ABSTRACT

Eukaryotic organisms package their genetic material into linear chromosomes. Telomeres are the ends of linear chromosomes and consist of terminal G-rich repetitive sequences. These structures play a key role in genetic stability protecting chromosome ends from degradation and end-to-end fusions. Telomeres are maintained by the specialized enzyme reverse transcriptase telomerase. The mayority of human somatic cells have telomerase inactivated, thus with every round of replication, telomeres shorten. As chromosome ends shorten, genomic instability increases to a point at which ten cell enters a senescing phase, leading to cell death. Human telomeres are long enough to prevent this senescence during our lifespan. Yet, it has been observed that 90% of all cancer cells switch on telomerase, due to their high rate of cell divisions. Contrary, the other 10% of cancer cells and immortalized cell lines, instead of switching on telomerase, use the alternative lengthening of telomrese pathway (ALT) to maintain their telomeres.

Yeast express telomerase every cell generation. Thus, telomerase can be inactivated *via* gene disruption, generating ALT yeast cells which mimic (to some extent), the process of cell senescence and instability caused by telomere shortening in mammalian somatic ALT cells. Telomerase-deficient yeast enter a senescing phase, causing the death of most of the colony, yet a minor subpopulation of the cells survive. This investigation consists in the analysis of the genetic rearrangements that occur in these post-senescent survivors. These surviving cells take one of two pathways to overcome the resulting senescence: type I cells survive by the amplification of the subtelomeric Y' sequence, while type II survivors arise through addition of long tracts of telomere repeats, similar to human ALT cells.

Assesment of telomere length variation revealed that different *S. cerevisiae* isolates had overall, very homogeneous telomere lengths, while *S. paradoxus* strains had much more

heterogeneous telomeric tracts. The *S. paradoxus* sequenced strain harbouring short telomeres senesced after 60 generations, while a Far East isolate with long telomeres entered senescence after 20 generations. This faster rate of senescence in the Far East isolates is counter-intuitive, as longer telomeres should result in a longer buffer period in the absence of telomerase. Similarly, shorter telomere cells should senesce earlier.

In search for genetic variation in the *Saccharomyces sensu stricto* complex, an *S. paradoxus* Y' deficient strain was found. Disruption of RAD50 (a gene determining one of the ALT recombination pathways) in this strain produced two types of survivors: type I survivors which amplify some subtelomeric sequence other than Y', and type II survivors that are RAD50 independent. A 50/50 ratio was observed between these two survivor types.

Finally, the insertion of a telomere tag into two strains of *S. paradoxus* was achieved. Chromosome XV was tagged in both, the CBS432 strain characterized by short telomeres (400 bp), and the Far East Y' deficient isolate harbouring long telomeres (800 bp). Mitotic propagation indicated that the cells from the Far East strain had added telomeric repeats, while the telomeric ends of the CBS432 strain had shortened. The artificial telomere obtained species-specific length. A single recombination event did not alter telomere length.