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Do telomere dynamics link lifestyle and lifespan?

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Identifying and understanding the processes that underlie the observed variation in lifespan within and among species remains one of the central areas of biological research. Questions directed at how, at what rate and why organisms grow old and die link disciplines such as evolutionary ecology to those of cell biology and gerontology. One process now thought to have a key role in ageing is the pattern of erosion of the protective ends of chromosomes, the telomeres. Here, we discuss what is currently known about the factors influencing telomere regulation, and how this relates to fundamental questions about the relationship between lifestyle and lifespan.

Life-history tradeoffs and telomeres

The concept of tradeoffs is central to our understanding of the evolution of life histories. Because resources, and the time it takes to acquire them, are limiting, high expenditure on one life-history trait has negative consequences for other traits requiring the same resources. Explanations of differences within and among species in life-history strategies are generally framed in terms of differences in the optimal resource investment in growth, reproduction and self-maintenance. Hence, we have differences in size, reproductive rate and lifespan [1].

For most organisms, managing the prevention, occurrence and repair of damage to the body is played out against a backdrop of developmental processes being followed, apparently inevitably, by degenerative processes. Additional complexity is introduced when traits or patterns of investment that are beneficial at one life-history stage are detrimental later in life; that is, they are antagonistically pleiotropic. Such traits might still be favoured by selection depending on the overall balance of fitness benefits and on the proportion of the population that survives to experience the negative effects late in life. Thus, the benefits of a particular trait or pattern of resource allocation might not be evident at all life-history stages.

Although life-history theory is built on the idea of tradeoffs, we know little about the processes that mediate these or produce the interlinking of events across life-history stages. Whereas at one time, the mechanistic level of understanding was not considered important in evolutionary ecology, there is an increasing recognition that we need to reunite studies of proximate mechanisms (which inform us on capacities and constraints) with studies of function (which tell us about fitness costs and benefits) to understand how life histories evolve.

Here, we focus on one fundamental cellular process that appears to have a major role in underpinning the links between organismal growth and degeneration: telomere dynamics. Telomeres are specialized areas at the ends of chromosomes that prevent chromosome degradation and maintain genome integrity. The level of investment in maintaining the health of somatic tissue is crucial in determining lifespan, and the pace of degeneration is expected to be influenced by lifestyle [2]. In addition, environmental circumstances during early growth can have long-term consequences for the rate of degeneration in later life [3,4]. Could such links be mediated, at least in part, by telomere length changes? Might telomeres mediate the tradeoff between growth and lifespan and, via oxidative damage, link reproductive rate and energy expenditure to lifespan? Do telomere lengths provide us with useful information about the state of individuals? To answer such questions, we need studies that span different biological levels, and link cellular process to organismal and evolutionary biology.

What are telomeres and what do they do?

Telomeres form the ends of eukaryotic chromosomes (Box 1) and are currently thought to have a variety of functions. They provide the cell with a mechanism for distinguishing between real chromosome ends and those arising from chromosome breaks that need to be repaired. They also protect the encoding parts of the chromosomes from the loss of nucleotides that occurs because chromosome ends are not replicated completely during cell division (Box 2). Telomeres also appear to have a role in the alignment and segregation of chromosomes during meiosis [5]. Long telomeres are associated with the reversible silencing of genes near telomeres [6], a positional effect that provides a mechanism for the modification of gene expression by telomeres [6,7]. Long telomeres might also provide a cellular signal that induces stress resistance in cells and tissues [8].

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Box 1. What are telomeres?

