terminal shortening that would otherwise accompany semiconservational replication of linear molecules. Second, they play a critical role in preventing telomeres from being recognized as DNA breaks or other forms of DNA damage.

Consequently, there are two general mechanisms by which dysfunctional telomeres can arise. One is via defects in telomere replication, which can occur due to alterations in the enzyme telomerase or factors that regulate its activity. In either yeast or human cells in which telomerase is not expressed, there is a gradual loss of telomeric DNA, until a point is reached at which telomeres can no longer sustain proper end protection function, and further proliferation is blocked. In budding yeast, the catalytic core of the enzyme is composed of the Est2 reverse transcriptase protein and the TLC1 RNA subunit, while Est1p and Est3p are additional subunits of the holoenzyme that contribute to in vivo regulation of enzyme function (reviewed in Dubrana et al. 2001). Defects in any one of these subunits result in a severe telomere replication defect and an accompanying senescence phenotype (Lendvay et al. 1996).

Despite the proliferation defect that is brought about by a telomerase deficiency, rare populations of cells are able to acquire the ability to maintain their chromosome termini via a recombination mechanism. In budding yeast, two pathways have been described to generate these survivors. Both pathways require RAD52 but otherwise have distinct genetic requirements and are therefore thought to utilize different recombination substrates (Lundblad and Blackburn 1993; Le et al. 1999; Teng and Zakian 1999; Ghien et al. 2001). Telomeres in one class of survivors (often called type I) are characterized by extensive amplification of both subtelomeric Y′ elements and flanking short G-rich stretches, although the terminal G-rich tracts remain short. A second class of survivors (called type II) maintain their telomeres via recombination of the terminal TG13 telomeric repeats, giving rise to very long and heterogeneous terminal G-rich tracts. In both cases, recombination, instead of telomerase, replenishes telomeric G-rich sequences, thereby permitting continued viability (reviewed in Lundblad 2002). Telomerase-independent telomere maintenance can also occur in human cells, and increasing evidence indicates that this also occurs via a recombination-based mechanism (Dunham et al. 2000; Varley et al. 2002).

The second mechanism by which dysfunctional telomeres can be generated is via defects in the telomeric nucleoprotein complex that protects the natural chro-
mosome ends. Uncapped telomeres become subject to DNA degradative activities and are also sensed as DNA damage, thereby triggering a DNA damage response (Garvik et al. 1995; Barnes and Rio 1997; van Steensel et al. 1998; Karlseder et al. 1999; Maringele and Lydall 2002). Defects in this essential telomere function result in immediate effects on cell viability, in contrast to the delayed phenotypes displayed by a telomerase deficiency. For example, in budding yeast, loss of the single-strand telomere DNA-binding protein Cdc13 results in rapid and extensive resection of the C-rich telomeric strand, leading to cell cycle arrest and, ultimately, irreparable DNA damage and cell death (Garvik et al. 1995; Booth et al. 2001). The Ku heterodimer (which is composed of ~70- and 80-kD subunits, encoded by YKU70 and YKU80, respectively) also protects the C strand from resection. Unlike cdc13A strains, which are inviable, yku70A and yku80A strains are viable at 30°C, although the length of the duplex telomeric tract, as well as the extent of the terminal single-stranded G-rich overhang, is perturbed (Boulton and Jackson 1996; Porter et al. 1996; Gravel et al. 1998; Polotnianka et al. 1998). This altered terminal structure has suggested that Ku also regulates the access and/or activity of a telomere-processing entity.

Accumulating evidence indicates that one activity that is restricted by the Cdc13 and Ku end protection factors is the 5' to 3' exonuclease, Exo1 (Maringele and Lydall 2002). Exo1 has been previously characterized for its role in a variety of DNA repair processes, including meiotic recombination, double-strand-break repair, and repair of UV-damaged DNA (Moreau et al. 2001 and references therein). Recent observations suggest that Exo1 also functions at telomeres. When yku70A strains are propagated at high temperatures, there is enhanced resection of the C strand of the telomere, which leads to inviability within ~10 generations. Strikingly, both resection and inviability are rescued by a null mutation in EXO1 (Maringele and Lydall 2002). Similarly, the sequence loss and resulting inviability that occur in response to loss of Cdc13 function are also at least partially Exo1 dependent (Maringele and Lydall 2002; E. Pennock, E. Mandell and V. Lundblad, unpublished observations).

