

Review



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Experimental manipulation of telomere length: does it reveal a corner-stone role for telomerase in the natural variability of individual fitness?

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Telomeres, the non-coding ends of linear chromosomes, are thought to be an important mechanism of individual variability in performance. Research suggests that longer telomeres are indicative of better health and increased fitness; however, many of these data are correlational and whether these effects are causal are poorly understood. Experimental tests are emerging in medical and laboratory-based studies, but these types of experiments are rare in natural populations, which precludes conclusions at an evolutionary level. At the crossroads between telomere length and fitness is telomerase, an enzyme that can lengthen telomeres. Experimental modulation of telomerase activity is a powerful tool to manipulate telomere length, and to look at the covariation of telomerase, telomeres and individual life-history traits. Here, we review studies that manipulate telomerase activity in laboratory conditions and emphasize the associated physiological and fitness consequences. We then discuss how telomerase's impact on ageing may go beyond telomere maintenance. Based on this overview, we then propose several research avenues for future studies to explore how individual variability in health, reproduction and survival may have coevolved with different patterns of telomerase activity and expression. Such knowledge is of prime importance to fully understand the role that telomere dynamics play in the evolution of animal ageing.

This article is part of the theme issue 'Understanding diversity in telomere dynamics'.

1. Introduction

Whether telomere length is correlated to proxies of individual performance (from organismal health to individual survivorship) is presently a hot topic in evolutionary biology. The question is well discussed in the literature with studies reporting both the presence and the absence of a correlation [1,2]. However, a key step for future studies will be to establish whether the relationships, if any, are causal between telomere length and fitness. An early revelation about telomeres was that telomere loss due to the end-replication problem can be balanced by the addition of telomere sequences onto chromosomes by the enzyme telomerase [3]. Most of our current knowledge about telomerase comes from genetically modified laboratory models studied in a medical context [4,5]. Although telomerase has been reported as one modulator of cellular senescence and potentially of healthy ageing (i.e. sustained capacity to adapt to environmental challenges with age), how telomerase affects organismal ageing is still not completely understood. For instance, some cell and physiological changes observed when telomerase gene expression is experimentally modified are not associated with telomere lengthening [6]. Importantly, in wild species almost nothing is presently known about the link between telomerase and fitness-related traits and few

studies have tried to assess how experimentally manipulated telomerase activity may modulate individual performance and health (see electronic supplementary material, S1). Telomerase has a long ancestral history and was discovered in protozoans [7,8]. The universal presence of telomerase or telomerase-like activity (i.e. reverse transcriptase) among eukaryotes, and the recent rise of studies experimentally modulating telomerase activity in laboratory models have triggered a large interest across biomedical and evolutionary research fields. This opens interesting avenues of research to address very specific questions of key importance in our understanding of evolution and variability in individual ageing: (i) Do genetic and environmentally derived differences in telomerase activity exist among individuals? (ii) Is variation in telomerase activity related to fitness? and (iii) Does telomerase affect other cellular processes beyond telomere maintenance? Hereafter, we present current knowledge about how telomerase activity can be assessed (electronic supplementary material, S2), about the nature of experiments used to modulate telomerase (electronic supplementary material, S3) and about how telomerase may enhance certain physiological pathways. Our specific aim here is to inspire future experimental designs that manipulate telomerase and address its role in trade-offs where body maintenance and ageing are balanced with investment in costly life-history traits.

2. Telomerase and its role in anti-ageing through telomere maintenance

Telomerase is a ribonucleoprotein composed of an RNA template domain (TERC) and a reverse transcriptase catalytic protein (TERT). Telomerase is principally active in very early life and is maintained over adulthood mostly in germline, in stem and in some immune cells in humans [9], but this is not a general pattern across species [9,10]. The best-characterized constraint of maintaining high levels of telomerase activity is the risk of cell immortalization and cancer incidence (to date, there do not appear to be energetic costs to increasing telomerase activity, e.g. [11]). Thus, its crucial role in ageing and cancer, and the potential intervention of both (reviewed in [12]), drives general interest in telomerase-related questions. Because of its essential role in tissue renewal capacity, telomerase activity may be a promising therapeutic target for prolonging lifespan [13]. In particular, experimental telomerase activation may restore ever-shortening telomeres and allow a reduction to the pool of senescent and dysfunctional cells [14]. Seminal studies showing the beneficial impact of experimentally increased telomerase activity within normal human cells occurred 20 years ago [15]. The proof of a beneficial health impact of telomerase engineering at the whole organism level was first shown in genetically modified mouse strains [16], extending lifespan by up to 40%. Most of these rejuvenating properties of telomerase were obtained in transgenic mice that constitutively expressed telomerase subunits via virus-mediated induction [17,18]. Despite the need to balance these telomerase-based interventions with pro-tumorigenic pathways [12], telomerase research has aroused substantial medical interest, notably through the utilization of therapeutic molecules (electronic supplementary material, S3) that trigger telomerase activity either for disease treatment or as a preventive medical strategy to prolong a healthy lifespan.