Telomere structure

Telomeres (Figure I) are the ends of eukaryote chromosomes. They are made of non-coding DNA sequences, usually consisting of repeats of a short sequence of nucleotides that is rich in guanine (G). In general, the telomere complex comprises double-stranded DNA [the G-rich strand and its complementary cysteine (C)-rich strand]. At the very end, however, the G strand protrudes alone, giving a single-stranded overhang. The length of this overhang, and whether it is created by extension of the G strand (e.g. as appears to occur in mammals) or degradation of the C strand by exonucleases (e.g. in yeast and ciliates), appears to differ across taxa, as does the precise structure of the telomere [53,54]. In vertebrates, the relatively long overhang is thought to fold back on itself and tuck into the double-stranded section, forming what is known as the t-loop. This, together with several specific and non-specific proteins, creates the telomeric cap at the end of chromosomes that is essential to telomere function [9,55]

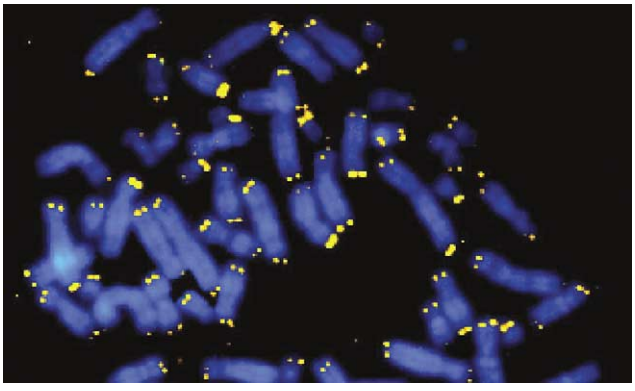

Figure I. Human chromosomes. The telomeres are seen *in situ* on the chromosome ends in yellow, as shown with the use of fluorescent *in situ* hybridization (FISH) [10,69]. Reproduced with permission from Chris Counter.

Variation among species

The telomeric nucleotide sequence itself is simple, ancient and highly conserved [54]. In most organisms studied to date, telomeric sequences usually consist of five–eight base pairs repeated in tandem. In vertebrates, the sequence is typically TTAGGG, in many insects TTAGG, whereas in many plants it is TTTAGGG. Species in a few groups of insects and plants have a quite different telomere structure from that most commonly found, but comparative information is limited [54]. The typical number of sequence repeats (i.e. the telomere length) also varies among species. In some ciliates, the telomere is only 20 base pairs long, in some yeasts a few hundred base pairs, whereas in vertebrates, it can be >100 000 base pairs long [54]. Telomere length can be readily measured by a variety of techniques [10,56].

Variation within species

Within species, telomere lengths and their rate of shortening vary among chromosomes, tissues, individuals and also with age [46]. In vertebrates, telomere length during embryonic development is similar in the cells of most tissues [57], but after birth, telomeres progressively shorten in proliferative somatic cells [58]. Some tissues, such as intestinal mucosa and peripheral blood cells, have rapid turnovers that require high cell proliferation and thus show greater telomere shortening [59,60]. Conversely, other tissue types, which are predominantly mitotically inactive, such as muscle and the brain, have fairly stable telomere lengths [61]. Still other tissues, such as the liver and renal cortex, show telomere shortening with age, despite little mitotic activity [62]. Furthermore, it appears that, even in stem cells, some telomere loss also occurs [22].

Consequences of telomere loss

Telomere loss puts a finite limit on the reproductive life of cells. Each time a cell divides, the telomeric DNA on its chromosomes gets shorter (Box 2), unless it is restored; once some crucial length is reached, the telomere becomes dysfunctional and the protective capping of chromosome ends provided by telomeres (Box 1) no longer operates. Telomeres can also become dysfunctional owing to direct DNA damage or changes in the telomere-associated proteins [9]. Dysfunctional telomeres make the genome of the cell unstable, because true chromosome ends on the 'uncapped' chromosomes can no longer be distinguished from double-stranded breaks, which results in the activation of the DNA damage machinery of the cell. This usually leads to one of two outcomes under normal circumstances; cell death (apoptosis) or replicative senescence, whereby the cell remains alive but stops dividing [9].

Telomere length measurements are usually based on averages taken across the chromosomes and cells present in the tissue sample [10]. Telomeres can also be measured on specific chromosomes [10], but little is currently known

Box 2. What causes telomere loss?

