Current Zoology 56 (6): 714–727, 2010

Telomeres: Linking stress and survival, ecology and evolution

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Abstract Telomeres are protective structures at the ends of eukaryotic chromosomes. The loss of telomeres through cell division and oxidative stress is related to cellular aging, organismal growth and disease. In this way, telomeres link molecular and cellular mechanisms with organismal processes, and may explain variation in a number of important life-history traits. Here, we discuss how telomere biology relates to the study of physiological ecology and life history evolution. We emphasize current knowledge on how telomeres may relate to growth, survival and lifespan in natural populations. We finish by examining interesting new connections between telomeres and the glucocorticoid stress response. Glucocorticoids are often employed as indices of physiological condition, and there is evidence that the glucocorticoid stress response is adaptive. We suggest that one way that glucocorticoids impact organismal survival is through elevated oxidative stress and telomere loss. Future work needs to establish and explore the link between the glucocorticoid stress response and telomere shortening in natural populations. If a link is found, it provides an explanatory mechanism by which environmental perturbation impacts life history trajectories [*Current Zoology* 56 (6): 714–727, 2010].

Key words Corticosterone, Stress, Survival, Telomeres

1 Introduction

The birth of telomere biology began with a small group of scientists studying the functional elements found at chromosomal ends. But over the past century, the study of telomeres has moved into the mainstream, connecting diverse fields like cellular biology, aging, cancer, ecology and evolution. In 2009, Elizabeth Blackburn, Carol Greider, and Jack Szostak were awarded the Nobel Prize in Physiology and Medicine for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase. It is not our intention to cover the breadth of telomere biology in this review, but instead, our goal is to specifically address how the study of telomeres can provide insight into ecology and evolutionary biology. The measurement of telomeres has become a valuable tool in these fields (Nakagawa et al., 2004; Monaghan et al., 2006), as telomere dynamics are related to survival (Haussmann et al., 2005; Bize et al., 2009; Salomons et al., 2009), reproductive success (Pauliny et al., 2006), physiological stress (Epel et al., 2004; Kotrschal et al., 2007) and growth (Jennings et al., 1999; Hall et al., 2004).

In this review, we concentrate on the relevance of telomere biology to studies in ecology and evolution. We begin by briefly outlining telomere structure and function, and then discuss the main mechanisms by which telomere length is regulated. We cover both events that lead to telomere loss and also mechanisms that allow for telomere restoration. This is followed by a discussion of how telomeres are related to survival and lifespan, which focuses heavily on data from wild populations. It is important to note that currently there is not a consensus on whether telomeres are a cause of aging or merely a consequence of it (Hornsby, 2006). However, we explore how telomere measurements provide important information in ecological studies by serving as a measure of chronic oxidative stress – a process central to aging (Finkel et al., 2000) and a mediator of life history trade-offs (Costantini, 2008; Monaghan et al., 2009). We finish by highlighting interesting new connections between telomeres, oxidative stress, and the glucocorticoid stress response. While the idea that chronic stress can accelerate the aging process has permeated gerontology for a century, only recently has a link between chronic stress and cellular aging been established. Because organisms making their living in the wild experience a wide range of stressors, telomeres and oxidative stress may act as an underlying mechanism connecting the glucocorticoid stress response and survival in natural populations.

Received Jan. 16, 2010; accepted May 10, 2010

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2 Telomeres and Oxidative Stress

2.1 The discovery of telomeres and telomeric function

Gene expression and proper cell functioning depend upon the maintenance of chromosomal integrity. Early cytogenetic work in the 1930s by Hermann Muller and Barbara McClintock showed that chromosomes damaged by X-rays resulted in products of broken chromosomes that had joined together. McClintock further observed that the resulting chromosome instability was detrimental to cells often resulting in cell death. Both Muller and McClintock discovered that chromosome products only formed between newly broken ends, but in no case did a new broken end of one chromosome attach to the original, intact end of another. This suggested that something was inherently different about the original ends of chromosomes, and J.B.S. Haldane named the end of chromosomes the telomere. While X-rays were used as a tool by early cytogeneticists to explore chromosome structure by damaging DNA, cells are naturally bombarded by a number of damaging agents that can cause DNA double-stranded breaks. DNA repair machinery recognizes these breaks and functions to join broken chromosomes back together. All linear eukaryotic chromosomes then pose a general problem: how to distinguish between DNA that is a natural chromosome end and DNA double strand-breaks that require repair? Although there are many different solutions to this problem, one of the most ubiquitously employed is telomeres.