These observations suggest that Exo1 might be a general mediator of telomere dysfunction. To address this idea further, we examined whether an Exo1 deficiency affected telomere maintenance in both telomerase-proficient strains and recombination-dependent survivors from telomerase-deficient strains. Analysis of the consequences of an EXO1 deficiency in strains that lack YKU80, telomerase, or both activities indicates that Exo1 action is detrimental to cells with partially uncapped telomeres, presumably due to increased production of single-stranded DNA. Conversely, however, Exo1 activity positively contributes to telomere maintenance in the absence of telomerase, by promoting the formation of telomerase-independent survivors, presumably by providing a substrate for telomere recombination. These observations indicate that one task of telomere end protection is to prevent illegitimate access of Exo1 to telomeres and may provide insights into the mechanism by which an alternative pathway(s) for telomere maintenance is engaged by telomerase-negative cancer cells.

**MATERIALS AND METHODS**

**Yeast strains and plasmids:** All *Saccharomyces cerevisiae* strains used in this work are isogenic derivatives of YPH275. The yku80Δ::kan6, est2Δ::URA3, tle1Δ::LEU2, and rad52Δ::LYS2 mutations have been previously described (Lendvay et al. 1996; Nugent et al. 1998; Bertuch and Lundblad 2003). The exo1Δ::kan6 disruption removes amino acids 6-698 of the 702-amino-acid Exo1 open reading frame (ORF), and the yku80Δ::LEU2 disruption deletes the entire YKU80 ORF plus 78 and 76 bp of upstream and downstream sequences, respectively. Diploid strains YVL234 (MATa/α tle1Δ::LEU2/TEL1 yku80Δ::kan6/YKU80), YVL1068 (MATa/α est2Δ::URA3/EST2 rad52Δ::LYS2/RAD52 yku80Δ::kan6/YKU80) (ura3-3B/CEN4/D8B), YVL2305 (MATa/α est2Δ::URA3/EST2 exo1Δ::kan6/YKU80Δ::LEU2/YKU80), and YVL2350 (MATa/α est2Δ::URA3/EST2 exo1Δ::kan6/EXO1 yku80Δ::LEU2/YKU80), and YVL2359 (MATa/α est2Δ::URA3/EST2 exo1Δ::kan6/EXO1 yku80Δ::LEU2/YKU80) were constructed by standard techniques (by introducing the relevant gene deletions by one-step gene disruption or by mating isogenic freshly generated haploid strains of the appropriate genotype). All diploid strains have the following isogenic genetic background: ura3Δ-52/ura3Δ-52 lys2-801/lys2-801 ade2-101/ade2-101 trp1Δ/Δ trp1Δ his3Δ200/Δ his3Δ200 leu2Δ1/Δ leu2Δ1.

**Genetic methods:** All incubations were performed at 28°C, except where otherwise noted. Diploid strains were sporulated at room temperature. Yeast genomic DNA preps and telomere Southern blots were performed as previously described (Lendvay et al. 1996). The serial dilution plate assay was performed by resuspending dissected plate colonies in their entirety into 200 μl of water. Tenfold serial dilutions were plated on YPAD and incubated for 2 days at 28°C. Serial liquid culture experiments were performed similarly to methods previously described (Le et al. 1999; Rizki and Lundblad 2001). Each spore colony in its entirety was inoculated into YPAD and grown for 1 day at 28°C. Cell counts were determined; the cultures were subsequently diluted into fresh media, to 1 x 10⁶ cells/ml, and incubated for 22 hr; and cell counts were performed. The protocol was repeated every 22 hr. Several independent isolates for each genotype were analyzed.

**Telomeric G-strand overhang analysis:** The extent of single-stranded G-rich terminal sequences was determined as previously described (Bertuch and Lundblad 2003). Briefly, the strains were grown to midlog phase in rich media. Genomic DNA was isolated as previously described (Hoffman and Winston 1987), and equivalent amounts (10 μg) were XhoI-digested and hybridized to a 5' 32P-end-labeled dCCACCACA CACACCCACCC probe. The samples were resolved by agarose gel electrophoresis, and the gel was subsequently dried and exposed to detect single-strand telomeric DNA (native gel in Figure 1B). To detect the full complement of telomeric DNA (denatured gel in Figure 1B), the gel was denatured and rehybridized with the same 32P-labeled dG3A probe, as previously described (Dionne and Wellinger 1996). The average single-strand telomeric DNA content for two independent isolates of each strain was quantified by normalizing, relative to the average for the YKU80 isolates, the ratio of the native gel signal to the denatured gel signal for the Y-containing
RESULTS

