Telomere shortening rate predicts species life span

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Telomere shortening to a critical length can trigger aging and shorter life spans in mice and humans by a mechanism that involves induction of a persistent DNA damage response at chromosome ends and loss of cellular viability. However, whether telomere length is a universal determinant of species longevity is not known.

To determine whether telomere shortening can be a single parameter to predict species longevities, here we measured in parallel the telomere length of a wide variety of species (birds and mammals) with very different life spans and body sizes, including mouse (Mus musculus), goat (Capra hircus), Audouin’s gull (Larus audouinii), reindeer (Rangifer tarandus), griffon vulture (Gyps fulvus), bottleneck dolphin (Tursiops truncatus), American flamingo (Phoenicopterus ruber), and Sumatran elephant (Elephas maximus sumatranus). We found that the telomere shortening rate, but not the initial telomere length alone, is a powerful predictor of species life span. These results support the notion that critical telomere shortening and the consequent onset of telomeric DNA damage and cellular senescence are a general determinant of species life span.

Humans have relatively short telomere lengths from 5 to 15 kb (1–3), and yet humans have much longer life spans than mice, which can start with telomere lengths around 50 kb (4, 5). Previous studies have suggested that the telomere shortening rate rather than the initial telomere length is the critical variable that determines species life span (4, 6–10). In particular, we have previously shown that human telomeres shorten at a rate of ∼70 bp per y (1), which is in line with the rate published by other authors (3, 11–14), while mice telomeres shorten at a rate of 7,000 bp per y (4). These different rates of telomere shortening between human and mice could explain the different longevities of mice and humans. However, the telomere shortening rate has been investigated to date in few species (4, 6–10, 15, 16), and using different techniques, which has prevented side-by-side comparisons of telomere shortening rates in phylogenetically distant species with different body sizes and life spans.

Here, to address whether telomere length and/or telomere shortening rates could explain species longevity, we measured telomere length in peripheral blood mononuclear cells from individuals of different species of birds and mammals at different ages in parallel, and calculated the telomere shortening rate per year in each species. A longitudinal study of telomere length was not considered here owing to the very different longevities of the species included in this study. Future studies warrant this approach to the telomere shortening rates in these species.

We next investigated relationships between telomere length, telomere shortening rate, and species life span. For the species maximum life span, we used the AnAge database (20). The average life spans were obtained from various sources (SI Appendix, Table S1). First, we did not find any correlation between the telomere | life span | species

<table>
<thead>
<tr>
<th>Species</th>
<th>Telomere shortening rate (bp per y)</th>
<th>Initial telomere length (kb)</th>
<th>Life span (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottlenose dolphin</td>
<td>105</td>
<td>19.8</td>
<td>80</td>
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<tr>
<td>American flamingo</td>
<td>21.0</td>
<td>19.8</td>
<td>80</td>
</tr>
<tr>
<td>Sumatran elephant</td>
<td>100</td>
<td>19.8</td>
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Significance

The exact causes of aging are still not understood, and it is unclear why some species live less than 1 d, while others can live more than 400 y. Research suggests that telomeres are related to the aging process, but a clear relationship between the life span of a species and initial telomere length has not been observed. Here, we measure the telomere lengths of a variety of different species. We find that, in fact, there is no strong correlation between the life span of a species and initial telomere length. However, we find a strong correlation between the telomere shortening rate and the life span of a species.
estimated initial telomere length and species longevity (Fig. 2A–D). In particular, a graph of the species maximum life span versus the estimated initial telomere length resulted in an \( R^2 \) value of 0.0190 with a linear regression curve (Fig. 2A), and an \( R^2 \) value of 0.0407 with a power law regression curve (Fig. 2B). A graph of the species’ average life span versus the estimated initial telomere length resulted in an \( R^2 \) value of 0.125 with a linear regression curve (Fig. 2C), and an \( R^2 \) value of 0.145 with a power law regression curve (Fig. 2D). Note that there is even a trend for shorter life spans with longer initial telomere lengths with the low \( R^2 \) values just mentioned, and negative slopes in the regression line equations (Fig. 2A–D). Also note that the inverse correlation between average life span and initial telomere length (\( R^2 = 0.145 \); Fig. 2C) was better than that between maximum life span and initial telomere length (\( R^2 = 0.019 \); Fig. 2A). These findings agree with a previous study that compared telomere length in more than 60 different species (21). Although the telomere shortening rate was not measured in that study, the authors concluded that the life span of a species could not be predicted from the initial telomere length and that there was a trend for short-lived species to have longer telomeres (21).

Interestingly, when we plotted maximum life span versus the rate of telomere shortening for the different species, we obtained a power law curve with an \( R^2 \) value of 0.829 (Fig. 2E). The equation from this curve can be used to predict the life span of a species when given the telomere shortening rate without using any information about the initial telomere length with an \( R^2 \) value of 0.782 (Fig. 2F). The same graphs can be made using the average life span instead of the maximum life span (Fig. 2G and H), and in this case the power law curve \( R^2 \) value is 0.934. The observation that life span versus telomere shortening rate fits a power law curve is in agreement with many natural phenomena fitting either a power law or exponential curve such as population growth, temperature cooling/heating, city sizes, species extinction, body mass, individual incomes, and the number of connections to nodes in a scale-free network, among others (22–25).