The end-replication problem

Part of the loss of telomereic sequences occurs because the process of DNA replication is incomplete. DNA molecules are made up of two strands of nucleotide sequences that run antiparallel to one another; one strand runs 5′→3′, whereas the other runs 3′→5′. During DNA replication, the DNA strands unzip, and each is used as a template for producing a new strand of DNA. DNA synthesis by DNA polymerase runs continuously along the 3′→5′ template strand, which is then replicated in full (the leading strand). For the 5′→3′ template strand, DNA synthesis proceeds in small discontinuous steps, and involves a primer that attaches to the strand at various points (the lagging strand) . Because of the presence of the primer, this strand is not fully replicated: when the primer dissociates, the last (end) part to which primer was attached is not replicated and the newly formed strand is slightly shorter than the template strand. Thus, each time a cell divides, some of the nucleotide sequences at the chromosome ends are lost.

Oxidative damage

In human cells, telomeres shorten by 30–200 base pairs per cell division, but only approximately ten base pairs of this reduction is thought to be due to the end-replication problem [63]. The remaining loss is partly due to reactive oxygen species, often implicated in studies of ageing [2] and which increases telomere shortening *in vitro* [14]. Furthermore, compared with other regions of DNA, the G-rich telomeric sequence appears to be particularly vulnerable to certain types of oxidative damage caused by free radicals [64]. It has also been suggested that telomeres serve an additional function, by acting as sentinels of the general level of DNA damage occurring in the cell; high levels of damage to telomeric DNA would be indicative of high levels of damage to the coding sequences. It appears that the amount of unrepaired damage to the telomeres influences the magnitude of telomere loss at the next cell division [14]. This 'score board' function of telomeres would provide a mechanism to ensure that cells with high levels of DNA damage soon cease division [65].

Box 3. Telomere restoration

The enzyme telomerase

Permanent telomere loss at cell division is not inevitable. Telomere length can be restored by telomerase, first discovered in the ciliate *Tetrahymena* [5]. Telomerase is a ribonucleoprotein consisting of an RNA template, which contains the information needed for replicating the telomere sequence, and protein components, which contain the replicative machinery. Telomerase is very widespread and conserved in the eukaryote lineage, although there are a few exceptions: *Drosophila melanogaster* (which has a telomere structure and mechanism for telomere length control that differs from most other eukaryotes [66]) is a particularly ironic one given the amount of detailed genetic work that has occurred in this species, and its widespread use as a model organism [67].

Pattern of telomerase activity

Telomerase is generally active in the germ line and in embryonic tissue, but is also variably active in different organisms, in different tissues throughout the body (being most active in those with high cell turnover [46]) and at different life-history stages [67]. Telomerase enables the replicative potential of germ cells to persist, and can reset telomere length during early embryogenesis [68].

Although not usually active in somatic cells, telomerase could lengthen telomeres during adult life in some cell types by turning back the cellular clock and delaying cell senescence [67]. In the African clawed frog *Xenopus laevis* for example, telomere length appears to be longer in the adult spleen than in testes or early embryos [69], and it has recently been suggested that telomere length in human sperm increases with male age [52]. In one species of bird, telomere length in blood cells appears to increase with age (Figure 1a, main text), and this pattern has also been found in samples of root tissue from the bristlecone pine *Pinus longaeva*, one of the longest lived eukaryotes on Earth [42]. Such positive relationships between age and telomere length could be due to differential survival of individuals with very long telomeres rather than telomere elongation within individuals. In the absence of longitudinal studies of the same individuals, we cannot distinguish between the two. However, telomerase has been found to be active in adults in both long-lived birds and long-lived pines [42,45], so actual elongation could occur. Overall, however, the regulation of telomere length during development and in later life is still poorly understood.

about inter-chromosomal variability in telomere length and loss rates. It is still unclear whether telomere length on some chromosomes is more important than on others [11], as well as what determines either the threshold at which cell division ceases, or whether cell apoptosis or replicative senescence occurs. Recent evidence suggests that telomere lengths on groups of chromosomes, rather than on single chromosomes, are important in arresting cell division [12]. The progressive loss of telomeric DNA in the absence of any restoration is thought to be a major determinant of the limited number of divisions (i.e. the Hayflick number) that cells are capable of *in vivo* and *in vitro* [13]. Even within clonal cell cultures, there is substantial variation in the replicative potential of cells, some of which could be explained by telomere-independent factors, and some of which might be due to variation in the rate of telomere loss [14,15].