(a) Does activating telomerase specifically impact immune function?

The immune system serves as a particularly attractive candidate for exploring the consequences of telomerase activation, for several reasons. Lymphoid immune cells are a highly proliferative tissue that can be easily and non-destructively sampled in blood. Furthermore, immune protection is intricately linked to fitness and immunosenescence is an important mechanism of ageing (e.g. [19]). Accordingly, short telomeres in leucocytes or defects in telomerase activity are associated with immune dysfunction (reviewed in [20]). Although a recent study failed to confirm the reliability of white cell telomere length as an indicator of the age-related decline of immune activity [19], other studies have shown that the immune system benefits from up-regulated telomerase activity. For instance, *in vivo* maturation of naive T cells into memory T cells is accompanied by telomere loss, but in the same cells short telomeres trigger senescence *in vitro* [21]. This suggests that a fine-tuned control of telomere loss is important to preserve an optimal functioning of lymphoid-based immunity, and the fact that telomerase activation rescues T-cell telomere length, prolongs their proliferation capacity and reduces the number of circulating senescent T cells is of central interest [22,23]. Therefore, the degree of telomerase activity in T cells may be one key regulating factor maintaining their responsiveness via high replicative potential, besides the reduction of auto-immune damage and tumour development [20]. The observation that T-cell telomere length is highly variable in humans is even more interesting in an evolutionary context [24]. Whether such natural variation exists in other species and may be based, at least partly, on the level of telomerase activity is a promising hypothesis to explore in wild animals (see below).

(b) The old-age-specific impact of telomerase activation: a key point for evolutionary biologists?

An interesting observation is that experimental triggering of telomerase activity in adult and old mice delays ageing without increasing cancer risks [25]. For evolutionary biologists, a late-life beneficial effect of telomerase is particularly interesting in relation to senescence. Senescence is defined as the reduction of reproductive success and survival rates with age. Several studies have highlighted an inverted U-shaped curve in fitness proxies with age, such as foraging efficiency in animals living under natural conditions (e.g. [26]). However, the mechanistic explanations of such a decrease in performances do not always match with a deterioration of physiological proxies (e.g. immunity, hormones, oxidative stress; [26]). Therefore, our understanding of the origin of individual differences in fitness depends, in part, on the onset of senescence at different ages within a population. As mice that benefit from an experimental rise in telomerase activity display higher metabolic responses (e.g. to glucose challenge), improved physical performance and even higher cognitive ability [27], we may find that natural among-individuals variability in telomerase expression is one important determinant of individual fitness. This may also be the case if telomerase is triggered during or after a stressful event [28], enabling the organism to restore homeostasis, or during a key period of the species' natural cycle like moult [29]. Future studies using long-term records should relate longitudinal variation in telomerase expression or activity profiles to individual variance in lifetime

reproduction success and health status. For instance, telomerase reactivation in mice eliminated degenerative phenotypes in organs such as testes [30], suggesting that fertility could be maintained in old individuals thanks to preserved telomerase activity in old age. A similar observation could be made for cognitive-dependent impacts on fitness, because restoration of telomerase activity induced neurogenesis and preserved cognitive abilities in mice [17,30].

