EXO1 Plays a Role in Generating Type I and Type II Survivors in Budding Yeast

Laura Maringele¹ and David Lydall²

School of Biological Sciences, University of Manchester, Manchester M13 9PT, United Kingdom

Manuscript received June 26, 2003
Accepted for publication November 24, 2003

ABSTRACT

Telomerase-defective budding yeast cells escape senescence by using homologous recombination to amplify telomeric or subtelomeric structures. Similarly, human cells that enter senescence can use homologous recombination for telomere maintenance, when telomerase cannot be activated. Although recombination proteins required to generate telomerase-independent survivors have been intensively studied, little is known about the nuclease that generate the substrates for recombination. Here we demonstrate that the Exo1 exonuclease is an initiator of the recombination process that allows cells to escape senescence and become immortal in the absence of telomerase. We show that EXO1 is important for generating type I survivors in yku70Δ est2Δ cells and type II survivors in tlc1Δ cells. Moreover, in tlc1Δ cells, EXO1 seems to contribute to the senescence process itself.

Budding yeast cells, like almost all immortal eukaryotic cells, use telomerase to maintain the end of their chromosomes. Cells lacking telomerase components, for example, estΔ, est2Δ, or tlc1Δ mutants, have been engineered and studied for their ability to escape replicative senescence (a state of cell cycle arrest caused by short and/or defective telomeres) and survive indefinite periods of time using a secondary mechanism of telomere maintenance. This mechanism appears to be based on break-induced replication (BIR), a variant of the main DSB-repair mechanism in budding yeast, homologous recombination, and is associated with amplification of telomeric or subtelomeric structures (Lundblad and Blackburn 1993; McEachern and Blackburn 1995; Teng and Zakian 1999). One major difference between BIR and the telomerase-based way of maintenance is that recombination can copy and amplify only preexistent structures, while telomerase can synthesize a telomere de novo.

Long-term survival without evidence of telomerase activity was found in ~15% of malignant tumors, where it was termed alternative lengthening of telomeres (ALT; Grobelny et al. 2001; Henson et al. 2002). Recent data report a higher incidence of ALT (25–66%) in some types of cancer like glioblastoma and osteosarcoma (Hakin-Smith et al. 2003; Ulaner et al. 2003). Because the mechanism of ALT is practically indistinguishable from BIR (Reddel et al. 1997), recombination at telomeres in budding yeast is considered a good model for understanding the telomerase-independent survival in mammalian cells.

It is clear that genes involved in recombination at double-strand breaks are also responsible for amplification of telomeres in the absence of telomerase (Li et al. 1999; Sugawara et al. 2000; Chen et al. 2001). But, perhaps with the exception of RAD52, which is essential for recombination, all other recombination genes seem to be divided into two distinct pathways, depending on what kind of structure is amplified. One pathway is responsible for amplification of subtelomeric Y’ regions and is dependent on RAD51, RAD54, RAD55, RAD57, and RAD52 (Chen et al. 2001). In yeast, approximately two-thirds of the chromosomes have one to four homologous subtelomeric Y’ regions, adjacent to telomeres (Przyde and Louis 1997). Cells that escape senescence by amplifying Y’ regions are termed type I survivors (Lundblad and Blackburn 1993; Teng and Zakian 1999; Chen et al. 2001). Another pathway involves the RAD50/MRE11/XRS2 complex together with RAD52, RAD55, SRS2, SGS1, and TID1 and drives the amplification of TG-telomeric repeats to generate type II survivors (Chen et al. 2001; Signon et al. 2001).

There are subtle differences between the recombination-amplification induced by telomere shortening and the recombination-repair induced by a double-strand break, despite the fact that they involve the same genes. For example, initiation of (sub) telomeric amplification is a relatively rare event, with approximately one in a million cells able to complete this process; in contrast, double-strand breaks (DSBs) are highly efficiently repaired.

Although recombination proteins required to generate telomerase-independent survivors have been inten-
EXO1 encodes a 5′ to 3′ exonuclease with FLAP endonuclease activity (Tran et al. 2002). EXO1 is involved in resection of meiotic DSBs (Tsoubochi and Ogawa 2000), mitotic DSBs (Fiorentini et al. 1997), and unprotected telomerases (Maringele and Lydall 2002; Lydall 2003). In addition, EXO1 affects meiotic recombination (Khazaneddari and Borts 2000; Kirkpatrick et al. 2000; Symington et al. 2000) and is part of a complex with mismatch repair proteins (Tishkoff et al. 1997; Sokolsky and Alani 2000; Tran et al. 2001).

