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Rif1/2 and Tel1 function in separate pathways during replicative senescence

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Telomeres, the physical ends of linear eukaryotic chromosomes, are bound by proteins that protect chromosome ends from being recognized as DNA double-stranded breaks.[1] Telomeres shorten due to incomplete DNA replication and nucleolytic degradation. This shortening is counteracted by a specialized reverse transcriptase called telomerase.[2] In the budding yeast *Saccharomyces cerevisiae*, deletion of any of the genes encoding telomerase components—such as the *EST2* gene, which encodes the protein catalytic subunit[2]—leads to an **e**ver **s**horter **t**elomere (EST) phenotype and replicative senescence within 60–80 generations.[3] Rare cells can escape senescence to become survivors, maintaining their telomeres via DNA recombination.[3]

There are many genes involved in the regulation of telomere length homeostasis, including the phosphoinositide-3-kinase-related kinases Tel1 and Mec1, yeast orthologs of human ATM (ataxia telangiectasia mutated) and ATR (ATM and Rad3-related), respectively. Yeast cells lacking Tel1 have short but stable telomeres, while mutation of *MEC1* confers a mild telomere shortening phenotype.[4] Cells lacking both Tel1 and Mec1 have a senescence phenotype similar to that of telomerase-negative strains.[4] Telomeric DNA repeats are bound by Rap1, which in turn recruits Rif1 and Rif2 to the telomeres.[5] Deletion of *RIF1* or *RIF2* leads to elongated telomeres, but further deletion of *TEL1* largely yields telomeres that mimic those in a *tel1*Δ single mutant.[6] Consistent with these genetic results, Rif1 and Rif2 inhibit the localization of Tel1 to telomeres,[7] preventing the further recruitment of telomerase.[8]

We recently found that telomerase-negative strains lacking Rif1 or Rif2 exhibit accelerated senescence.[9] In contrast, deletion of *TEL1* delays the senescence of a telomerase-negative strain.[4] These observations indicate that Rif1, Rif2 and Tel1 have functions outside the regulation of telomerase. One explanation for these observations is that the Rif proteins prevent accelerated senescence by inhibiting Tel1. To test this hypothesis, we assayed senescence of telomerase-negative *rif*Δ *tel1*Δ strains. We sporulated an *est2*Δ/+ *rif1*Δ/+ *rif2*Δ/+ *tel1*Δ/+ diploid and monitored the growth of the telomerase-negative (i.e., *est2*Δ) haploid meiotic progeny in liquid culture by dilution of cells to 5×10^4 cells/ml every 24 h (**Fig. 1**). In this assay, telomerase-negative strains show a progressive loss of viability followed by the appearance of survivors, which restores the growth rate of the cultures.[10] We find that in the absence of telomerase, *rif*Δ mutants senesce after fewer population doublings than *RIF* cells while *tel1*Δ mutants senesce after more population doublings than *TEL1* strains (**Fig. 1**), consistent with previous observations.[4,9] Surprisingly, the effects of the *rif*Δ and *tel1*Δ mutations on the rate of senescence are additive. In the absence of telomerase, a *tel1*Δ deletion delays senescence by approximately 10 generations, which is also true in a *rif1*Δ, *rif2*Δ or double *rif1*Δ *rif2*Δ background (**Fig. 1**). Therefore, although the Rif proteins regulate telomerase-mediated telomere length homeostasis by inhibiting Tel1, the Rif proteins do not prevent accelerated senescence via the inhibition of Tel1.

Interestingly, we find that deletion of *MEC1* partially suppresses the accelerated senescence of telomerase-negative *rif*Δ strains (data not shown). This observation is consistent with previous studies showing that Mec1 is required to induce a cell cycle arrest during senescence.[11] Thus, in the absence of telomerase, the Rif proteins prevent accelerated senescence through the inhibition of Mec1, not Tel1.