Telomeres and immortality

Telomeres can be restored by telomerase or its equivalent (Box 3). So, why then do all cells not maintain telomere length and, therefore, their replicative potential? Multicellular animals contain in their soma variable amounts of post-mitotic cells, which do not divide, and mitotically active cells that do. In relatively simple organisms, such as *Caenorhabditis elegans* and *Drosophila melanogaster*, nondividing cells predominate in the adult body. In more complex groups, such as vertebrates, many tissues are constantly renewing through cell division and recruitment of stem cells, with presumably beneficial effects for tissue function. It appears that, following the uncapping of telomeres, there is a third potential outcome for the cell in addition to replicative senescence or apoptosis. In simple terms, the mechanisms that arrest cell division do not engage, and the genomically unstable cell somehow activates telomerase, or acquires alternative mechanisms for maintaining telomere length, and becomes a malignant tumour cell. Turning off telomerase and therefore curtailing the replicative potential of cells appears to be a mechanism whereby the young animal is protected against cancer.

Sadly then, eternal youth with ever-renewing cells would come with high risks: avoiding cell senescence could bring about the downfall of the whole system through a high incidence of malignancy [9,16–18]. Later in life, the same mechanism that protects the young organism appears to be disadvantageous, because the accumulation of senescent cells can impair tissue function and even increase the risk of malignancy developing. The relationship between telomere dynamics and cancer is an active area of research and it appears that the presence of senescent cells in tissues might promote malignancy in pre-cancerous cells. This could explain the increased incidence of cancer in old age [19]. There might therefore be an antagonistically pleiotropic effect [17,20]. The optimal resolution of this tradeoff between the early- and late-life effects of limiting cell replicative potential will depend on the overall lifetime fitness benefits.

Telomeres and age

Telomere shortening used to be thought of as occurring at a constant rate, hence the suggestion that telomeres represented a 'mitotic clock', the reading of which would give information about the replicative past and future of a cell [15]. It was therefore hoped that telomere length could be used as an indicator of the age of an organism [21]. In several organisms, including humans, there is some correlation between age and average telomere length in at least some tissues (e.g. Figures 1,2). Interestingly however, there is generally substantial unexplained variation in the relationship between telomere length and age. In some long-lived birds, although there is a difference in the average blood cell telomere lengths of chicks and adults, there is no correlation between telomere length and age in the adults themselves; moreover, in two long-lived species, the relationship is positive rather than negative (see Figure 1 and Box 3).

The absence of the expected negative correlations between telomere length and age, and the variation in

