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Telomeres, Aging, and Plants: From Weeds to Methuselah – A Mini-Review

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Abstract
The process of aging affects most, if not all, living creatures, from single celled yeast to multi-cellular mammals and plants. The DNA end-replication problem along with the tissue-limited expression of telomerase led to the telomere hypothesis of aging, where limits on cellular proliferation are genetically encoded in the lengths of a cell's telomeres. Support for this hypothesis has been found in several organisms, from worms to mice to humans. While development, and therefore the process of aging, is quite different between plants and animals, telomere biology between these organisms is fundamentally the same. Do telomeres, then, also play the role of a molecular clock in plants? In this review, we explore the current knowledge of the relationship between telomeres and aging in plants in three specific cases: leaf senescence, aging of perennials and seed longevity.

What limits organisms' lifespans and what causes them to deteriorate as they grow old is one of the most fundamental questions of biological research. There is no universal definition that fully encompasses the different aspects of aging across all species. Aging is generally thought of as the time-dependant deterioration of an organism, resulting in an increased susceptibility to disease and environmental stress, age-related physiological changes, reduced fertility and increased mortality. As time marches on, organisms accumulate stochastic damage to DNA, proteins and other macromolecules that, when left unrepaired, impairs important biological functions. Although the rate of accumulation of such damage should be relatively similar across organisms, the broad spectrum of lifespans and rates of senescence apparent among different species [1] predicts that aging is not just 'wear and tear', but that its onset is genetically encoded. Comparative studies and experiments with animal models have hinted at several genetically controlled mechanisms that have a considerable effect on organismal aging and longevity. One group of mechanisms involves cell non-autonomous endocrine signaling that controls growth, energy metabolism and sexual reproduction [2]. These mechanisms likely affect lifespan and aging through regulating programs that optimize the allocation of resources between reproduction, growth and self-maintenance under adverse environmental conditions.

Replicative cellular senescence is another mechanism strongly implicated in organismal aging. This phenomenon was first described by Hayflick and Moorhead [3], who noticed that human fibroblasts undergo a limited number of cell divisions before they irreversibly arrest. Fibroblast proliferation is accompanied by telomere

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Fig. 1. An example of aging in plants. This oak, *Quercus robur*, located in a riparian forest in southern Moravia (Czech Republic), is approximately 280 years old.

shortening due to the end-replication problem (discussed below); this may eventually lead to telomere dysfunction that triggers cellular senescence [4, 5]. The limitation of cell proliferation capacity through insufficient replication of telomeres provides a very attractive model for the age-dependent decline of tissue renewal and organ function [6, 7].

As humans have always sought the fountain of youth, it is understandable that the major concepts in aging research were developed primarily with humans and animals in mind. However, a simple walk through a park or a weekend stroll through the woods makes it clear that plants age as well. Everyone can easily distinguish an old tree from a young one (figure 1). Similar to animals, plants exhibit a wide range of lifespans from a few weeks to as long as millennia. In fact, a bristlecone pine named Methuselah is currently the oldest living individual organism known on earth, at the ripe age of 4,841 years it germinated some 200 years before the construction of the first pyramids in Egypt [8]. The rate of senescence also varies dramatically; it is most notable in monocarpic plants, which undergo abrupt senescence after a single reproductive cycle, and is almost negligible in long-lived trees [9]. Thus, like in animals, the rate of senescence and length of lifespan in plants are species-specific, and therefore genetically determined. This may imply that the concepts and mechanisms underlying aging in animals can also be applied to plants. However, plants have adopted fundamentally different life and developmental strategies, and even seemingly simple terms such as aging, lifespan or individual may sometimes be difficult to clearly define [for excellent reviews on this topic see 10, 11]. In this review, we briefly discuss various aspects of aging in plants and examine whether telomere metabolism may play a role in plant aging and development.

Telomeres and the Telomere Hypothesis of Aging

Eukaryotic chromosomes are formed of a single linear DNA molecule. Breakage of this molecule is a severe threat to the integrity of a chromosome, as it can result in a genetically unstable acentric fragment or inappropriate repair leading to genomic rearrangements. Life in all its forms has evolved sophisticated machinery, involving hundreds of proteins, whose major function is to efficiently recognize and properly repair DNA damage. This machinery also mediates the DNA damage response (DDR) by eliciting transient DNA checkpoints. Severe DNA damage in animal cells can further trigger two specialized cellular programs, apoptosis and cellular senescence [12]. While apoptosis removes damaged cells via programmed cell death, senescent cells irreversibly arrest proliferation, though they remain metabolically active and can stay alive for long periods of time [7, 12].