Rif1 and Rif2 inhibit the localization of Tel1 to telomeres.[7] The Rif proteins also inhibit 5' exonucleolytic degradation of telomere ends mediated by the MRX complex.[12] MRX-mediated nucleolytic degradation would expose single-stranded DNA (ssDNA) that can become coated with RPA; RPA-ssDNA complexes recruit Mec1/ATR to initiate a DNA damage checkpoint response.[1] We propose that while the Rif inhibition of Tel1 localization to telomeres is important to regulate telomerase-mediated telomere length homeostasis, the Rif inhibition of the MRX complex is important to prevent Mec1-mediated accelerated senescence. In telomerase-negative strains lacking Rif1 and Rif2, senescence is accelerated likely because MRX-mediated telomere uncapping occurs earlier after loss of telomerase. Our model suggests that in the *rif*Δ mutants, the enhanced exonucleolytic degradation triggers a Mec1-dependent DNA damage signal, which helps induce the accelerated senescence.

Our findings uncover a new twist in the relationship between the Rif proteins and the Tel1/ATM kinase, but what is the Rif-independent role of Tel1 in delaying senescence? Tel1 is not required for the telomere checkpoint response in senescing cells.[11] Insight into the function of Tel1 during senescence would be aided by identifying its relevant substrates. It will also

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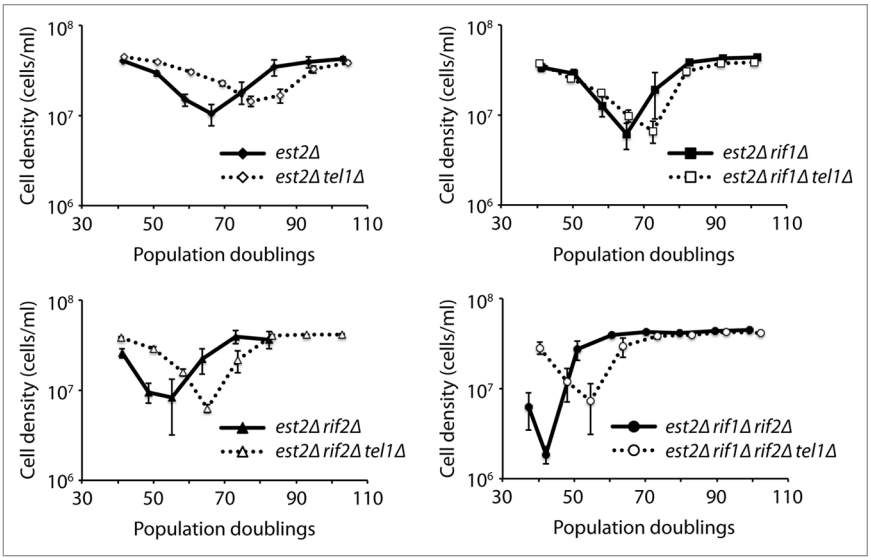

Figure 1. Rif1/2 and Tel1 function in separate pathways during replicative senescence. An *est2*Δ/+ *rif1*Δ/+ *rif2*Δ/+ *tel1*Δ/+ diploid was sporulated and haploid meiotic progeny were isolated. Spores were allowed to form colonies on YPD agar plates after 2 d of growth at 30°C, or approximately 25 population doublings. Cells from these colonies, with the indicated genotypes, were serially passaged in liquid YPD media at 24 h intervals. For each passage, the cell density in each culture was determined and the cultures were diluted back into fresh YPD media at a cell density of 5 x 10^4 cells/ml. The mean cell densities and standard errors of the means for at least three independent spore isolates for each genotype are shown. Similar results were seen starting with a *tlc1*Δ/+ *rif1*Δ/+ *rif2*Δ/+ *tel1*Δ/+ diploid (data not shown). *TLC1* encodes the RNA subunit of telomerase.[10]

be interesting to compare and contrast the telomeric functions of yeast Tel1 and Mec1 with mammalian ATM and ATR, which are also inhibited at telomeres.[1]

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