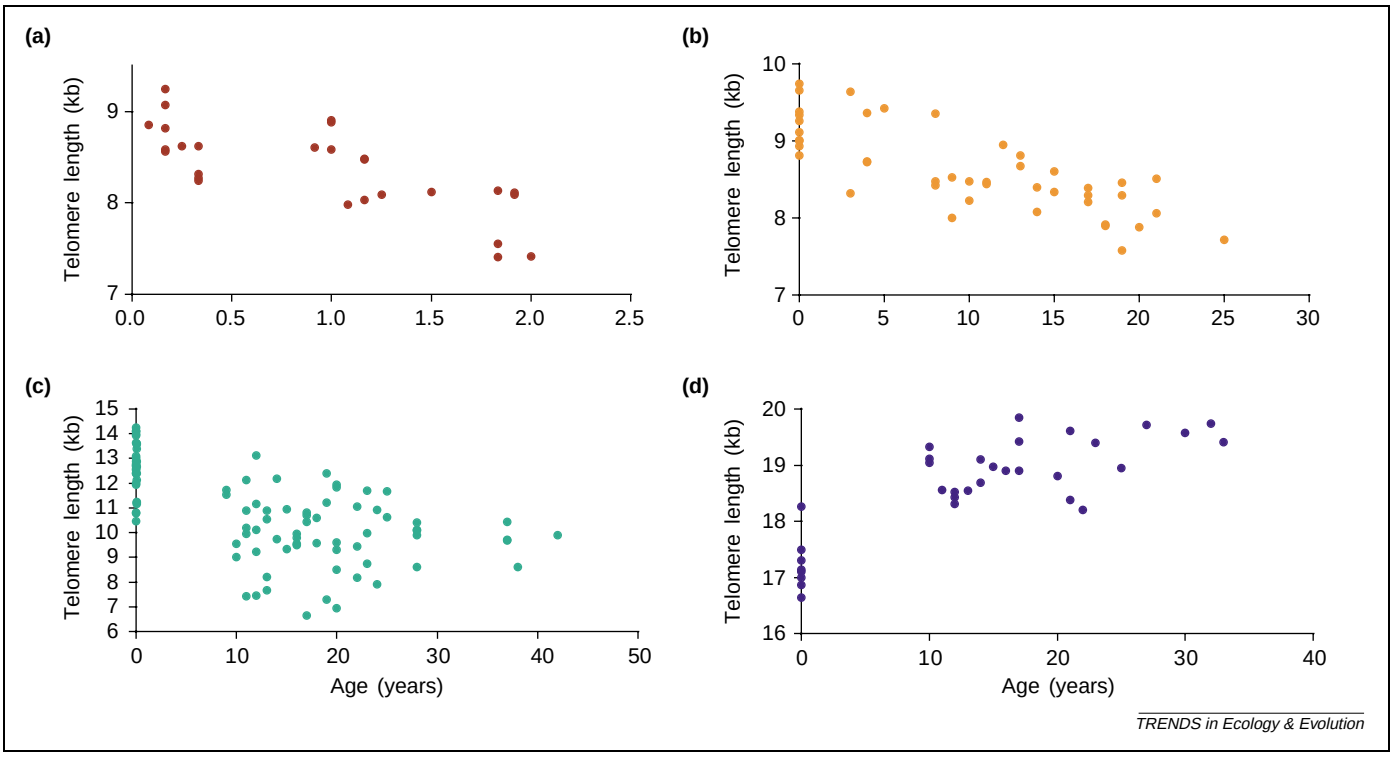

Figure 1. The relationship between red blood cell telomere length and age in a range of birds, the non-mammalian vertebrate group in which most cross-sectional work over a wide range of ages has been done to date [24,43]. Avian red blood cells are nucleated, and small blood samples thus provide a rich source of DNA. Telomere length decreases with age in zebra finches *Taeniopygia guttata* ($P<0.001$, $r^2=0.54$) **(a)** and in common terns *Sterna hirundo* ($P<0.001$, $r^2=0.61$) **(b)**. Although there is a difference in telomere length between chick and adult wandering albatrosses *Diomedea exulans* ($P<0.001$), there is no relationship between age and telomere length in the adult birds ($P<0.52$, $r^2=0.08$), although maximum lengths appear to be shorter in the oldest birds **(c)**. In Leach's storm-petrel *Hydrobates pelagicus*, telomere length appears to increase rather than decrease with age ($P<0.001$, $r^2=0.66$) **(d)**. This relationship remains even when chicks are excluded, but is weaker ($P<0.01$, $r^2=0.23$).

telomere length among individuals, could be a consequence of many processes, such as differential telomere restoration by telomerase, different starting lengths, different rates of telomere loss through DNA damage, different cell division rates or of course differential survival with respect to telomere length. The few species in which the same individuals have been sampled at different ages do show loss within individuals (cats *Felix domesticus* [22], humans [23], shags *Phalacrocorax aristotelis* [24] and tree swallows *Tachycineta bicolour* [25]), but also suggest that loss is fastest during early growth. More within-individual studies are needed from a wider range of taxa, but for long-lived organisms, the lifespans of which can stretch from decades to thousands of years, such information is difficult to obtain.