EXO1 contributes to telomere shortening in a yku80Δ strain: To determine whether EXO1 contributes to telomere length homeostasis, telomere length in exo1Δ, yku80Δ, and exo1Δ yku80Δ telomerase-proficient strains was analyzed. Consistent with a previous report (Moreau et al. 2001), no detectable alteration in telomere length was observed in exo1Δ mutants (Figure 1A). However, when the telomere length of EXO1 yku80Δ and exo1Δ yku80Δ strains was compared, a small but reproducible EXO1-dependent effect was observed. Loss of YKU80 results in substantial telomere shortening (Boulton and Jackson 1996). Notably, the telomere length defect observed in an exo1Δ yku80Δ strain, however, was not as severe as that of a yku80Δ strain (Figure 1A; see also the denatured gel in Figure 1B). Thus, Exo1 partially contributes to the telomere maintenance defect that occurs when Ku function is absent, presumably due to a skew in the balance between telomerase-mediated telomere-elongation and telomere-shortening activities.

Strains that are deficient for YKU80 or YKU70 also exhibit altered regulation of the single-stranded overhang, such that extended single-stranded termini are detectable throughout the cell cycle (Gravel et al. 1998; Polotnianka et al. 1998). To examine whether Exo1 contributes to the formation of these abnormal termini, the extent of the single-stranded G-rich overhang in yku80Δ and exo1Δ yku80Δ strains was assessed by native gel analysis. Strikingly, the readily detectable increase in terminal single-strandedness that is observed in a yku80Δ strain was substantially diminished in an exo1Δ yku80Δ strain (Figure 1B). Quantitation of the extent of single-strandedness indicated that Exo1 was responsible for most, although not all, of the increased G-rich single-strand signal detected in yku80Δ strains (Figure 1B). Therefore, the combined effects of an EXO1 deficiency on telomere length and the terminal overhang in yku80Δ strains argue that the loss of end protection that occurs when Ku is absent also affects telomere length maintenance. These observations about the consequence of Exo1 action in the absence of Ku function are also concordant with prior results reported by Maringele and Lydall (2002), who demonstrated that the much more extensive resection of chromosome termini that occurs when yku70Δ strains are propagated at high temperatures is similarly Exo1 dependent.

The lethality of a yku80Δ est2Δ strain is partially relieved by loss of EXO1: Previous work has shown that loss of Ku function confers rapid lethality in a strain that also lacks telomerase (Gravel et al. 1998; Nugent et al. 1998). In contrast to an est2Δ strain, which initially exhibits healthy growth immediately upon sporulation of a heterozygous diploid, a similarly generated yku80Δ est2Δ strain gives rise to a colony that consists largely of dead cells, incapable of further propagation (Nugent et al. 1998; Figure 2A). This synthetic lethality has been proposed to be due to a severe end protection defect, whereby the accelerated telomere shortening—due to the simultaneous loss of both Ku and telomerase—
rapidly leads to telomeres that are incapable of binding end protection factors.

As discussed above, the data shown in Figure 1 indicate that an exo1Δ mutation partially alleviates the telomere shortening observed in a yku80Δ strain, as well as rescuing the Ku-specific end protection defect. We therefore asked whether loss of Exo1 would similarly influence the phenotype of a yku80Δ est2Δ double-mutant strain. Freshly generated yku80Δ est2Δ and yku80Δ est2Δ exo1Δ spore colonies were resuspended in their entirety and assayed for viability by plating serial dilutions and examining growth after 2 days of incubation. Figure 2A shows that an exo1Δ mutation partially rescued the lethality displayed by the yku80Δ est2Δ double-mutant strain. This rescue was characterized by increased microcolony formation, indicating that the exo1Δ mutation rescued some, but not all, of the block in growth potential. Loss of EXO1 function similarly rescued a yku80Δ est1Δ strain, deleted for the Est1 component of the telomerase holoenzyme (data not shown).