Natural ends of eukaryotic chromosomes are shielded from being recognized as damaged DNA by telomeres. Telomeres form a specialized heterochromatin that consists of telomeric DNA, a specific set of telomeric DNA binding proteins, DNA repair complexes and general

chromatin factors. Together, these components contribute to the assembly of a protective structure that inhibits natural chromosome termini from triggering a full-blown DDR. Telomeres serve an additional function: their unique mode of synthesis compensates for the inability of conventional DNA polymerases to fully replicate chromosome termini. The unidirectional nature of DNA replication and the requirement for a primer to initiate synthesis predict that chromosome ends will lose a stretch of DNA sequence at every cell division. This end-replication problem is, in most eukaryotes, solved by telomerase, a reverse transcriptase that adds telomeric DNA to the chromosome termini. Telomerase inactivation leads to a replication-dependent attrition of telomeric DNA in a variety of experimental systems including human cells, mouse, yeast, plants and worms [13]. Telomerase is typically down-regulated in most human tissues [14]. Together with the observation that telomere size declines in cultured somatic cells, this provided a basis for the hypothesis that replication-dependent telomere shortening serves as a mechanism limiting cell proliferation capacity [7, 15]. Importantly, ectopic over-expression of telomerase reverse transcriptase (TERT) in cultured cells led to telomere elongation, allowing human fibroblasts to escape replicative senescence [5]. It is now commonly accepted that replicative cellular senescence can be triggered by a DDR initiated at only one or a subset of telomeres that shorten below a critical length insufficient to support the assembly of a fully protective structure. Therefore, maintenance of an average telomere length is not as critically important as maintaining all telomeres above a minimum threshold.

Telomeres have also been proposed to act as a sensor of general DNA damage induced, for example, by oxidative stress [16]. Telomere-binding proteins have an apparent anti-checkpoint function which may limit the capacity of the general DNA repair system to recognize and efficiently repair DNA lesions occurring within telomeric DNA [15]. The sensitivity of telomeres to DNA damage may further be exaggerated by the inherent propensity of the G/C rich telomeric repeats to form secondary structures that impede progression of DNA polymerases. Thus, DNA lesions could preferentially accumulate over time at telomeres, accelerating the loss of telomeric DNA.

The dependency of cellular senescence on elapsed cell divisions raised the question of whether telomere shortening also contributes to organismal aging. This idea gained support through a number of observations that reported an inverse correlation between telomere length and human age, although the extent of telomere loss is variable between cell types and tissues [7, 15]. In addition, some human progeroid syndromes that are characterized by accelerated aging, such as dyskeratosis congenita or Werner's syndrome, are accompanied by deficiencies in telomere maintenance or telomerase activity [15, 17]. The role of telomeres in organismal aging is further supported by experimental studies in animals with altered telomere maintenance. Elongation of telomeres by over-expressing a protein involved in telomere length regulation in *Caenorhabditis elegans* extends the lifespan of the transgenic animals and makes them more resistant to heat stress [18]. In mice deficient in telomerase, progressive shortening of telomeres recapitulated many pathologies associated with human aging [17]. In the reverse experiment, over-expression of TERT in mice delayed aging and extended the lifespan of transgenic animals [19]. The evolutionary benefit of telomerase limitation in most human adult tissues may be explained in terms of antagonistic pleiotropy. While putting limits on cell division provides protection against unchecked proliferation of somatic cells, and therefore cancer, it will eventually impede tissue renewal and regeneration potential and directly contribute to organismal aging [6].

Peculiarities of Plant Aging

Plant development differs fundamentally from animals, and these differences must be taken into consideration when discussing aging and mechanisms associated with age-related changes in plants. Animal lifespan is clearly defined by the survival of a whole body that consists of various organs, most of which are indispensable for life. Animal body plans are established during embryogenesis, and post-embryonic development is usually limited to the maintenance and enlargement of pre-existing structures. In contrast, only a very rudimentary body plan is developed during plant embryogenesis, and virtually all plant structures and organs are formed by the indeterminate proliferation of meristematic cells throughout adult life. Another feature that distinguishes plants from animals is their modular growth. This provides plants with enormous developmental plasticity, allowing them to efficiently explore available environmental niches. Individual modules (root or shoot branches, inflorescences, leaves) are dispensable for the survival of the organism, and their function can be replaced by tissues newly differentiated from meristems. In this regard, plant lifespan is largely defined by the indeterminate growth of vegetative meristems. This particularly applies to iterop-

arous perennial plants that maintain indeterminate growth beyond one reproductive season. This mode of growth, however, blurs clear boundaries defining individual plants, as vegetative meristems can take up their own fate and regenerate into a new organism. Clonal propagation is very frequent in plants and there are examples of entire forests being formed by clonal propagation of a single organism [8]. These groups of clonal plants may, in fact, represent the oldest currently living organisms on earth, with some estimated lifespans on the order of tens of thousands of years [11]. If perennial plants age, this process should be primarily manifested through a systemic cessation of meristematic activity in a whole plant.