Telomeres and lifespan

The big question in the context of life-history tradeoffs is how these changes at the cellular level influence organism performance. Telomere loss and maintenance both have costs and benefits that need to be balanced (Figure 3), and we need to understand the extent to which managing the pattern and pace of telomere loss and restoration is likely to be important in determining lifespan. The replicative potential of cells in culture is positively related to the longevity of the species from whence they came [26]. The picture within species is less clear; correlations have occasionally been found between donor age and cell replication *in vitro* [27]. That the link between the replicative potential of cells in culture and donor age seems weak or absent is perhaps not surprising, given the number of factors in addition to telomeres that can influence division in cultured cells. The relationship between cell replicative potential and telomere length itself is stronger [27].

Linking cell and organism senescence

The links between cell replicative senescence or apoptosis and tissue function are crucial in determining the link

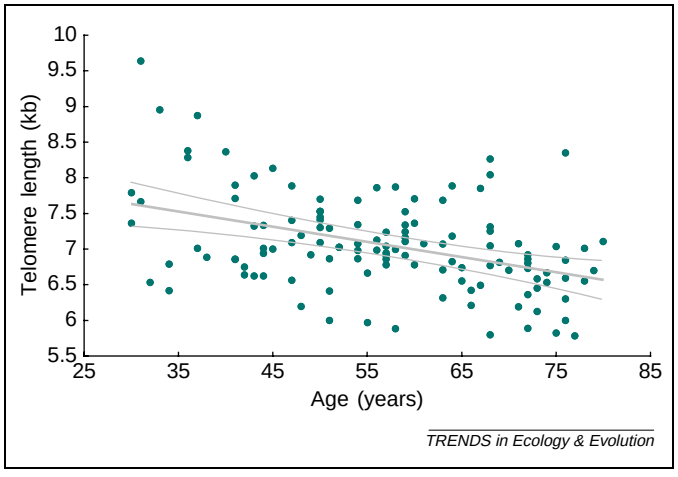

Figure 2. The relationship between age and telomere length in white blood cells in a sample of 125 randomly selected, healthy humans ($P<0.0001$, $r^2=0.18$) [52]. The grey lines indicate the regression line and confidence limits. Although there is a negative relationship between telomere length and age, there is wide variability; some individuals in their early 30s have shorter telomere lengths than do those in their late 70s.

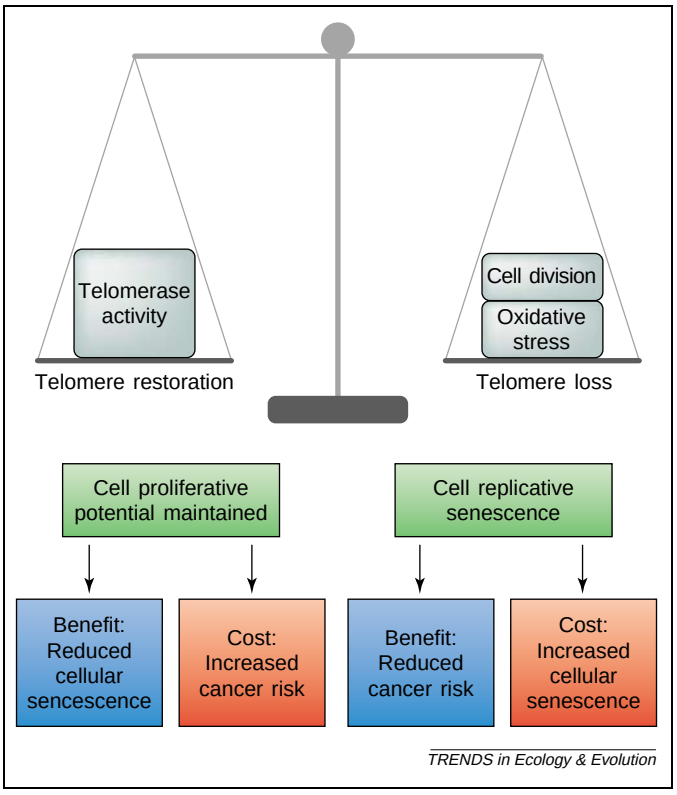

Figure 3. The potential costs and benefits of telomere loss and restoration. The optimal balance of costs and benefits will vary among tissues, life-history stages and species.