In contrast, loss of EXO1 activity did not have a notable effect on the growth phenotype of a strain that lacks only telomerase. To assess this, est2Δ and est2Δ exo1Δ mutant strains were compared in a liquid growth assay. Both strains exhibited a comparable loss in growth potential after 5–6 days of liquid propagation, as a consequence of critical telomere shortening and the resulting proliferation defect (Figure 2B). Following this growth nadir, however, the proliferative potential increased for both strains, such that both cultures were eventually overgrown by telomerase-independent survivors. The recovery period for the est2Δ exo1Δ isolates appeared to

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**Figure 2**—Exo1 contributes to the lethal phenotype of a yku80Δ est2Δ strain. (A) Haploid strains of the indicated genotype were generated by sporulation of the diploid strain YVL2359. Spore colonies were grown for 4 days at 28° and resuspended in their entirety and 10-fold serial dilutions were incubated for 2 days at 28°. (B) Single colonies of freshly generated EST2 EXO1, est2Δ EXO1, est2Δ exo1Δ, and yku80Δ est2Δ exo1Δ haploid strains were resuspended in their entirety, inoculated into YPAD, and grown for 24 hr at 28°. Cell counts and serial dilutions were performed as described in MATERIALS AND METHODS. Three EST2 EXO1 isolates and seven isolates each of the est2Δ EXO1, est2Δ exo1Δ, and yku80Δ est2Δ exo1Δ strains were examined. (C) Diploid strain YVL2303 was sporulated to generate yku80Δ est2Δ exo1Δ isolates. Isolates were propagated by single-colony isolation, for up to eight streak-outs. Each streak-out was incubated at 28° for 2 days and then stored at 4°. Colonies from the stored streak-outs were restreaked, as shown, and incubated for 3 days at 28°.
be slightly delayed (see the last two time points in Figure 2B), suggesting that loss of EXO1 might have a slight impact when telomeres become critically short. However, this difference did not appear to be highly significant, arguing that loss of EXO1 function does not have a robust effect on the growth phenotype of a telomerase-defective strain that retains YKU80 function.

Recombination-dependent survivors obtained from a ykuΔ estΔ strain are promoted by EXO1: Although the ykuΔ estΔ exoΔ strain did not grow as well as a YKU80 EST2 EXO1 strain, this triple-mutant strain was nevertheless capable of continuous long-term propagation, although growth at successive time points was still relatively poor. Successive plating by propagation on solid media were characterized by microcolony formation and poor plating efficiency, although there was a gradual increase in colony size at later time points (Figure 2C). Liquid serial culture propagation gave similar results: the ykuΔ estΔ exoΔ strain could be stably and continuously propagated, albeit with an extremely low doubling rate, although at later time points, recombination-dependent survivors overtook the culture (Figure 2B and see below). Therefore, loss of EXO1 was sufficient to allow long-term growth of a ykuΔ estΔ mutant strain, consistent with the premise that the severe end protection defect characteristic of this double-mutant strain had been partially rescued.

However, although a ykuΔ estΔ strain initially exhibited a much higher degree of cell death, when compared to a ykuΔ estΔ exoΔ strain (Figure 2A), survivors with a healthy growth characteristic could be recovered from the ykuΔ estΔ strain, even after only limited propagation (Figure 3; Grandin and Charbonneau 2003). For this analysis, both ykuΔ tlcΔ and ykuΔ estΔ strains were examined. When freshly generated ykuΔ tlcΔ and ykuΔ estΔ spores were restreaked for single colonies, no growth was initially observed after 2 days of incubation, a time period that was sufficient to allow both TLC1 and tlc1Δ strains to form full-sized colonies (Figure 3A). However, following an additional 4-day incubation, a small number of heterogeneously sized colonies appeared on these ykuΔ tlc1Δ streak-outs. These colonies, as well as similarly obtained ykuΔ estΔ colonies, were capable of subsequent long-term propagation, with a growth phenotype that was comparable to that of survivors recovered from a YKU80 estΔ strain (Figure 3B and data not shown). Appearance of these survivor colonies was also dependent on RAD52, because they failed to arise in ykuΔ estΔ rad52Δ mutants (Figure 3C). This suggests that these ykuΔ estΔ survivors arise by the same well-characterized recombination-dependent mechanisms that give rise to telomerase-defective survivors (Lundblad and Blackburn 1993).

EXO1 alters the pattern of survivors recovered from telomerase-defective strains: The results shown in Figures 2 and 3 demonstrate that, although EXO1 contributes to the initial lethality of a ykuΔ estΔ strain, EXO1 conversely promotes the appearance of survivors in this same strain. Exo1 could contribute to this process by increasing the degree of single-strandedness at telomeres, thereby providing a substrate for recombination between telomeres. This also suggests that Exo1 action at telomeres might influence the types of telomerase-independent survivors recovered. To address this possibility, the types of survivors that were recovered in EXO1 vs. exo1Δ strains were determined.