Another peculiarity of plant aging is the disparity between cell death and death of the organism. The biomass of trees, for example, consists primarily of dead cells which form a scaffold for a thin layer of newly emerged organs. The most dramatic manifestation of cell death is leaf senescence. While in animals the term senescence usually refers to the deteriorating effects of aging, in plants, senescence refers to a highly regulated physiological process that leads to death at the organ and tissue level [20]. In annual plants, leaf senescence is tightly associated with death of the whole plant. In perennials, leaf senescence occurs multiple times throughout the lifespan, painting the spectacular scenes of colored leaves on autumn trees. Leaf senescence does not represent unregulated decay, but is rather a precisely orchestrated, and at least partially reversible, process that starts with the breakdown of protoplasts followed by degradation of other organelles and cellular structures. The purpose of this catabolic activity is to convert cellular materials into portable nutrients that can be remobilized to support developing seeds or to be stored in sink tissues helping a plant resume growth after adverse conditions have passed [20, 21]. In this respect, senescence cannot be viewed as an age-related process but rather as the last step of leaf development.

Excluding senescence as a sensu stricto aging process and considering the enormous plasticity of plant development that is coupled with a seemingly everlasting capacity of indeterminate growth, one has to ask the question: do plants really age on the organismal level and if yes, how? There are numerous reports documenting that with increasing age and size trees tend to grow more slowly and are more likely to succumb to biotic or abiotic stress. This age-related decline has been associated with reduced photosynthesis, decreased water and nutrient availability, and altered levels of phytohormones [22]. An age-dependent decrease in physiological and reproductive performance and increased mortality has also been observed in herbaceous perennials [23, 24]. These data show that plants also experience age-related declines in fitness akin to what is observed in animals; in short, they also grow old. What, then, are the causes of plant aging and what leads to the dramatic differences in lifespan and onset of aging among plant species? While leaf senescence was thoroughly investigated in well established monocarpic models such as *Arabidopsis* and tobacco [20, 21], there are only a few studies that address the mechanisms of plant aging other than those that are coupled with programmed senescence. Studies based on grafting experiments indicate that age-related changes in the growth of trees are mainly caused by a physiological burden, such as the higher demand for water and nutrients, that is correlated with the increasing size of trees [25, 26]. However, some of the grafting experiments are consistent with the view that cell intrinsic processes, such as accumulation of genome damage and somatic mutations, may also affect meristematic activity of aging plants [27, 28]. Telomeres are, once again, a particularly attractive molecular sensor of aging because of their ability to 'record time' in a chronological as well as in a replication-dependent manner. In the following section we review the current knowledge on telomeres in plants that is relevant to a discussion of aging.

Telomeres and Plant Senescence

Telomeres in most plant species are formed of tandem arrays of TTTAGGG repeats that can range from a few to hundreds of kilobases. These arrays are maintained within a species-specific range by telomerase [for recent reviews on plant telomeres see 29–32]. Strikingly, the expression pattern of telomerase in plants closely resembles the situation in humans. In monocarpic annual plants, telomerase activity appears to correlate with cell proliferation, as the highest activity is detected in meristematic tissues and reproductive organs, while no or low activity is found in endosperm, leaves and stems [33–37]. This raises the obvious question of whether the limited expression of telomerase activity contributes to the onset of senescence in monocarpic plants. Several lines of evidence argue against this. First, the presence of telomerase is not necessary in most fully developed plant tissues, such as leaves and stems, because they largely consist of non-dividing post-mitotic cells. Analysis of tobacco cell cultures revealed that telomerase activity is specifically expressed

during S-phase [38]. Thus, the absence of telomerase in adult plant tissues may simply reflect the very low level of proliferation in these tissues. Secondly, studies in the monocarpic plants *Arabidopsis*, tobacco and white campion indicate that telomeres do not undergo detectable replicative shortening during development [35, 39, 40]. The notable exception is barley, which was reported to exhibit dramatic changes in telomere length in the order of tens of kilobases between different tissues, a change that is unlikely to solely be due to the end-replication problem [41]. The final argument against the role of telomeres in plant senescence is the fact that telomere dysfunction in *Arabidopsis* does not lead to a massive early onset of leaf senescence. In contrast, examination of *Arabidopsis* mutants deficient in the *TERT* gene revealed that plants with critically short telomeres, despite severe developmental defects, remained green longer and had substantially longer lifespans than their wild-type counterparts [42]. This was apparently caused by the abortive sexual reproduction and diminished seed set of the *tert* mutants, as seed production is tightly coupled with leaf senescence in monocarpic plants. Thus, telomeres seem not to play an important role for leaf senescence in monocarpic plants.