Here we present evidence that EXO1 is important for generating recombination-dependent, type I and II survivors, in two different types of senescent mutants: telomere capping defective (yku70Δ mre11Δ) and telomerase-negative (tLc1Δ) cells. We show that deletion of EXO1 delays the appearance of survivors for ~30–40 generations. Therefore, EXO1 appears to be one of the “initiators” of the recombination process that allow rare cells to escape senescence and become immortal in the absence of telomerase.

MATERIALS AND METHODS

Yeast strains: All strains used in this study are isogenic, in the W303 background, and RAD5. To construct strains, standard genetic procedures of transformation and tetrad analysis were followed (Adams et al. 1997). Since W303 strains contain an ade2-1 mutation, YPD (yeast extract, peptone, and dextrose) medium was routinely supplemented with adenine at 50 μg/liter. All yku70Δ mre11Δexo1Δ strains and their derivatives were made by crossing DLY131 (mre11Δ::hisG::URA3) with DLY1408 (yku70Δ::HIS3 exo1Δ::LEU2). All tLc1Δ exo1Δ strains were made by crossing DLY1628 (tLc1Δ::HIS3 pTLC1::URA3 from D. Gottschling) with DLY1948 (exo1Δ::LEU2 rad52Δ::TRP1). Diploid cells that had lost the pTLC1 plasmid were sporulated, dissected, and germinated.

Growth rate assay: Cells of the appropriate genotype (four independent strains for each genotype of interest) were isolated from a fresh germination plate and grown in YPD medium overnight. The following morning, cells were counted using a hemocytometer, diluted to 1 × 10^6 cells/ml, and then incubated at 23°, with aeration. Every 23 hr, cell densities were measured and then the culture was diluted with fresh YPD liquid medium to a density of 10^6 cells/ml. During counting, cells were put on ice. This cycle was repeated for several days. At indicated time points, samples were collected for telomere length analysis.

Streak assay: Cells of the appropriate genotype were picked from a fresh germination plate and streaked onto YPD plates. After incubation for 4 days at 23°, similar amounts of cells (~1 × 10^6 cells) from several independent colonies were picked and restreaked on YPD plates. This restreaking was repeated several times to allow senescence and appearance of survivors.

Microcolony assays: Colony-purified yeast strains were inoculated into 1 ml YPD and grown overnight with aeration at 25° until they reached a concentration of ~8 × 10^6 cells/ml. Cells were sonicated briefly and spread on plates. The plates were incubated at 22°. After an appropriate length of time, the colonies were photographed and the cell numbers in 100 colonies were counted/estimated.

Telomere and subtelomere Southern blots: Southern blot analyses were performed to examine telomere and Y lengths. Each time, cells from three or four independent mutants with the same genotype were pooled prior to DNA extraction, except for Southern blot analysis of single colonies. Yeast genomic DNA was isolated and ~20 μg of DNA was digested with XhoI and separated on a 0.8% agarose gel. DNA was then transferred to a Magna Nylon membrane (Genetic Research Instrumentation) and UV crosslinked. The membrane was then hybridized with a Y′-TG probe (pHT128; Tsoubochi and Ogawa 2000) and hybridization was detected using a nonradioactive detection kit (Amersham, Arlington Heights, IL).

RESULTS

EXO1 is important for generating survivors in yku70Δ mre11Δ senescent cells: Initiation of (sub)telomeric amplification in the absence of telomerase is a rare event. Factors involved in the initiation of survivors were still unknown, but we speculated that EXO1 might be one, because of its role in degradation of unprotected telomeres (Maringele and Lydall 2002). Our previous experiments have demonstrated that defective telomeres in ade13-1 and yku70Δ mutants were targeted by the Exo1p exonuclease. EXO1 was important for resecting the 5′ AC strand, from telomeres toward centromeres, leaving behind a detectable 3′ overhang, up to 1 kb long in yku70Δ strains (Maringele and Lydall 2002). One consequence of Exo1p activity at defective telomeres was arrest of the cell cycle before anaphase (Maringele and Lydall 2002). The ability to degrade unprotected telomeres in vivo is not a common feature of all exonucleases (M. Zubko, unpublished data) suggesting that EXO1p has a special function at telomeres.