between telomere dynamics and organismal ageing, although we need to be careful in assuming that cellular senescence is equivalent to organismal senescence. Changes in tissue function with age are likely to be a consequence of the interplay among many contributing factors, including the rate of cell division and cell death, recruitment of stem cells, telomere maintenance and the proportion of senescent cells. So how might senescent cells promote ageing? One possibility is that the accumulation of senescent cells directly impairs tissue function in later life. Senescent cells do not simply remain quiescent. Once replication is arrested, the phenotype of the cell changes; it becomes more flattened, and secretes high levels of degradative enzymes, inflammatory compounds and certain growth factors [9,17]. Accumulation of such cells, which can be detected by histochemical staining, has been reported in multiple tissues of rodents and humans in old age, and especially at sites where age-related deterioration in performance at the organismal level occurs, such as arthritic joints. Senescent cells are thus present at 'the expected time and place' [17].

Linking telomere length and organism senescence

The effect at the organismal level of telomere loss rates in different tissues will depend on many factors, including the extent to which new progenitor cells are recruited. Cell death, replicative senescence and loss of proliferative potential could all have adverse effects on organism performance. In humans, a recent study, in which individuals were followed over a 20-year period, reported that people over 60 years of age with shorter-than-average telomeres (measured in whole blood samples) have higher mortality from heart and infectious diseases than do people of the same age with longer-than-average blood cell telomeres [28]. Interestingly, two human syndromes in which premature ageing occurs (Werner syndrome and dyskeratosis congenita) appear to involve short telomere length [9]. Telomere-induced changes might matter in some tissues more than in others. The immune system is likely to be important in this context because the ability of the organism to combat disease could become impaired by telomere loss [29–31]. Wound healing deficits and immunosenescence have been linked to reduced telomere length [9].

In *C. elegans,* the effect of telomere length on lifespan has been examined using a transgenic line in which the expression of a protein involved in telomere lengthening was increased, giving rise to worms with longer telomeres. These worms lived longer and were more resistant to heat stress than were those with shorter telomeres [8]. This suggests that telomere length is important to organism function even when the adult body comprises mainly post-mitotic cells.

Telomeres and interspecific variation in lifespan

One might expect that particularly long-lived species would have relatively long telomeres. However, there has been little comparative work examining the links between telomere length and interspecific variation in lifespan. No connection appears to exist between mean telomere length and lifespan across those vertebrate groups that have been examined [32–34]. Within the primates studied, humans have relatively short telomeres, but the longest lifespan [35], and there is no relationship between absolute telomere length and lifespan in mammals [36] or birds [37]. A variety of mouse species and strains show wide variability in telomere length (10–200 kb), but none of this variation is linked to lifespan [38,39]. Interestingly, the length of time that the mice have been domesticated appears to correlate positively with the length of telomeres [39–41], suggesting a need for caution when using inbred mice as a model for the telomere biology of other species. However, a recent study of telomere length early in the life of various species of pine trees, in which maximum lifespan varies from 100 to 5000 years, has found that, in root (but not needle) tissue, the longest-lived species have the longest telomere lengths [42].