Examination of the telomeres of ykuΔ estΔ survivors revealed long, heterogeneous telomeres, with a pattern...
tern that was roughly reminiscent of type II recombination (Figure 4A; see also Grandin and Charbonneau 2003). These yku80Δ est2Δ survivors differed from those recovered from est2Δ strains in one notable fashion. Only type II-like survivors could be recovered from a yku80Δ est2Δ strain, whereas both type I and type II survivors were recovered from an est2Δ strain. Over 20 yku80Δ est2Δ survivors generated by serial single-colony isolation were examined, and all exhibited telomeric restriction fragment profiles characteristic of type II survivors, similar to that shown in Figure 4A. In contrast, 2 of 5 survivors isolated in a similar manner from an est2Δ strain were of the type I class, indicating type I survivors could be readily recovered from an est2Δ strain when Ku proficient.

The absence of type I survivors suggested that the severe telomere uncapping defect displayed by the yku80Δ est2Δ strain influenced the type of recombination events—and hence the types of survivors that could be recovered—when telomeres become precipitously short in this double-mutant strain. To ask whether EXO1 action influenced this process, survivors from yku80Δ est2Δ exo1Δ strains were similarly isolated. Although this triple-mutant strain was characterized by a prolonged period of microcolony formation and poor plating efficiency, eventually discrete small colonies could be recovered and analyzed for telomere structure. In sharp contrast to the type II telomere profile exhibited by every survivor recovered from yku80Δ est2Δ strains, both type I and type II types of survivors could be isolated from yku80Δ est2Δ exo1Δ strains (Figure 4B). The yku80Δ est2Δ exo1Δ survivors shown in lanes 3 and 4 of Figure 4B have the telomere profile of type II survivors, but additional survivors isolated from this triple-mutant strain exhibited the characteristic features of type I recombination, with extensive Y′ amplification and a short terminal TG1-3 tract (Figure 4B, lanes 1, 2, 5, and 6). This bias held up even if yku80Δ est2Δ exo1Δ survivors were isolated following serial liquid culturing. Previous work has shown that when an est2Δ strain is propagated in liquid, only type II survivors are eventually recovered, due to the selective advantage of this class relative to type I survivors (Teng and Zakian 1999). However, even when yku80Δ est2Δ exo1Δ strains were grown in liquid culture until each culture was overgrown with survivors, type I survivors could be identified (Figure 4B, lane 9).

To determine whether this EXO1-dependent bias was specific only to strains that were defective for both telomerase and YKU80, the consequences of an exo1Δ mutation on the type of survivors that emerged from an est2Δ strain following serial liquid culture were assessed. Although these conditions should favor the outgrowth of type II survivors, loss of EXO1 shifted the telomere profile exhibited by an est2Δ strain from the exclusively type II pattern to a predominantly type I pattern (Figure 4C). Eighteen of 18 est2Δ survivors displayed the heterogeneous, long telomeres characteristic of type II survivors, whereas 13 of 16 est2Δ exo1Δ survivors exhibited a type I pattern, with Y′ amplification and a short terminal G-rich telomeric tract. Therefore, just as was observed for a yku80Δ est2Δ strain, Exo1 influences the form of telomere recombination utilized for survival in telomerase-deficient mutants, resulting in an increased relative frequency of type I survivors in its absence.
DISCUSSION

Exo1 influences the balance between elongation and shortening activities at telomeres: Telomere length homeostasis is a genetically regulated process that maintains chromosome termini within a carefully controlled length range. Careful analysis of individual telomeres, however, has revealed that the length of telomeric ends can vary, even in telomerase-proficient cells, resulting in a certain degree of length heterogeneity (Shampay et al. 1984; Lansdorp et al. 1996). This clonal variation in the length of individual telomeres is thought to be the consequence of a balance between shortening and lengthening processes that can occur during each round of replication (Blackburn 2001).

In wild-type cells, telomerase is the primary activity responsible for elongating telomeres, whereas incomplete replication and potential nuclease-mediated degradation have been proposed to contribute to telomere shortening. In yku70Δ and yku80Δ cells that express telomerase, the balance between shortening and lengthening activities is shifted, such that telomeres are maintained at a much shorter mean length. Part of this telomere length decline is due to loss of an interaction between Ku and a 48-nucleotide stem-loop of the yeast telomerase RNA. This interaction facilitates telomerase-mediated telomere elongation, by contributing to either telomerase recruitment or activation (Peterson et al. 2001; Stellwagen et al. 2003). However, loss of the Ku heterodimer also results in an end protection defect that is manifested by increased single-stranded regions at chromosome termini (Gravel et al. 1998; Polottnianka et al. 1998). The action of various nucleases on these partially exposed termini might be expected to contribute to telomere shortening in yku70Δ and yku80Δ strains.