We, therefore, tested the role of EXO1 in senescence and survival of cells with telomere defects caused by the absence of telomere protective genes YKU70 and MRE11. This model of senescence and survival is based on the observation that yku70Δ mre11Δ double mutants senesce very rapidly, during the early growth of germinated spores (Ritchie and Petes 2000; Dubois et al. 2002; Maringle and Lydall 2002).

We monitored growth of yku70Δ mre11Δ double mutants and yku70Δ mre11Δ exo1Δ triple mutants. Cells were taken directly from the germination plate (four independent strains of each genotype) and incubated in liquid at 23° for several days. At 23°, wild-type strains would divide ~10 times a day. Cell densities were scored every 23 hr, followed by dilution to 10^5 cells/ml and continued incubation. At 23°, yku70Δ and yku70Δ exo1Δ cells reached and maintained densities of ~0.5 × 10^6 cells/ml, similar to the wild type, while other control mutants, mre11Δ and mre11Δ exo1Δ, reached densities 5-fold lower than those of the wild type (Figure 1a). In contrast, yku70Δ mre11Δ and yku70Δ mre11Δ exo1Δ cells were in crisis during the early days of the experiment, when they had densities 200-fold lower than those of
the wild type, consistent with early entry into senescence (Figure 1a).

Both types of senescent mutants, yku70Δ mre11Δ (EXO1+) and yku70Δ mre11Δ exolΔ, were able to generate survivors, but with different dynamics. While EXO1+ survivors escaped senescence early (days 4–5) and grew, after 2 days, at growth rates (determined by cell density after 23 hr growth) similar to those of mre11Δ mutants, exolΔ survivors appeared more slowly and gradually reached, after 15 days in liquid culture, a 5-fold lower cell density, compared with mre11Δ cells (Figure 1a).

The data above suggest that EXO1 plays a significant, but not essential, role in generating survivors. Another possibility would be that EXO1 is important for cell vitality in an mre11Δ background, even in the absence of senescence. When growth rates of mre11Δ exolΔ cells are compared with those of mre11Δ mutants, it appears that exolΔ deletion did indeed cause a kind of crisis during the first 4 days, when mre11Δ exolΔ mutants daily reached only ~25% of the cell density of mre11Δ strains (Figure 1a; Moreau et al. 2001). However, growth of mre11Δ exolΔ strains improved during the following generations, oscillating between 55 and 98% of the mre11Δ growth. Thus, the requirement for EXO1 was stronger in yku70Δ mre11Δ survivors than in mre11Δ mutants. Interestingly, deletion of EXO1 in yku70Δ cells shows an effect opposite to that in mre11Δ cells, because exolΔ mutation improves the growth rate of yku70Δ mutants ~15% at 23° (Figure 1a). At 37°, an exolΔ even rescues the inviability of yku70Δ mutants (Maringele and Lydall 2002).

The requirement for EXO1 in recovery from senescence was also tested by growth on plates, when comparable amounts of cells from germination plates were spread on fresh YPD plates (passage 1) and incubated at 23° for 4 days (Figure 1b). Several passages were performed consecutively. This experiment confirmed that early generations of yku70Δ mre11Δ (EXO1+) and yku70Δ mre11Δ exolΔ mutants were in a state of minimal growth, presumvably senescent (passage 1), and that EXO1+ strains generated survivors more rapidly (passage 2) compared with exolΔ strains (passage 3). Also, EXO1+ survivors grew notably better than exolΔ survivors (passage 3), and a difference in growth was maintained even after 15 passages, which is equivalent to 600 wild-type generations (Figure 1d, compare size of individual colonies; data not shown).

EXO1 is required for maintenance of survivors: The continued growth defect in yku70Δ mre11Δ exolΔ could have many possible explanations, such as slow metabolic rate, longer cell cycle, decreased viability, high rate of resenescence, and/or delay in reappearance of survivors. It is known that in telomerase-defective (estΔ) survivors, the senescence phenotype reappears in some subclones (Lundblad and Blackburn 1993). We monitored the ability of "established" survivors (with a common history of 32 days in culture, therefore longer than 200 generations) to form colonies by microcolony assay, spreading similar amounts of cells on plates after a short sonication and then incubating them for 24 hr at 23° (Figure 2).