Although absolute telomere length might not explain differences among species in longevity, the rate at which telomere erosion occurs might be more important. The rate of telomere shortening in blood cells is inversely correlated with longevity in birds and mammals [43]. There are problems associated with measuring rates of telomere loss, because this is not constant throughout life [23,24,44]. Thus, the age composition of the individuals sampled will influence the estimate of loss rate, as will the tissue used (Box 2). Despite this added noise, long-lived bird and mammal species appear to lose fewer telomere repeats with age than do short-lived species [43]. This could involve either differences in the factors responsible for telomere loss (Box 2) or telomere restoration (Box 3) (Figure 3). In the long-lived bird species studied, bone

marrow telomerase is expressed throughout life, whereas in short-lived bird species, expression is reduced after fledging [45]. This suggests that telomerase activity in bone marrow contributes to the different telomere shortening rates found in red blood cells from bird species with different lifespans. However, telomerase activity does not always vary with lifespan. For example, inbred mouse strains express telomerase in many tissues at many ages, whereas human telomerase activity is absent in most somatic tissues [46]. More comparative work is needed to determine the patterns of telomere shortening and telomerase activity in other taxonomic groups, and whether this is related to longevity.

Telomeres and lifestyle

The rate of telomere loss is sensitive to cell division rates and to environmental circumstances in the cell, particularly the level of oxidative stress (Box 2), and this potentially provides an important link between lifestyle and senescence. Telomeres are not simple cell division or time counters. The absence of tight relationships between age and telomere length within species is due at least partly to the fact that telomeres give a handle on biological (rather than chronological) age at the organismal level, indicative of the current position of the individual in its journey through life. This bio-chronometer can run faster in some individuals than in others.

Evidence is increasing that individual life histories can be related to telomere dynamics. Circumstances early in life, when telomere loss is likely to be highest, might be particularly important. Growth acceleration following a period of poor nutrition has been linked to accelerated telomere shortening in rats *Rattus norvegicus* [47], and greater telomere loss has been found to be associated with faster growth rates in a long lived bird species, the shag *Phalacrocorax aristotelis* [24]. Changes in telomere lengths might therefore provide the link between early growth conditions and the pace of deterioration in later life [3,4]. One-year-old female tree swallows with shorter-than-average telomere lengths have recently been found to have lower survival rates than do individuals with longer-than-average telomeres; that such effects are evident even early in life also suggests that telomere shortening is linked to individual quality or lifestyle [25]. Conditions later in life will also have an effect. High rates of environmentally induced psychological and oxidative stress in the lives of women have been found to be associated with shorter telomeres, indicative of a 9–17-year acceleration in the degree of ageing [48]; cigarette smoking and obesity have also been linked to shorter telomeres in women [49]. Such environmental factors are likely to induce a multitude of physiological effects that could influence telomere loss.

What next?

Measures of relative telomere lengths could offer organismal biologists a molecular marker of individual history and current state, and a useful measure of the effects of various investment patterns on potential lifespan. More information is needed on what causes telomere changes in different tissues and how this affects organism survival prospects. Although our understanding of processes at the cellular level is moving forward fast, from an evolutionary and life-history perspective, there is a clear need to broaden the range of organisms studied in order to encompass more-variable life histories. Studies in a greater diversity of animals could also benefit the biomedical community, for example by providing information on novel mechanisms that have evolved to enable organisms to circumvent some of the costs associated with high cell division rates or telomerase activity in somatic cells. More longitudinal studies of individuals experiencing different environmental circumstances are also needed, and this should involve experimental manipulations if causal relationships are to be uncovered. The effects of factors such as early growth conditions, dietary antioxidants, demands on the immune system, reproductive rate and energy expenditure on telomere length are likely to be particularly interesting for ecologists. There is also much potential for mechanistic-based life-history models that use telomere dynamics to examine the optimal balance of telomere erosion and repair in a given set of circumstances and to model links across life-history stages [50].

Important advances in behaviour and evolutionary ecology will come from linking studies at different biological levels. To do this, biologists from different disciplines must cooperate, and learn each other's language. Studies of telomere dynamics that link the molecular to the organismal require a leap across 'a vast interdisciplinary canyon' [51]. The leap is risky, the landing likely to be bumpy, but if a bridge can be built then the benefits for our understanding of life-history tradeoffs will be huge.

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