Genome Maintenance and Sustained Meristematic Activity in Perennials

In perennials, meristems proliferate for the entire lifespan of the plant, which can be millennia in some long-lived trees. Thus, meristematic cells in perennials may undergo thousands of divisions, a number sufficient to result in a replication-dependent loss of telomeric DNA if telomere maintenance is impaired. In addition, environmental stress and impaired physiological conditions associated with aging may result in increased DNA damage, particularly at telomeres. Plant cells respond to persistent DNA stress, such as telomere dysfunction, oxidative DNA damage and replication stress by losing their competence to divide, which may cause meristem arrest [43]. Replication may also lead to the accumulation of somatic mutations and chromosomal aberrations that would eventually compromise the growth and integrity of meristematic tissues. Could such mechanisms be, at least in part, responsible for the age-dependent growth cessation observed in many perennials? How efficient are telomere maintenance and DNA repair mechanisms in meristems, and is their activity constant or do they respond to physiological and environmental conditions?

There are a limited number of studies that examined telomere dynamics in correlation with age in perennials. Although a comparative study that included six tree species with different lifespans found a positive correlation between telomere length and life expectancy, no age-dependent decline was detected in extremely long-lived bristlecone pines [44]. In fact, telomeres in root samples slightly increased with age. This is in accordance with data indicating that performance of shoot apical meristems in bristle-cone pines does not decline with age [9]. A similar trend of telomere length dynamics was also found in *Ginkgo biloba* trees that ranged from 1 to 1,400 years, where older trees showed a slight increase in telomere length [45, 46]. This is consistent with the ubiquitous expression of telomerase activity that was detected in needles, roots and even in samples taken from the trunks of several gymnosperm trees [44]. These studies demonstrate that average telomere length in long-lived trees can efficiently be maintained during vegetative growth for millennia. Data is almost non-existent on the effects of clonal propagation on telomere length. In perhaps the only study examining this question, tobacco leaves were clonally propagated through two cycles of callus formation followed by regeneration into plants [39]. During these two cycles, there was no significant change in telomere length. However, de-differentiation into callus is not a normal part of the clonal propagation process, and reactivation of telomerase in callus may have compensated for any loss of telomeric DNA during this experiment.

DNA damage could be another cell intrinsic impetus for age-dependent decline in meristem proliferation. The major source of DNA damage in plants are reactive oxygen species produced in mitochondria and chloroplasts, as well as genotoxic environmental agents such as solar UV irradiation [47]. The level of everyday exposure of plant cells to DNA damage may be enormous, as demand for sunlight and photosynthetic activity are defining features of the plant kingdom. Interestingly, however, a recent evolutionary study indicated that the rate of molecular evolution in trees and shrubs with long generation times is slower when compared to related herbaceous plants with shorter generation times [48]. This indicates that somatic mutations that may have occurred during prolonged mitotic growth do not compensate for the effect of generation time on the rate of evolution. One explanation for this observation is that plant meristematic cells possess robust DNA repair and genome maintenance mechanisms. Indeed, transcriptional profiling revealed increased expression of genes involved in chroma-

tin modification, DNA repair and telomere synthesis in the stem cell niche of the *Arabidopsis* shoot apical meristem [49]. Furthermore, meristematic stem cells appear to be particularly sensitive to DNA damage and succumb to cell death even under relatively mild genotoxic treatments [50]. Thus, massive investment in genome maintenance and strict genome quality control mechanisms may be the key rejuvenation strategies of meristematic cells. This raises the question of whether long-lived plants have evolved strategies to more effectively protect their DNA than short-lived species.

Telomeres and Seed Longevity

Seeds represent the dormant form of a plant that, in their fully desiccated state, have very low metabolic activity and an extremely high tolerance to harsh environmental conditions. Efficient seed formation, dissemination and germination are crucial for the reproductive success of most plant species. Many plants can spend a substantial part of their life in the form of a seed, in some cases allowing the plant to survive for centuries or millennia in the dormant stage [51]. Thus, seed longevity should also be considered when discussing plant aging and lifespan. Even if stored under favorable conditions, seed viability declines over time. Loss of seed viability is accompanied by increasing fragmentation of chromosomal DNA in embryos, and one of the earliest events in germination, occurring within minutes after water uptake by the seed embryo, is repair-associated DNA synthesis [52]. It is assumed that most of the DNA damage that occurs during seed aging is a result of reactive oxygen species that are produced during desiccation and prolonged storage.