Consistent with such a prediction, this work demonstrates that the severe telomere length defect displayed by Ku-deficient cells is partially rescued in an exo1Δ background. In parallel, the end protection defect of a yku80Δ mutant strain, both type I and type II patterns can be observed in Ku-defective strains. One possibility is that any increase in telomere length resulting from an Exo1 deficiency may be only transient in YKU80 cells, due to cis-inhibition of telomerase action on these slightly elongated telomeres in subsequent cell divisions (Marcand et al. 1997, 1999). In contrast, telomere length regulation via telomerase has clearly been compromised in Ku-defective strains, and hence reduced resection by Exo1 would result in a detectable change in telomere length.

Exo1 mediates telomerase-independent proliferation: The above observations indicate that, in cells that express telomerase, Exo1 action opposes telomere elongation. In contrast, Exo1 appears to directly contribute to telomere maintenance when telomeres are maintained by recombination. The effect of Exo1 on telomerase-independent pathways for telomere maintenance is twofold. First, in a strain that is defective for both telomerase and the Ku heterodimer, Exo1 promotes the formation of telomerase-independent survivors. Second, in telomerase-defective strains that also lack Exo1, there is a shift in the type of recombination-dependent survivors that are recovered: whereas liquid propagation of est2Δ strains yields only survivors with the characteristic type II telomeric pattern of rearrangements, both type I and type II patterns can be observed in est2Δ exo1Δ survivors.

Telomere maintenance in the absence of telomerase has been proposed to employ break-induced replication (BIR; reviewed in Kraus et al. 2001). Two similar genetic pathways have been described for telomerase-independent telomere maintenance and BIR, one that is RAD50/ RAD59 dependent (type II) and a second pathway that is RAD51 dependent (type I). An early step in BIR is end resection by a 5’ to 3’ exonuclease, thereby generating a single-stranded recombination substrate capable of
strand invasion. In the case of telomerase-independent telomere maintenance, the recombination processes appear to involve different regions of homology. For type I recombination, BIR is likely initiated either between terminal and TG_{1,3} repeats present between subtelomeric Y' elements or between subtelomeric repeat elements. In contrast, for type II recombination, BIR appears to be initiated between terminal TG_{1,3} repeat sequences themselves. Therefore, the impact of the presence or absence of Exo1 on the types of telomerase-independent survivors recovered from et2Δ strains may reflect differences in the rate and the nature of the single-stranded termini generated, which are capable for strand invasion. In the absence of both Exo1 and telomerase, resected termini may not arise until there has been substantial reduction in duplex length perhaps to a point immediately adjacent to the subtelomeric repeat elements. Thus, the resulting single-stranded termini capable of strand invasion would encompass either subtelomeric repeat or internal TG_{1,3} sequences, thereby favoring type I recombination. In the absence of Ku, the enhanced action of Exo1 would lead to rapid generation of single-stranded termini encompassing the very terminal telomeric repeats, thereby favoring type II recombination. Notably, in the absence of Ku, the normal requirement of RAD59 for the type II pathway is alleviated (Grandin and Charbonneau 2003). Alternatively, the influence of Exo1 may be a later event, differentially influencing the robustness and/or maintenance of type I vs. type II survivors and, hence, their ability to be recovered.

Conclusions and perspectives: Increasing evidence indicates that processing of the C strand is a regulated component of yeast telomere function, just as elongation of the G strand by telomerase is highly regulated. Interestingly, in contrast to these observations in yeast, there is no evidence as yet for a mammalian C-strand processing activity, although there is an active mechanism that protects the G-strand overhang in mammalian cells (van Steensel et al. 1998; Smogorzewska et al. 2002). A potentially integrated view has recently been provided in work by Price and co-workers, which indicates that specific nuclease processing events must occur on both the G strand and the C strand of the telomere (Jacob et al. 2003). While these processing activities are required to generate the terminal DNA structure necessary to allow assembly of the telomere end protection complex, failure to regulate their activity may have untoward effects on the ability of the telomeres to carry out their essential function or may positively influence the establishment of alternative pathway(s) of telomere maintenance in telomerase-negative cancer cells.

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