3. Beyond the telomere-maintenance role of telomerase

Telomerase may influence organismal performance through effects on telomeres, via other pathways or both. Beyond its ability to lengthen telomeres, telomerase has also been shown to have several other effects. For example, it is generally recognized that elevated telomerase activity is associated with cell growth promotion and cell protective effects [31]. However, telomerase expression has also been found to occur without concurrent notable enzymatic activity. For instance, tissue localizations of the protein and RNA components of telomerase (i.e. telomerase gene expression) do not always match detectable levels of telomerase activity [32], and telomerase activity is often low in adult somatic stem cells although TERC and TERT subunits are expressed [33]. Therefore, it is questionable whether the main mechanism that confers the exceptional proliferative capacities of stem cells is more related to the expression of those telomerase components than to the maintenance of telomere length itself. In other words, these results seem to suggest that TERT or TERC impacts cell functions despite the fact that telomerase activity is undetectable. This question applies also to our understanding of the influence of telomerase on individual fitness, opening unexplored avenues of research to distinguish between telomere-related and non-telomere-related effects of telomerase.

(a) Non-telomere-related cell renewal control by TERT

The origin of the *telomerase–fitness* relationship was tested experimentally using transgenic mice models either lacking telomerase expression (e.g. *Tert*^{-/-} strains) or presenting an overexpression of telomerase in given cell types [4]. Tissue fitness may be promoted by two main mechanisms: (i) a rapid mobilization of the stem cell pool to replace damaged cells and (ii) a higher protection of mature somatic cells. In a previous study where telomere length in epidermic and hair follicle cells stayed apparently unchanged over the experiment, overexpression of TERT promoted stem cell mobilization [34]. Therefore, cell proliferation from stem to mature somatic tissue compartments appears to be, at least partly, under the control of TERT [35,36]. More precisely, TERT seems to interfere with the expression of proteins involved in the control of cell-cycle division or of cell differentiation mechanisms [31,37]. More direct evidence comes from experimental transfections of TERT cDNA within somatic cells. Human TERT-transfected lens epithelial cells presented a delayed apoptotic response to an experimental oxidative stress challenge, characterized by an attenuated activation of the pro-apoptotic caspase-3 activity [38]. These results suggested that hTERT cDNA displays some functions beyond telomere synthesis, inducing delayed cell senescence, possibly attributed to a downregulation of apoptotic gene expression [31] even in telomerase-negative cells [39].

Other key and well-conserved ageing pathways are suggested to be under the influence of TERT or TERC expression. The insulin-like growth factor pathway, for instance, was upregulated in cells overexpressing TERT [40]. Conversely, the suppression of hTERT expression in human fibroblast disrupts DNA damage response, suggesting that hTERT is implicated in the regulation of the DNA repair machinery and/or of the chromatin structure [41]. A DNA protection role of telomerase was also found in the zebrafish [42] and in yeast, where telomerase appeared necessary to avoid DNA replication stress [43]. In addition, the progeny of stem cells overexpressing TERT presented a higher resistance to oxidative stress, another crucial component-controlling senescence [44]. An additional age-related modification of interest which also seems sensible to the presence of TERT is the progressive decline in the degree of methylation of the genome [45]. By stabilizing the DNA methyltransferase, TERT could counteract the age-related DNA methylation pattern [46]. Importantly, all these data hint at the exciting possibility that TERT and TERC may be involved in a large panel of ageing pathways that are not directly related to telomere erosion. The next step will be to establish whether those telomerase components directly modulate cellular processes known to drive organism performance, such as mitochondrial functioning.

(b) Telomerase as a direct modulator of energy balance processes?

This idea has been partly tested by looking at intra-cell distribution of TERT in cells exposed to oxidative stress [47]. In those cells, TERT was excluded from the nucleus but localized within the mitochondria, a phenomenon concurrent with the existence of improved mitochondrial functioning markers (mtDNA protection, increased mitochondrial potential and lower free radical production or oxidative damage). Accordingly, the hTERT sequence contains a mitochondrial N-terminal localization peptide signalling the protein to the mitochondria [48]. Two mechanisms have been suggested so far to explain the interaction between TERT and the mitochondria. First, TERT binds to mitochondrial DNA, protecting it from DNA-specific damaging elements [49]. Interestingly, 13 mitochondrial proteins are encoded by the mtDNA, including complex I of the electron transport chain (ETC) and the ATP synthase, suggesting that gene protection by TERT is a key property to evaluate in the near future. Second, TERT mitochondrial localization increases ETC activity which is crucial for the cellular ATP production, and specifically protects protein complex I [49]. Complex I is the major site where the deleterious and pro-ageing reactive oxygen species are produced. More generally, mitochondria stand at the crossroads between metabolism and ageing [50], and mitochondrial dysfunction is a supposed pre-requisite for the onset of the ageing process [51]. Therefore, TERT could have a role in mitochondrial protection when the cell is exposed to disruption of the oxidative balance, providing an interesting mechanistic research avenue that may lead us to explain individual variability in metabolic efficiency and ageing rate. Fitness differences among individuals may be easy to characterize when environmental circumstances are stressful. Individuals of high intrinsic quality (e.g. in terms of tissue renewal rate) should perform better under such conditions. In this context, non-canonical expression of telomerase should be plastic and