Although a number of nondividing cells (single-cell "colonies") or cells with a large bud (two-cell colonies) were microscopically detected after 24 hr in all mutants with a mre11Δ background, the percentage of these one- or two-cell colonies in yku70Δ mre11Δ survivors was simi-
EXO1 is required for the maintenance of survivors. Small amounts of cells from the same passage (eight, corresponding to 32 days in culture) were diluted to the same concentration, briefly sonicated, spread onto a YPD plate, and then incubated for 23 hr at 23°C before being photographed.

EXO1 increases the rate of Y' amplification in yku70Δ mre11Δ survivors (type I survivors): Some cells can escape senescence and generate survivors. In yeast, presumably also in mammalian cells, this occurs by correction of the telomeric defect. Repair proteins that belong to the recombination pathways are able to amplify sub-telomeric regions (in yeast, Y' regions), generating type I survivors, or amplify terminal telomeric TG repeats, generating type II survivors. It is known that Rad50p, which acts in a complex with Mre11p, is required to maintain type II survivors, because in a rad50Δ background, type I survivors replace type II survivors very early (Chen et al. 2001). Since Mre11p and Rad50p function together, type I survivors would be expected to predominate in an mre11Δ background as well.

Also, the fact that exo1Δ survivors appear late and maintain a growth defect, compared with the EXO1Δ strains, raised the possibility that exo1Δ survivors were using a different survival mechanism. To address the mechanism, we performed Southern blots to detect Y' or TG amplifications (Figure 3). Cells were collected every 3 days and DNA was cut with XhoI and probed with a Y'-TG probe; that gave three fragments, ~6.5, 5.5, and 1.3 kb in wild-type cells. The two larger fragments correspond to repetitive Y’s, while the shortest fragment is the terminal 1 kb of Y' and also contains ~350 bp of telomeric TG repeats (Figure 3a).

In mre11Δ control strains, the telomeric fragment was shorter than that in wild type (Figure 3b), consistent with previous data (Boulton and Jackson 1998; Chamankhah et al. 2000). Deletion of EXO1 from the control mre11Δ strains, maintained by telomerase, apparently does not influence the length or intensity of (sub)-telomeric fragments, consistent with earlier observations (Figure 3b; Tsubouchi and Ogawa 2000; Moreau et al. 2001). We observed that growth of mre11Δ exo1Δ strains improved after several days in liquid culture (Figure 1a), but this growth improvement did not correlate with any notable change at the telomeres of these strains (Figure 3b).

However, in senescent yku70Δ mre11Δ cells, the effect of exo1Δ was obvious. During the first 3 days in culture, yku70Δ mre11Δ cells were senescent and the larger frag-
ments corresponding to Y' looked similar to wild type, while the short fragment appeared less intense, presumably due to loss of telomeric sequences in many of these cells (Figure 3b). But after 6 days, yku70Δ mre11Δ cells had already amplified the Y' repeats (type I survivors), and this amplification apparently remained constant afterward (days 6–12; Figure 3b). Deletion of EXO1 from yku70Δ mre11Δ cells considerably slowed the rate of Y' amplification. Amplification occurred slowly, but progressively during 3–12 days in culture; however, even after 12 days, the Y' amplification was less pronounced in yku70Δ mre11Δ exo1Δ cells than in yku70Δ mre11Δ cells after 6 days in culture (Figure 3b). Therefore, cells lacking EXO1 had difficulty in generating type I survivors. The difference in dynamics of Y' amplification, in exo1Δ vs. EXO1+ survivors, is not a consequence of poor cell growth, because similar amounts of DNA were analyzed in Southern blot assays (see DNA loading control, Figure 3b) and, initially, Y' amplifications occur during senescence. Rather, the growth defect might be explained by difficulties and delays in amplification of Y' regions in yku70Δ mre11Δ exo1Δ strains due to the absence of EXO1.