Alternatively, more linear life span predictions can be made using both the initial telomere length and the rate of telomere shortening. In this case, it seems unlikely that species die when their telomeres are completely eroded since the life spans predicted by complete telomere erosion are longer than the observed life spans for most species (SI Appendix, Table S1). Instead, we find here that...
the length of the telomeres when species die at the age of the maximum life span appears to be \(\sim 50\%\) of the original telomere length for that particular species, when considering the average of all of the species measured (SI Appendix, Table S2). Interestingly, when considering the timepoint of the average life span, the telomere length appears to be \(\sim 75\%\) of the original length (SI Appendix, Table S2). Therefore, we can calculate the life span of a species if we assume that telomeres shorten with a constant linear rate and that the time of death will occur once the telomeres have shortened to 50% or 75% of the original telomere length. The equation of the estimated life span if telomeres shortened to 50% of the original length is as follows: 

\[
\text{Estimated life span} = \left( \frac{\text{Initial telomere length} - \text{Initial telomere length} \times 0.5}{\text{Telomere shortening rate}} \right)
\]

A plot of the estimated life span at 50% original telomere length vs. the maximum life span yields an \(R^2\) of 0.565 (Fig. 3A). The estimated life span at 50% original telomere length vs. the average life span yields an \(R^2\) of 0.694 (Fig. 3B). Similar graphs are presented for 75% original telomere length (Fig. 3C and D). With this dataset, the
Although the weight with an average life span provides the most accurate results with an $R^2$ of 0.694. Although the $R^2$ value is the same as the value in Fig. 3B, this graph also has a slope that is closer to a value of 1, indicating a smaller shift between the actual and estimated life spans. Note that better correlation coefficients are obtained with the power law regression curves using the telomere shortening rate without taking the initial telomere length into consideration (Fig. 2 E–H).

Another trait that correlates with life span is body mass (26). In general, larger species such as elephants and whales have longer life spans than small species such as mice and rabbits. One investigation compared the mass and life span of 1,456 different species, and found a trend for longer life span with larger mass ($R^2 = 0.397$) (26). With the species in our dataset, we also observed a correlation between mass and life span (SI Appendix, Table S3).

The species telomere shortening rate also correlated with body weight with an $R^2$ of 0.413 (SI Appendix, Fig. S1). Species with higher body weights tend to have lower telomere shortening rates and longer life spans.

Some authors have shown an inverse correlation between life span and heart rate, a variable related to organismal metabolism (27, 28), although more extensive studies do not seem to support this notion (29). Here, we set to address a potential correlation between heart rate and telomere length. First, we observed a correlation between life span and heart rate with our dataset (SI Appendix, Table S3). We also found a linear correlation between the telomere shortening rate and the heart rate with an $R^2$ of 0.974 (SI Appendix, Fig. S2 A and B).

Next, to investigate the effect of the multiple variables on life span when combined into the same model, we performed a multivariate linear regression. The input variables of telomere shortening rate, initial telomere length, body mass, and heart rate were fit to either the average life span or the maximum life span. The data used for the regression are presented in SI Appendix, Table S4. The log value of all of the data points was used for the regression

Fig. 3. Species life span predictions with telomere parameters II. (A) The estimated life span if telomeres shortened to 50% of the original length vs. the maximum life span. (B) The estimated life span if telomeres shortened to 75% of the original length vs. the average life span. (C) The estimated life span if telomeres shortened to 50% of the original length vs. the maximum life span. (D) The estimated life span if telomeres shortened to 75% of the original length vs. the average life span. The estimated life span is calculated using the following equation: $(\text{Initial telomere length} - (\text{Initial telomere length} \times 0.50)) / \text{Telomere shortening rate}$. (E) Graphical illustration which shows the main finding from this paper, which is that faster telomere shortening rates result in shorter species life spans.
instead of the original values. Each variable vs. the average life span or maximum life span had either a higher linear $R^2$ correlation coefficient when using log-transformed data, or there was no noticeable change in the correlation coefficient in the case of the initial telomere length variable. The model fit to the average life span resulted in an $R^2$ value of 0.997 and an adjusted $R^2$ value of 0.992 (SI Appendix, Table S5), demonstrating that these variables can predict the average life span. The $P$ values (listed in the Pr$(> |t|)$ column) were statistically significant for all variables. The telomere shortening rate was the most statistically significant variable ($P = 0.000422$). The model fit to the maximum life span resulted in an $R^2$ value of 0.950 and an adjusted $R^2$ value of 0.884 (SI Appendix, Table S6), demonstrating that the variables can also predict the maximum life span. In this case, only the telomere shortening rate variable was statistically significant ($P = 0.0218$).