The first link between telomeres and seed aging was provided by an analysis of telomere structure in wheat embryos from dry seeds [53, 54]. These studies showed a loss of telomeric sequences that was accompanied by an increase in extrachromosomal telomeric DNA as seeds aged. The seed-age-associated fragmentation appeared to be specific to telomeric DNA and not to other repetitive loci. Extrachromosomal telomeric DNA was not genetically stable and rapidly disappeared after germination. Progressive excision of chromosomal telomeric sequences which correlated with decreased germination was also observed during storage of rye seeds [55]. As there is no proliferation activity in quiescent seeds, the loss of telomeric DNA must be replication independent and, thus, cannot simply be due to the end-replication problem. Likely mechanisms involve nucleolytic cleavage or intrachromatid recombination elicited by the accumulation of DNA lesions in telomeric regions. Intrachromatid telomeric recombination may have deleterious consequences for plant viability, and research in *Arabidopsis* suggests that such events are inhibited by the Ku70 and Ku80 DNA repair proteins [56]. Interestingly, seeds of *Arabidopsis* mutants deficient for the Ku70 DNA repair protein are extremely sensitive to DNA damage caused by methyl methanesulfonate, although germinated seedlings show no sensitivity to this genotoxic drug when compared to wild-type plants [57]. Thus, the function of the Ku70 protein may be particularly important in dry seeds, where it might compensate for the increased vulnerability of telomeres to structural damage.

It is currently not clear whether telomere fragmentation is a cause or a consequence of seed aging. Nevertheless, restoration of telomeres at chromosome ends occurs early during seed imbibition or germination. In this regard, it is interesting to mention that low levels of telomerase activity were detected in dry seeds of white campion [35]. Thus, telomerase activity may be an important factor influencing seed viability during long periods of storage. Further studies using mutant plants deficient in various aspects of telomere maintenance may be instrumental in examining the contribution of telomeres to seed aging.

Conclusions

Though the precise definitions may vary, organisms as diverse as yeast and humans eventually age, and plants are no exception. While there is abundant evidence that telomere function plays a role in mammalian aging, experiments designed to specifically address this question in plants remain limited. Despite the lack of data, it is clear that the role telomeres play in plant aging is profoundly different from what is observed in mammals.

One of the most obvious forms of plant aging, leaf senescence in monocarpic species, is almost certainly unrelated to telomere function. However, the definition of aging in this context is quite different from that commonly used in humans. In this case, senescence is clearly the final developmental program of the organ and plays an important function in sexual propagation of the organism. Interestingly, this developmental program can be overridden by over-expressing particular plant hormones [21]. It would be interesting to examine whether telomerase is required for this override, and whether the absence of telom-

erase in this context would lead to a phenotype more similar to that described for mammalian aging.

In the case of perennial plants, the definition of aging more closely resembles that commonly used for mammals. While there is no correlation between telomere length or telomere function and plant age, there are several key experiments that remain untested. While, in the tested cases, average telomere length remains relatively unchanged, data from mammals suggest that a single critically short telomere can lead to a DDR and cellular senescence. Experiments of this type have not yet been conducted in perennials. Quantitative fluorescence in situ hybridization, a cytological method for measuring the length of individual telomeres within a single cell, would provide a valuable tool for addressing whether older plants possess a normal average telomere length but harbor some critically short telomeres that would go unnoticed by conventional Southern blotting. Further, to our knowledge there have been no tests comparing the ability of young and old plants to respond to DNA damage. Finally, most of the experiments testing the relationship between telomere length and age in plants have been conducted in plants with relatively long lifespans. As longer-lived plants appear to have developed mechanisms to reduce their genotoxic load, it would be interesting to repeat these experiments in shorter-lived plants, where the machinery dedicated to the DDR may not be as robust.

Finally, in the case of seed aging, there is stronger evidence suggesting that telomere function may play a role. However, the data gathered to date are largely correlative. Direct tests examining the role of telomeres in the aging process of seeds are becoming more feasible as the catalogue of mutants affecting telomere function in plants grows.

Despite the readily apparent differences between plants and animals, and although even the definitions of what it means to age are different, both types of organisms have species-specific and, therefore, genetically-determined lifespans. Although the function of telomeres in the aging process of plants may not be as important as for humans, the question of how plants age, and even what it means to age, is fundamentally intriguing. At the ripe age of 4,841 years, the bristlecone pine Methuselah has certainly found a fountain of youth that many humans would be excited to discover.

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