

# Telomerase Expression Is Differentially Regulated in Birds of Differing Life Span

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**ABSTRACT:** Cellular senescence caused by telomere shortening has been suggested as one potential causal agent of aging. In some tissues, telomeres are maintained by telomerase; however, telomerase promotes tumor formation, suggesting a trade-off between aging and cancer. We predicted that telomerase activity should vary directly with life span. We determined telomerase activity in bone marrow in cross-sectional samples from two short-lived bird species and two long-lived bird species. The two short-lived species had high telomerase activity as hatchlings but showed a sharp downregulation in both the young and old adults, whereas the two long-lived species had relatively high telomerase activity in bone marrow that did not decrease with age. In zebra finches, the age-related change in telomerase activity varied in different tissues. Telomerase activity increased late in life in skeletal muscle, liver, and gonad, but not in blood or bone marrow.

**KEYWORDS:** telomerase; life span; bird; aging; telomere

One cannot consider the wide variation in life spans of different species without reflecting on the underlying physiological and molecular mechanisms. One potential mechanism is telomere regulation. Cellular replicative senescence caused by the shortening of telomeres has been suggested as a causal agent of aging and age-related diseases.<sup>1</sup> Organismal aging is normally accompanied by telomere shortening, which has been shown in several species including birds and mammals.<sup>2</sup> In some tissues, telomeric repeats are maintained by telomerase, a ribonucleoprotein capable of maintaining telomeres.<sup>3</sup> Expressing telomerase is associated with a cost, however, because its presence promotes tumor formation.<sup>4</sup> The association between aging and cancer suggests a trade-off. Downregulation of telomerase and the subsequent ero-

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sion of telomeres with age protects organisms by decreasing the possibility of runaway proliferation and tumor formation. This mechanism acts as an antagonistic pleiotropy, affording beneficial effects early in life, but eventually contributing to senescence.<sup>5</sup>

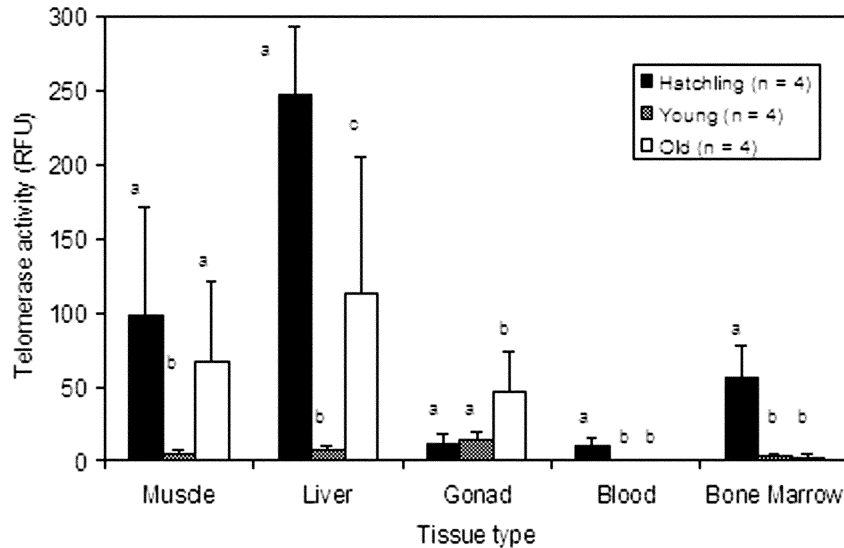
We have shown that birds and mammals with longer life spans lose telomeric repeats slower than species with shorter life spans.<sup>2</sup> This suggests that telomerase expression has been adjusted through natural selection to alter the rate at which telomeres shorten and thereby contribute to life span modification. We predict that evolutionary strategies in telomerase activity may vary with life span. Species with a short life span and rapid rate of telomere shortening due to lack of telomerase expression should be protected from tumor formation until the end of life. At this point, critically short telomeres may be rescued by forced telomerase expression, which leads to an increased incidence of cancer. Species with long life span and a slow rate of telomere shortening due to tightly regulated expression of telomerase should be at risk for tumor formation throughout life but have delayed cellular replicative senescence compared with short-lived species.