modified by stress factors if implicated in mechanisms enabling the individuals to maintain their level of performance. Rapid mobilization of telomerase subunits (TERT) towards mitochondria in response to stress may contribute to such individual differences.

(c) Does activating telomerase improve metabolism while ageing?

The utilization of TA-65, extracted from roots of *Astragalus membranaceus*, has been shown to trigger telomerase activity both *in vitro* and *in vivo* ([25,52], but see [53]). Health proxies listed to be improved by TA-65 encompass glucose tolerance, skin maintenance, osteoporosis (in mice) or glucose and lipid metabolism (in humans) ([22,25], reviewed in [54]). Still, the underlying mechanisms linking telomerase activity and intermediate metabolism remain unknown. For instance, physical performance and energy metabolism are also dependent on mitochondrial and cardiac functioning. Physical performance has been linked with telomere dysfunction via PGC-1 signalling inducing impaired mitochondrial biogenesis [51]. While we lack information on how experimental telomerase activation may change mitochondrial functioning, we know that telomerase can migrate into the mitochondria, and protect mitochondrial DNA and function [47,49]. Interestingly, telomerase knockout mice suffered from a reduction of cardiac myocyte proliferation and had increased rates of heart failure [55]. Furthermore, the impact of enhanced telomerase activity on heart functioning was experimentally tested in humans using a 5-year dietary supplementation of TA-65, resulting in a reduction in systolic and diastolic blood pressures [22]. In the same context, resveratrol administration enhances the expression of hTERT in endothelial cells, potentially preserving angiogenesis for tissue regeneration [12,56]. A similar beneficial impact has been observed on neuromuscular coordination of skeletal muscles [27]. Although it is too early to extrapolate any conclusions about how this may influence the individual fitness of free-living animals, these data do suggest that telomerase may be involved in a broad array of pathways affecting energy metabolism and physical performance.

In summary, accumulating evidence suggests that TERT and TERC may slow the ageing process through mechanisms other than telomerase activation per se [31,57] (but see [58] for an alternative view). Protein and RNA components of telomerase are implicated in the DNA damage repair response; in the control of stem cell proliferation; in the regulation of close to 300 genes involved in cell-cycle regulation, growth, signalling, differentiation, metabolism and apoptosis; and in epigenetic regulation of chromatin structure [59]. Although the fitness consequences of telomerase and its TERT/TERC components at the cellular level are not always uncoupled from the regulation of telomere length by telomerase, the existence of TERT within the mitochondria should attract our interest. And, looking more closely into how non-canonical expression of telomerase explains individual variability in fitness proxies in the wild is a next essential step to investigate. While there are multiple potential targets, the main connections (including shortened telomeres, well-conserved apoptotic pathways and mitochondrial dysfunction) constitute a promising integrated axis to begin an exploration of the telomerase–fitness relationship in multiple organisms [60].

4. Perspectives: how to explore the link between telomerase and fitness

Our understanding of the evolution of telomerase expression patterns and how they covary with telomere length and organismal fitness remains poor, particularly outside model systems. Experimental triggering of telomerase activity could be conducted on wild animal models. Despite limitations of genome-wide association studies, quantitative genetic approaches may be of interest to examine the links between telomere length, telomerase expression and fitness in free-living individuals. Such an approach, among other things, will require either a better phenotypic description of high/low telomerase activity levels, or characterization of the output of rare mutations in the telomerase gene within wild individuals of known pedigree [61]. Furthermore, given the variety of molecules with the potential to trigger telomerase (electronic supplementary material, S2), particularly in old age using statins [62] or AGS-499 [63], we may also be able to dig deeper into the cellular and physiological pathways that translate telomerase effects at the level of individual fitness.