YKU70 inhibits the maintenance of type I survivors: We have shown that Exo1 degrades telomeres in yku70Δ mutants (Marinelli and Lydall 2002). To see if Exo1 plays a role in generating type I survivors in the presence of YKU70, we analyzed tlc1Δ mre11Δ strains. These strains senesce due to the absence of the telomerase RNA template, Tlc1, while deletion of Mre11 ensures that only type I survivors can be generated. We monitored senescence and survival in experiments similar to those described in Figure 1. In this experiment, an exo1Δ decreased the density of tlc1Δ mre11Δ cells before and after senescence (Figure 4a). This is partially due to the synergic effect of exo1Δ and mre11Δ deletions in decreasing the cell viability, as previously mentioned. A second phenomenon was noted: during the early postsenescence period, tlc1Δ mre11Δ survivors grew poorly compared with yku70Δ mre11Δ survivors and did not reach the growth levels of mre11Δ single mutants, as yku70Δ mre11Δ survivors did (Figure 4a vs. Figure 1a). Also, the Y' amplification in tlc1Δ mre11Δ survivors was inferior to the Y' amplification in yku70Δ mre11Δ survivors (Figure 4b vs. Figure 3b). The data suggest that the presence of Yku70 makes the maintenance of type I survivors difficult and might explain why tlc1Δ cells preferentially maintain type II survivors.

EXO1 opposes adaptation and plays a role in generating type I survivors in tlc1Δ mre11Δ cells: We next addressed the question of whether EXO1 plays a role in generating type I survivors, in the presence of YKU70, in tlc1Δ mre11Δ mutants. Figure 4a shows that tlc1Δ mre11Δ exo1Δ survivors grew about fivefold less than tlc1Δ mre11Δ survivors, during the early days of the postsenescence period (days 9–12). This may be due to the synthetic effect of mre11Δ and exo1Δ in decreasing the cell viability. However, was the escape from senescence and early postsenescence growth of tlc1Δ mre11Δ exo1Δ strains due to recombination events that generated type I survivors? To test this, we deleted RAD52, which is essential for recombination. It is clear from Figure 4a that RAD52 recombination defective, tlc1Δ mre11Δ exo1Δ rad52Δ strains also escape senescence and their postse-
Figure 5.—**EXO1** is important for generating type II survivors in *tlc1Δ* cells. (a) Strains as indicated in the legend were grown as described in Figure 1a. (b) Telomeric length was monitored by Southern blot as in Figure 3b. On day 0 cells were taken directly from germination plates and grown for 4 hr in liquid culture, and DNA was prepared. (c) Y′ and TG amplification was monitored as in Figure 3b. (d) Germinated spores were grown and mass passaged daily on plates for 6 days. On the sixth day the majority of cells had entered senescence. By day 8 we were able to pick 12 colonies (A–L) that were still growing. These colonies were grown overnight in liquid culture before DNA was prepared. Y′ and TG amplification was monitored as in Figure 3b.

Nescent growth curve almost overlaps with the growth curve of *tlc1Δ mre11Δ exo1Δ* mutants, during the first 17 days in culture. By contrast, *tlc1Δ mre11Δ* rad52Δ cells did not escape senescence. Thus, *tlc1Δ mre11Δ* *exo1Δ* rad52Δ cells appeared to be adapting to the telomere defect, rather than using recombination to overcome senescence.

Thus, early postsenescent growth of *tlc1Δ mre11Δ exo1Δ* cells was *RAD52* independent, while growth of *tlc1Δ mre11Δ* cells during the same period was strictly *RAD52* dependent. Later on, *RAD52* strains amplified the Y′ repeats, *tlc1Δ mre11Δ exo1Δ* mutants to a lesser extent than *tlc1Δ mre11Δ* mutants (Figure 4b), while *rad52Δ* survivors showed no amplification of subtelomeric regions (data not shown). Adaptation is defined as escape from the cell cycle arrest without repair of the DNA damage (Toczyski et al. 1997; Lee et al. 1998). In this case it means to escape senescence with short telomeric regions.

Our interpretation is that **EXO1** is, indeed, required to generate type I survivors in *tlc1Δ mre11Δ* mutants, but also to suppress the adaptation to telomeric damage and the recombination-independent escape from senescence.

**EXO1 plays a role in generating type II survivors in *tlc1Δ* cells:** If **EXO1** plays a role in the amplification of subtelomeric repeats, presumably by generating long 3′ overhangs that would invade hom(e)ologous structures, what about its role in type II survivors? Type II survivors amplify the TG-telomeric repeats, with help from a nuclease/helicase complex (Rad50p/Mre11p/Xrs2p), a helicase (Srs2p or Sgs1p), and recombination proteins (Rad52p and Rad59p; Chen et al. 2001). We wondered if there was any requirement for Exo1p in generating type II survivors.