To initiate a test of these predictions, we determined telomerase activity in cross-sectional samples from zebra finches (*Taeniopygia guttata*), tree swallows (*Tachycineta bicolor*), common terns (*Sterna hirundo*), and Leach's storm-petrels (*Oceanodroma leucorhoa*). The reported maximum life spans in the wild for these species range from 5 to 36 years and includes two relatively short-lived species, zebra finch (5 years) and tree swallow (11 years), and two relatively long-lived species common tern (27 years) and Leach's storm petrel (36 years). Within each species, telomerase activity was measured in the bone marrow of hatchlings, young adults at first breeding (at ~10% of maximum life span), and old adults (at ~60% of maximum life span). In zebra finches, telomerase activity also was measured in blood, muscle, liver, and gonadal tissue to determine if telomerase expression was variable between tissues and at different ages.

Birds were euthanized and tissues were immediately snap-frozen in liquid nitrogen. Tissues were ground into cell lysates and their protein concentrations were determined by Bradford assay. Telomerase activity was detected using the PCR-based TRAPeze XL Telomerase Detection Kit (Intergen Company, Norcross, GA). The yield of the PCR was determined by measuring the fluorescence of the reaction in a spectrofluorometer.

Telomerase profiles in bone marrow varied with life span. The two short-lived species had high telomerase activity as hatchlings but showed a sharp downregulation in both the young and old adults (zebra finches  $F_{2,9} = 23.80$ ,  $P = .0003$ ; tree swallows  $F_{2,7} = 6.89$ ,  $P = .02$ ). The two long-lived species had relatively high telomerase activity in bone marrow that did not decrease with age (common terns  $F_{2,7} = 0.40$ ,  $P = .69$ ; Leach's storm-petrels  $F_{2,9} = 1.97$ ,  $P = .20$ ). In the various zebra finch tissues, telomerase activity varied with age ( $F_{2,56} = 8.1056$ ,  $P = .0008$ ). Telomerase activity in blood and bone marrow was relatively high in hatchling birds and then decreased in young and old adults (FIG. 1). In contrast, whereas telomerase activity in muscle, liver, and gonadal tissue was relatively low in young adults, it increased in old birds (FIG. 1).

These results are in agreement with our predictions for short- and long-lived organisms outlined above. In the long-lived common tern and Leach's storm-petrel, telomerase is expressed in bone marrow throughout life and telomeres of blood cells



**FIGURE 1.** Telomerase activity in muscle, liver, gonad, blood, and bone marrow of hatchling, mature, and old zebra finches measured in relative fluorescence units (RFU). Telomerase activity within each tissue varied with age (ANOVA,  $P < .0001$ ). Superscripts that differ denote significant differences within a tissue type (Student's  $t$  test,  $P < .05$ ).

shorten very slowly or not at all.<sup>2</sup> How these birds express telomerase throughout life and appear to avoid its tumor-promoting tendencies is of great interest. In contrast, in the short-lived zebra finch and tree swallow, telomerase expression in bone marrow is reduced after fledging, possibly to deter tumor production and blood cell telomeres shorten relatively quickly.<sup>2</sup> In young adult zebra finch, there was a sharp downregulation of telomerase activity in all tissues except gonad, but in old birds, telomerase expression increased in muscle, liver, and gonad. This upregulation may serve to rescue tissues with critically short telomeres, although it is also possible that this activity may indicate neoplastic tissue. It is interesting that telomerase activity does not increase in the bone marrow of old birds, and this may be caused by high tumor susceptibility in this tissue.