(a) Experimental manipulations

Experimentally manipulating telomerase expression (see electronic supplementary material, S3, for schematic of pathways) makes it possible to test the functional relationships among telomerase, telomere length and fitness. One promising avenue for future studies in a wide array of organisms is the use of TA-65. In mice, TA-65 exposure increased telomerase expression and average telomere length, and several aspects of general health, but importantly did not increase the incidence of malignancy [25]. There were, however, no significant effects of the treatment on longevity under these controlled laboratory conditions. Nevertheless, the sample sizes were relatively small ($n = 11–15$ per group) and additional studies are clearly needed. This approach was also successfully used in a recent captive study of zebra finches (*Taeniopygia guttata*), small songbirds. As in the mice, adult birds that received TA-65 had longer telomeres and were able to re-grow feathers more quickly, suggesting that they had enhanced tissue regeneration relative to controls [29]. In both of these studies, TA-65 was administered orally, and this manipulation may be well suited for field studies, particularly in species that are amenable to supplemental feeding. It may also be possible to modify TA-65 delivery so that it can be administered through slow release capsules, implants or skin patches as is often done for hormonal manipulations in physiological ecology, but this would require careful validation. Importantly, the long-term effects of TA-65 treatment on other health measures, longevity, cancer risk and fitness remain unknown and future studies in diverse organisms are urgently needed. Another important consideration for future TA-65 research will be the timing of exposure. Previous studies have focused exclusively on adult organisms, yet telomere attrition tends to be greatest during early life [64]. Thus, variation in telomerase expression during pre- and post-natal development could have a particularly large impact on telomere attrition and fitness. We have recently piloted the use of TA-65 in house sparrow chicks (*Passer domesticus*) still in the nest and found that chicks that received TA-65 during the growth period experienced less telomere attrition than controls (BJ Heindinger 2017, unpublished data).

Ongoing studies are examining the long-term phenotypic consequences of this treatment.

(b) Natural variation

Medical studies have previously looked for mutations in TERT and TERC genes and their association with different diseases. In addition to short telomeres, patients suffering from TERT/TERC-derived bone marrow failure are also characterized by low telomerase activity attributed to haploinsufficiency (reviewed in [6]). More interestingly, some of these mutations are responsible for a reduction in telomerase activity without inducing any pathological state [65]. This implies that individual variability in telomerase activity exists within human populations. Depending on how much telomerase expression is affected by these mutations, the phenotypic consequences may range from none (neutral mutations) to pathology (deleterious mutations), with potentially intermediate positive or negative effects (e.g. via proliferative stem cell capacities to anti-ageing shielding of the soma) that may contribute to the natural variance in individual fitness. An interesting study in yeast showed artificially deleted TERC RNA sequences produced mutants retaining the telomere maintenance capacity of telomerase, but with reduced fitness (population growth rate) when competing with wild-type yeasts [66].

Several recent genome-wide association studies in humans have established links between allelic variation in aspects of the telomerase complex, telomere length and longevity [67–70]. The use of these techniques is currently restricted to model organisms, but quantitative genetics is a promising approach that is increasingly used in physiological ecology studies [71] and will likely yield greater insight into the relationship between telomerase expression and fitness in diverse organisms (see [72] for a useful guide). In designing quantitative genetic studies, researchers will need to carefully consider several issues including how (electronic supplementary material, S1), when and in which tissue(s) to measure telomerase expression.

Telomerase expression is expected to be downregulated in most somatic tissues after embryogenesis, while remaining active into adulthood in highly proliferative tissues such as lymphocytes and germ cells [73]. However, this general pattern can vary among species [73,74]. For example, it was recently reported in a longitudinal study of the edible dormouse (*Glis glis*) that telomeres lengthen in old age, perhaps because telomerase expression is upregulated [75]. An important caveat is that it is possible that low telomerase expression can be missed if it is below the detectability of the assay (see electronic supplementary material, S1). As most tissues are expected to express telomerase during pre-natal development, variation at this time might have a particularly large impact on telomere length and fitness. Another interesting, but little explored possibility is that, given that telomerase remains active in germ cells into adulthood, it could have direct effects on the telomere length that offspring inherit, with fitness consequences for the next generation ([76]; for further discussion of this topic, see [77]).