In experiments similar to those described in Figure 1, we monitored the senescence and survival of *tlc1Δ exo1Δ* cells vs. *tlc1Δ* cells (Figure 5). The strains were germinated from spores that had inherited the normal telomere length from their parents. During the presemad period, *tlc1Δ exo1Δ* strains grew ~1.5-fold better than *tlc1Δ EXO1*Δ single mutants each day (Figure 5a; note the lack of overlap of error bars on days 3 and 4). This suggests that in the absence of telomerase, **EXO1** contributes to telomere shortening and senescence. However, this phenomenon was not observed in strains that lack *MRE11* (Figure 4a). It is possible that the synthetic effect of *mre11Δ* and *exo1Δ* mutations in decreasing cell viability (presumably for reasons other than telomere damage, because *mre11Δ exo1Δ* and *mre11Δ* cells have similarly short telomeres; Figure 3b) counteracts the effect of *exo1Δ* in presenescent *mre11Δ* cells.
As telomeres progressively shortened, cells became senescent. The vast majority of cells stopped cell division after 6 days in liquid culture, when tle1Δ and tle1Δ exo1Δ cells had 20- to 25-fold lower densities, compared with their growth rate from day 1 (Figure 5a). Soon after, tle1Δ cells generated survivors that reached their full growth potential within 2 days (day 8). By contrast, tle1Δ exo1Δ cells grew poorly and needed more time (11–12 days total) to achieve a better growth rate, still 1.6-fold less than that of tle1Δ or exo1Δ cells (Figure 5a). This indicates that EXO1 plays a role in generating survivors in a tle1Δ background.

Since telomerase-defective tle1Δ cells entered senescence more slowly than yku70Δ mre11Δ cells (compare Figures 1A and 5A) we were able to monitor the effect of EXO1 on telomere degradation as cells entered senescence. We purified DNA from cells as early as possible after they had germinated and every day thereafter. The effect of EXO1 on telomere shortening in presenescent cells was clear. In EXO1+ tle1Δ cells significant telomere shortening had occurred by the time we were first able to purify DNA (as the spores germinated and grew from a single cell to a colony of ~10⁶ cells). After this, during days 1–7, telomere shortening appeared to stop. In contrast, tle1Δ exo1Δ cells contained significantly longer telomeres at day 0 that slowly declined over the next 6 days, to reach a length similar to that in tle1Δ cells by day 7 (Figure 5b).

To understand the role of Exo1p during recovery from senescence, Southern blots were used to analyze the dynamics of telomere amplification in tle1Δ exo1Δ double mutants vs. tle1Δ single mutants (Figure 5c). After 6 days in culture, both types of mutants had short telomeres. After 8 days in culture, tle1Δ cells had amplified the telomeric TG sequences, generating type II survivors, while the short telomeres from tle1Δ exo1Δ cells looked unchanged (Figure 5c). After 10 days, tle1Δ exo1Δ cells showed little evidence of telomere amplification, and also their Y’ repeats were indistinguishable from wild-type Y’ repeats (Figure 5c). The tle1Δ exo1Δ cells significantly amplified their telomeres after 12 days in culture. The pattern of telomere amplification in tle1Δ exo1Δ survivors was also different from that in tle1Δ mutants: they seemed to maintain a fraction of short telomeres, together with amplified telomeric TG sequences (Figure 5c).

Cells containing short Y’ telomeres and extensive TG repeats are not commonly observed. We wondered if this pattern represented the initial phase in the generation of type II survivors. To test this we cloned 12 colonies from cells that had been passaged on agar plates for 8 days. At this point very few cells formed colonies. Four of 6 tle1Δ colonies were survivors: 2 (C and E) were type I, and 2 (A and F) were type II (Figure 5d). Both of these early type II survivors still contained a lower terminal Y’ fragment, similar to the tle1Δ exo1Δ strains at day 12 (Figure 5c). When we examined DNA from the tle1Δ exo1Δ cells, only 1 colony (G) had generated a type I survivor at this point, while the other colonies showed little (I and L have a weak 1.3-kb band) or no evidence for amplification and were, possibly, late senescent colonies (i.e., colonies derived from presenescent cells that have previously spent a long time in G₁, before Start, due to the caloric restriction caused by a large number of cells competing for nutrients on a limited agar surface).

**DISCUSSION**

With respect to its activity at defective telomeres, EXO1 encodes an exonuclease able to attack, degrade, and alert checkpoint pathways in yku70Δ and cdc13-1 mutants (MARINGELE and LYDALL 2002). Here we have...
shown that Exo1p activity at telomeres has consequences beyond damage generation and cell cycle arrest. Our experiments suggest that Exo1p is one of the initiators of a recombination process that allows only a “one in a million” cell to amplify its telomeres or its subtelomeric regions, to escape senescence and become immortal in the absence of telomerase.