Again, we found an inverse relation between average life span and initial telomere length with a $P$ value of $P = 0.0302$, with short-lived species having initial longer telomeres (SI Appendix, Table S5). Also, in the multivariate analysis, the relationship between the initial telomere length and the maximum life span was not significant in agreement with a weaker inverse correlation between initial telomere length and maximum life span compared with average life span (Fig. 2A and C). Thus, these findings confirm that the telomere shortening rate (negative correlation), initial telomere length (negative correlation), body weight (positive correlation), and heart rate (negative correlation) are significantly associated with the species' life span and that among these variables, the variable with the greatest power to predict the life span is the telomere shortening rate.

Finally, one caveat of studies with animals of different ages is that an effect can occur in which old animals with short telomeres selectively disappear due to death, and these telomeres are consequently not measured at older ages. Therefore, the telomere length could be artificially high at the older ages since only the animals with longer telomeres continue to survive at these ages. However, the fact that telomere shortening with age fits a linear regression in the majority of the species studied indicated that this phenomenon is not very distorting in our current study. Also, such disappearance of animals would only be expected to occur at very late ages, and the majority of the animals in this study were not extremely old (Methods).

Conclusions

Although a number of previous studies measured telomere length in different species (30–35), few of them determined the telomere shortening rates (4, 6–10, 15, 16). In this regard, some studies showed a relation between telomere shortening rates and species' life spans, including previous work from our group in mice and humans (1, 4, 6–10); however, these studies did not compare side-by-side telomere shortening rates in phylogenetically distant species by using a single technique to measure telomeres.

In our current study, telomere length and the rate of telomere shortening from multiple species with very different life spans, including birds and mammals, was acquired in the same laboratory by using the sensitive HT Q-FISH technique which allows to determine absolute telomere length values in units of base pairs as well as individual telomere signals. A limitation of the current study is, however, the few available individuals for some species.

The results shown here indicate that the telomere shortening rate of a species can be used to predict the life span of that species, at least with the current dataset (Fig. 3E). We observed that mean telomere length at birth does not correlate with species life span since many short-lived species had very long telomeres, and long-lived species had very short telomeres. Future studies warrant determination of telomere shortening rate in species such as the naked-mole rat or the bat, which do not match their predicted life span well according to their body size (26, 36).

Finally, the fact that the rate of telomere shortening can be used to predict life span suggests that the cellular effects induced by short telomeres, such as cellular senescence, may be the critical factor determining species longevity. In this regard, some studies correlate DNA repair ability to species longevity (37–39). In particular, the ability to repair UV-induced damage positively correlates with life span in different species, including primates (37, 38). Also, DNA repair rates are higher in longer living rodent species compared with rodent species with a shorter life span (39). It is interesting to note that short telomeres induce DNA damage, and in turn certain types of DNA damage, such as UV irradiation or oxidative stress, can also lead to telomere shortening (40–42).
plateucher with TBST (TBS [Tris-buffered saline, pH 7.0] with 0.08% Tween 20). Next, the plate was washed 1 x 5 min with a plate usher with TBST containing 1 g/μl DAPI (4′,6-diamidino-2-phenylindole, dihydrochloride; Life Technologies; catalog no. D-1306) to stain the nuclei. Then the plate was washed 1 x 5 min PBS and 50 μl of Mowiol solution (10 g of Mowiol [polyvinyl alcohol; Calbiochem; catalog no. 475904], 25 ml of 85% glycerol, 25 ml of H2O, 12 ml of 0.2 M Tris HCl, pH 8.5, and 2.5% [wt/vol] DABCO [1,4-diazaoctaoctane; Sigma-Aldrich; catalog no. D27802-25G]) was added. Plates were then sealed with aluminum foil lids (Beckman Coulter; catalog no. 338619) and stored at 4 °C in the dark. The plates were then processed by HT microscopy, as described in HT Microscopy, within 48 h.

**HT Microscopy.** Images were acquired on an Opera High Content Screening System (PerkinElmer) equipped with a UV lamp, 561-nm laser, and a 40×/0.9 N.A. water-immersion objective. Images were analyzed with Acapella Image analysis software (PerkinElmer). Data were analyzed with Microsoft Excel (Microsoft). Telomere fluorescence values were converted into kilobases by external calibration with the CCRF-CEM (7.5 kb), LS178Y-S (10.2 kb), and LS178Y-R (7.97 kb) cell lines (43, 44).

**Abundance of Very Old Individuals in Different Species.** We defined very old as the age above the value of 70% of the maximum life span for each species. For humans, this would correspond to an age of 122.5 ± 0.7 ± 73.5 y old. In our study, the number of old individuals (age greater than 70% of the maximum life span) spanned for each species is as follows: 0/7 (0%) for mice, 38/3 (37.5%) for dolphin, 0/15 (0%) for goat, 0/8 (0%) for reindeer, 0/16 (0%) for American flamingo, 0/6 (0%) for griffon vulture, 3/21 (14.3%) for Audouin’s seagull, and 0/4 (0%) for Sumatran elephant.

**Data Analysis.** Graphs were created and data analysis was performed in Microsoft Excel. Multivariate linear regression was performed in the R statistics software (45).

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