The pattern of telomerase expression is variable among species, in different tissues and at different developmental time points. In the chicken, telomerase activity was detected in early embryos and in all tissues throughout organogenesis. Subsequently, telomerase was downregulated in the majority of somatic tissues.<sup>6</sup> Chickens are a short-lived species that also display our short life span strategy in telomere expression.

Some of the findings in this study challenge current opinion.<sup>7</sup> Telomerase is thought to be highly expressed in almost all stem cells but was low in bone marrow of adult zebra finches and tree swallows. Telomerase should be very low or absent

in normal differentiated tissues, but increased in zebra finch muscle, liver, and gonad late in life. Telomerase also should gradually decrease with age, but its activity in bone marrow remained constant at all age classes in common terns and Leach's storm-petrels.

Long-lived bird species lose telomeres at a slower rate than short-lived birds,<sup>2</sup> and this may be caused by increased telomerase expression throughout life. Telomerase's ability to reduce telomere loss by elongating telomeres *de novo* is thought to extend the proliferative life span of cells,<sup>8</sup> and forced telomerase expression in normal human cells extends their proliferative life span.<sup>9</sup> Whether extended proliferative life span of cells translates into extended organismal life span is unknown at this time, and a link between cellular telomere length and organismal health has been proposed only recently.<sup>10</sup>

The major finding of this study is that telomerase profiles in bone marrow differ in short- and long-lived bird species. Whereas two short-lived species downregulated telomerase activity after neonatal life, two very long-lived species express telomerase at the same level throughout life. These results raise other interesting questions. Are these telomerase–life span relationships found in other taxa? How does increased telomerase activity throughout life influence life span, and how do the bird species that express telomerase throughout life avoid its oncogenic costs? These natural animal models for longevity deserve greater study for strategies and mechanisms that have evolved to delay the effects of aging.

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#### REFERENCES

1. VAZIRI, H., I. SCHACHTER, L. UCHIDA, *et al.* 1993. Loss of telomeric DNA during aging of normal and trisomy 21 human lymphocytes. *Am. J. Hum. Genet.* **52**: 661–667.
2. HAUSSMANN, M.F., D.W. WINKLER, K.M. O'REILLY, *et al.* 2003. Telomeres shorten more slowly in long-lived birds and mammals than in short-lived ones. *Proc. R. Soc. Lond. [Biol.]* **270**: 1387–1392.
3. GREIDER, C.W. & E. BLACKBURN. 1985. Identification of a specific telomere terminal transferase activity in *Tertahymena* extracts. *Cell* **43**: 405–413.
4. HARLEY, C.B., N.W. KIM, K.R. PROWSE, *et al.* 1994. Telomerase, cell immortality, and cancer. *Cold Spring Harb. Symp. Quant. Biol.* **59**: 307–315.
5. WEINSTEIN, B.S. & D. CISZEK. 2002. The reserve-capacity hypothesis: evolutionary origins and modern implications of the trade-off between tumor-suppression and tissue-repair. *Exp. Gerontol.* **37**: 615–627.
6. TAYLOR, H.A. & M.E. DELANY. 2000. Ontogeny of telomerase in chicken: impact of downregulation on pre- and postnatal telomere length *in vivo*. *Dev. Growth Differ.* **42**: 613–621.

7. ARAGONA, M., R. MAISANO, S. PANETTA, *et al.* 2000. Telomere length maintenance in aging and carcinogenesis (review). *Int. J. Oncol.* **17**: 981–989.
8. ENGELHARDT, M., R. KUMAR, J. ALBANELL, *et al.* 1997. Telomerase regulation, cell cycle, and telomere stability in primitive hematopoietic cells. *Blood* **90**: 182–193.
9. BODNAR, A.G., M. OUELLETTE, M. FROLKIS, *et al.* 1998. Extension of life-span by introduction of telomerase into normal human cells. *Science* **279**: 349–352.
10. CAWTHON, R.M., K.R. SMITH, E. O'BRIEN, *et al.* 2003. Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* **361**: 393–395.