We show that EXO1 plays three roles in cells that enter senescence. First, the presence of EXO1 in mre11Δ yku70Δ or tlc1Δ cells appears to contribute to entry into senescence. Second, the presence of EXO1 in the same cells contributes to rapid escape from senescence by generating type I and type II survivors. Thus, EXO1 accelerates entry into and exit from senescence. Third, EXO1 inhibits adaptation to senescence. All these effects of Exo1 can be explained by its 5’ to 3’ nuclease activity that generates single-stranded DNA overhangs at telomeres.

In yku70Δ mre11Δ cells, Exo1p is able to generate long 3’ overhangs in subtelomeric regions (Maringele and Lydall 2002). The experiments we report here further suggest that the long overhangs generated by Exo1p might serve as the invading strands in a recombination process that involves Rad52p and Rad51p. As a consequence, type I survivors are generated (Figure 6a). Although EXO1 is important for this process, it is not essential, because type I survivors are slowly produced in exo1Δ cells, presumably due to redundant nuclease activities.

In tlc1Δ cells, EXO1 is also important for generating type II survivors. Type II survivors might represent the product of a “disrupted” recombination pathway that had difficulty in progressing into the Y’ regions, due to the protective presence/activity of both Yku70p/Yku80p and MRE11/RAD50/XRS2 complexes, and that specializes in amplification of TG repeats. Exo1p activity is presumably required to degrade the telomeric 5’ strand produced by a helicase. It is known that Sgs1 and Srs2 are required for BIR and/or generation of type II survivors (Cohen and Sinclair 2001; Huang et al. 2001; Johnson et al. 2001; Signon et al. 2001). Degradation of the 5’ strand enables the 3’ strand to invade other homologous and homologous substrates. Subsequently, type II survivors are generated (Figure 6b).

In previous studies, an antirecombination activity has been attributed to the mismatch repair proteins in Escherichia coli (Petit et al. 1991) and Saccharomyces cerevisiae (Datta et al. 1996). Rizki and Lundblad showed that defects in mismatch repair, caused by deletion of MSH2, increased the survival rates of telomerase-defective cells. This was suggested to be due to increased telomeric recombination in msh2Δ strains (Rizki and Lundblad 2001; Lundblad 2002). Exo1p co-immunoprecipitates with Msh2p and is considered a mismatch repair exonuclease (Tishkoff et al. 1997; Sokolsky and Alani 2000). Interestingly, our experiments suggested that EXO1 promoted subtelomeric recombination, rather than opposed it. How is it possible that two interacting mismatch repair proteins have opposite effects toward recombination? Our previous studies demonstrated that Exo1p generates single-stranded DNA in subtelomeric regions (Maringele and Lydall 2002), but this activity did not require MSH2 (L. Maringele, unpublished data; M. Zubko, unpublished data). It is possible that a mismatch repair complex between Exo1p and Msh2p decreased the number of Exo1p proteins available to initiate homologous recombination. Thus, the positive effect of msh2Δ deletion on telomere recombination would be indirect, by increasing the amount of (un-bound) Exo1p.

In conclusion, EXO1 is important for generating type I survivors in yku70Δ mre11Δ cells and type II survivors in tlc1Δ cells. Moreover, EXO1 seems to contribute to the senescence process itself, in tle1Δ cells. We speculate that in human cells also, interference with EXO1 or other nucleases active at un-capped telomeres might slow down both the entry into senescence and the speed of generating recombination-dependent survivors.

We thank A. Bertuch and V. Lundblad for sharing unpublished data, R. Blankley for a tle1Δ deletion strain, D. Gottschling for the TlC1 plasmids, and H. Tsubouchi and H. Ogawa for the Y′-TG plasmid. The Wellcome Trust funded this work.

LITERATURE CITED


EXO1 and the Senescent Cell


Lundblad, V., 2001 The Saccharomyces cerevisiae EXO1, a gene encoding an exonuclease that -dependent single-stranded DNA at telomeres activates subsets of DNA damage and spindle checkpoint pathways in budding yeast yku70Δ mutants. Genes Dev. 16: 1919–1933.


Communicating editor: A. Nicolas