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Analysis of Telomere Length and Telomerase Activity in Tree Species of Various Lifespans, and with Age in the Bristlecone Pine *Pinus longaeva*

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ABSTRACT

Normal somatic cells have a finite replicative capacity, and with each cell division telomeres progressively shorten, unless the telomerase enzyme is present. The bristlecone pine, *Pinus longaeva*, is the oldest known living eukaryotic organism, with the oldest on record turning 4770 years old in 2005. The results from our study of telomere length and telomerase activity in samples (needle, root, core) from *P. longaeva* with age, and in other tree species of various lifespans, support the hypothesis that both increased telomere length and telomerase activity may contribute to the increased lifespan and longevity evident in long-lived pine trees (i.e., 2000- to 5000-year lifespan) compared with medium-lived (400- to 500-year lifespan) and short-lived (100- to 200-year lifespan) pine trees, as well as in *P. longaeva* with age.

MATERIALS AND METHODS

Samples (core, needle, and root) were collected with the permission of the United States National Arboretum in Washington, DC on July 25, 2003 from the following age-matched (18-year-old) tree species: The coastal redwood, *Sequoia sempervirens*, and the bristlecone pine, *Pinus aristata* (2000- to 5000-year lifespan: "long-lived trees"); the Western white pine, *Pinus monticola*, and the red pine, *Pinus resinosa* (400- to 500-year lifespan: "medium-lived trees"); and the loblolly pine, *Pinus taeda*, and the longleaf pine, *Pinus palustris* (100- to 200-year lifespan: "short-lived trees"). Samples (core, needle, and root) were collected with the permission of the Inyo National Forest in Bishop, CA on December 11, 2003 from the bristlecone pine *Pinus longaeva* from a total of nine *P. longaeva* trees of various ages (in years): 20 ± 5, 37, 54, 63, 180, 334, 1200 ± 100, 2000 ± 200, and 3500 ± 100. Measurements of telomere length and telomerase activity were performed as described.[1–4]

Telomeres are specialized structures at the physical ends of eukaryotic chromosomes and consist of highly conserved repeated DNA sequences. Telomeres continually shorten with each round of DNA replication because of the inability of DNA polymerase to completely replicate linear DNA molecules,[5] ultimately leading to cell senescence. Elongation of telomeres can occur by the ribonucleoprotein enzyme telomerase, which (in trees) adds tandem hepta-nucleotide repeats (i.e., TTTAGGG) *de novo* to 3′ ends of telomeres using its own RNA as a template.[6] The action of telomerase can compensate for the continual shortening of

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telomeres that would otherwise occur. For eons, the bristlecone pine trees (*Pinus aristata* and *Pinus longaeva*), have flourished in the harsh arid mountains of the Great Basin, which extend from California to Colorado. Collectively, the average age of the bristlecone trees is about 1000 years, with several specimens still alive at well over 4000 years of age. Indeed, the bristlecone pines are among the oldest known and longest-lived organisms on Earth.[7] In 1957, Schulman discovered the oldest living bristlecone pine on record (aptly named "Methuselah"), which turned 4770 years old in 2005. Although the name is Biblical, Methuselah predates Christ by nearly 3000 years and was a little over 200 years old when construction on the Great Pyramid of Giza in Egypt began in approximately 2550 BC.

RESULTS

Both needle and root samples in long-lived trees had higher average telomere lengths of the longest, mean, and shortest telomeres compared with both short- and medium-lived trees (except for the longest in needle samples). Additionally, in needle, root, and core samples, long-lived trees had higher average telomerase activity compared with both short- and medium-lived trees. Both telomere length and telomerase activity directly correlated with expected lifespan of each tree species, such that long-lived trees possessed longer average telomere lengths and exhibited higher telomerase activity compared with both medium- and short-lived trees.[4]

DISCUSSION

The present findings support the hypothesis that both increased telomere length and telomerase activity in long-lived trees, compared with medium- and short-lived trees, may play essential roles that directly or indirectly regulate lifespan in longer-lived tree species. Their increased expected lifespan may be caused by higher levels of telomerase activity, which could act to slow or prevent the attrition of telomeres (at least in needle and root samples, and to a possible lesser extent in core samples), thereby accounting for the presence of longer telomeres in long-lived tree species. Longer telomeres, in turn, may slow or prevent the subsequent entry of the associated cells into senescence (if occurring at all) that may otherwise occur in the absence of such telomerase activity.

In comparing samples collected from the bristlecone pine, *Pinus longaeva*, both telomere length and telomerase activity were found to exhibit a cyclical pattern with age. Importantly, for both telomere length and telomerase activity in both needle and root samples in *P. longaeva*, there was no evidence of overall telomere shortening, or decrease in telomerase activity, with age (up to 3500 years). In addition, telomerase activity was detected in all samples (needle, root, and core) and ages (up to 3500 years) analyzed, suggesting that active telomerase may be present to slow or prevent telomere attrition in the samples analyzed. In core samples, telomerase activity started at a high level and declined gradually with age, yet there is still detectable activity up to 3500 years of age. In both needle and root samples of *P. longaeva*, the pattern of telomere shortening and lengthening that occurred with age closely correlated to the concomitant decrease and increase in telomerase activity over the same time period. This suggests that telomerase activity may be capable of regulating telomere length with age in *P. longaeva*.[4]

Importantly, the current findings provide an exciting glimpse into the realm of tree telomere biology and support the hypothesis that telomere length and telomerase activity may play essential roles that directly or indirectly regulate lifespan and longevity in certain tree species, such as *P. longaeva*. Their long lifespan may result from the stable presence of telomerase activity (detectable in all samples analyzed up to 3500 years), which could act to slow or prevent the attrition of telomeres (at least in needle and root samples up to 3500 years of age, and possibly to a lesser extent in core samples because of the declining levels of telomerase activity evident within core samples with age), and thereby preserve telomere length as well as limit the quantity of critically short telomeres present with age. The absence or low quantity of such short telomeres may, in turn, slow or prevent the subsequent entry of the associated cells into senescence (if occurring at all) that may otherwise occur in the absence of such telomerase activity (and in the presence of critically short telomeres). As a result, if the individual cells within certain areas of a tree (e.g., needle, root, meristem) possess a long lifespan conferred by stable telomerase activity and preservation of telomere length throughout the centuries (as observed in *P. longaeva* with age), this, in turn, may result in the increased longevity of the organism as a whole.[8,9]

CONCLUSION

What secrets do the long-lived bristlecone pines possess that confer such great longevity and what are their limits, if any, in regard to maximum lifespan? These secrets remain hidden for now; however, finding the source of the great longevity evident in bristlecone pines (perhaps through the presence of stable telomerase activity and preservation of telomere length within its cells, especially within meristems, with age) may enable the development of a therapy to afford humans similar longevity (perhaps through preservation of telomere length in somatic cells via endogenous induction or exogenous delivery of telomerase). The implications of studying longevity in long-lived bristlecones is best summarized by Schulman[7] himself: "The capacity of these trees to live so fantastically long may, when we come to understand it fully, perhaps serve as a guidepost on the road to the understanding of longevity in general."

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