For telomerase expression to respond to selection some of that variation will need to be heritable and we currently know very little about the relative contributions of genetic and environmental effects to telomerase expression patterns. Recent evidence in adult mice suggests that telomerase expression is upregulated in response to stress exposure [51],

but whether this is sufficient to buffer telomere erosion or affect fitness is currently unknown. There is also both *in vitro* [78] and *in vivo* [79] evidence that sex steroids influence TERT expression. In humans, induced telomerase expression is highly heritable (0.814), suggesting that genetic effects account for much of the variation in this trait [80]. An important consideration though is that given that induced telomerase expression is a plastic trait, it might be more appropriate to treat it as a reaction norm than to simply measure the response to the environmental stimulus [81]. As the change in telomerase expression may vary with environmental conditions (i.e. stress, immune challenge) and life-history stages (i.e. moult, hibernation), it may be more appropriate to examine changes in the slope of telomerase expression across contexts rather than simply measure maximum telomerase expression. A similar approach could be used in free-living populations with known pedigrees or using experimental breeding designs [72].

(c) Comparative analyses

Comparative analyses are another approach that makes it possible to ask questions about telomerase expression, telomere length and longevity in a broad evolutionary context. Comparative analyses have been used in both mammals [73,82] and birds [10] and have yielded some interesting patterns. For example, Seluanov *et al.* [82] and Gomes *et al.* [73] used phylogenetically controlled analyses in mammals to examine the relationship between telomerase activity, body mass and lifespan. In long-lived organisms, telomerase activity is expected to be downregulated in somatic cells after embryogenesis as an anti-cancer protection mechanism [12]. However, across species, lifespan tends to be positively correlated with body size [83]. Interestingly, both Seluanov *et al.* and Gomes *et al.* found that telomerase activity coevolved with body mass rather than lifespan in mammals. Gomes *et al.* further found that telomere length, but not telomerase activity, has coevolved with lifespan. These results have interesting evolutionary implications because they suggest that selection to increase body size would have stronger indirect effects on telomerase expression than on telomere length, whereas selection to increase lifespan would have stronger indirect effects on telomere length than on telomerase expression. Moreover, depending on the genetic correlation between telomerase expression and telomere length, the independent evolution of these two traits could be facilitated or quantitatively constrained [84] (see [85] for further discussion on these ideas). By stark contrast, in a much more restricted analysis of four bird species, Haussmann *et al.* [10] found that long-lived species had higher telomerase expression throughout life and slower rates of telomere attrition than short-lived species [86]. Cancer is little studied in wild birds, but is thought to be relatively rare, particularly in long-lived seabirds [87]. Thus, although these results are based on a limited number of avian species, they provide tantalizing evidence that these two classes of vertebrates may resolve trade-offs between investment in telomere maintenance and malignancy risk in very different ways, and future comparative studies involving a greater array of vertebrates with diverse life-history strategies are critically needed. One particular focus should be on ectotherms (electronic supplementary material, S1), which are known to present very particular telomere length dynamics over life [88], and in which telomerase activity differences among individuals have recently been highlighted [89].

Future comparative studies should also include additional life-history and physiology traits expected to be related to telomerase expression, telomere length and longevity. In addition to body mass, Gomes *et al.* [73] also included oxidative stress resistance as these traits have been shown to covary with lifespan. Sex is another trait that should be incorporated into future comparative analyses as both size and lifespan can vary with sex, and sex has also been shown to influence both telomere length [90] and telomerase expression [91].

5. Conclusion

Taken together, we argue that studying telomerase is of prime interest from an evolutionary biology perspective for the following reasons. First, previous molecular and biomedical studies highlight that variation in telomerase activity can be found at all levels of organization. In addition, telomere length and longevity are related to genetic variation in the

telomerase complex and suggest that telomerase expression is heritable. Finally, an increasing number of studies show that telomerase modulates organism functioning in a way that benefits survival and longevity, and increased expression levels are correlated with improved health status of individuals. Whether prolonging healthy lifespan is associated with increased fitness is presently an assumption that needs evaluation in natural populations, but that telomerase expression is related to fitness is a credible hypothesis. In summary, all of the ingredients for a key role of telomerase in the evolution of life-history traits exist. Now, studies going forward need to begin to mix those ingredients in non-model, field organisms, and across life stages and multiple tissue types to determine the role that telomerase plays in organismal fitness.

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