Telomeres are repetitive, non-coding DNA sequences found at the termini of all linear eukaryotic chromosomes. In vertebrates, the telomere sequence is a repeat of 6 bases rich in guanine (TTAGGG/CCCTAA; Meyne et al., 1989). While the length of telomeres varies between chromosomes and species (Aubert et al., 2008), the sequence is similar across taxa (Bodnar, 2009) suggesting that telomeres are an evolutionarily conserved system to protect genomic integrity. At the distal end of the telomere, only the guanine-rich single-strand is present, forming a 'G-strand overhang'. This overhang, which has variable length on different chromosomes and in different species (Baird et al., 2003; Aubert et al., 2008), tucks back into the double-stranded telomere sequence effectively hiding the terminal tip of the chromosome in a 't-loop'. Chromosomes with sufficiently long telomeres form t-loops and are effectively 'capped'; it is this structure that prevents telomeric ends from being mistaken for double-stranded DNA breaks (di Fagagna et al., 2003). Proper t-loop formation requires 6 telomere-specific proteins called shelterin, and these proteins ensure that the DNA end is not inappropriately processed by DNA repair pathways (de Lange et al., 1999; Deng et al., 2009). Thus, if telomeres are long enough to form t-loops with associated shelterin the chromosomes are capped and protected from end-to-end fusions that hasten cell death. However, if telomeres shorten to a critical length, t-loops cannot form, chromosomes are uncapped, and chromosome instability and cell death results (Blackburn, 2000). An important question, then is: what events cause telomere shortening?

2.2 Telomere dynamics: The balance between loss and restoration

In the early 1960s, Leonard Hayflick made the discovery that cells in culture would only divide a finite number of times before ceasing cell division, a process known as replicative senescence. This led to the hypothesis that telomeres act as a mitotic clock, counting the number of cell divisions and eventually resulting in organismal aging and death. While the importance of replicative senescence to the aging phenomenon is still debated (Hornsby, 2006), we now know that telomeres shorten with each replication event, and when telomeres shorten to a critical length, they induce a permanent arrest in the cell cycle through a process called cellular senescence (Hornsby, 2003; Capper et al., 2007). Substantial evidence is accumulating that telomeres are important to the aging phenotype (Campisi, 2003; Patil et al., 2005). For example, senescent cells *in vivo* secrete degradative enzymes and inflammatory cytokines that disrupt nearby cells, contributing to aging and the threat of cancer (Wu et al., 2003; Campisi, 2005; Capper et al., 2007). Work in mice has demonstrated that short telomeres result in multiple organismal defects caused by defective tissue regeneration (Blasco et al., 1997), and telomere dysfunction in these mice contributes to the nonreciprocal translocations that are common in adult carcinomas (Capper et al., 2007). This pattern is also seen in human patients who have inherited genetic defects that limit telomere maintenance increasing their risk to a number of diseases (Finkel et al., 2007). Furthermore, short telomeres are a risk factor in cardiovascular disease (Samani et al., 2001), liver cirrhosis (Mason et al., 2005), pulmonary fibrosis (Armanios et al., 2007), diabetes (Valdes et al., 2005), stroke (Martin-Ruiz et al., 2006), and Alzheimer's disease (Honig et al., 2006).

Because the accumulation of short telomeres predis-

poses tissues to cancer and serves as a risk factor for many diseases, managing the pattern and pace of telomere loss and restoration is critical. Telomere length is a balance between shortening events (end-replication problem and oxidative stress) and restoration events (telomerase and recombination) and the changes in telomere length over time are termed telomere dynamics. The most relevant processes to telomere dyanmics are briefly described below.

2.2.1 Telomere loss: The End-replication problem (Fig. 1) Each time a cell divides; the telomeric DNA on its chromosomes gets shorter. This is because the DNA replication machinery cannot completely replicate the very ends of linear DNA (Fig. 1). This is due to both the requirement of an RNA primer to allow for polymerase to bind and properly function and because DNA polymerase can only elongate in the 5′ → 3′ direction. In the leading strand, replication is continuous and the strand is replicated in full. In the lagging strand DNA replication is discontinuous. At the end of the process the RNA primer is excised and is replaced with DNA by polymerase, but on the lagging strand, when the RNA primer dissociates from the most distal end, DNA polymerase is unable to fill in the gap because there is no double-stranded region to allow initiation. Thus, during lagging strand synthesis there is DNA sequence loss corresponding to where the most distal RNA primer was laid down. While this results in the generation of the G-strand overhang (Fig.1g), which is critical for t-loop formation (Fig. 1h), it also results in loss of telomeric DNA.

2.2.2 Telomere loss: Oxidative stress Aerobic species have evolved the capability of using oxygen for efficient energy metabolism. A consequence of this process is the formation of free radicals. While a small proportion of free radicals serve important functions as regulating mediators in signaling processing, at higher concentrations free radicals result in oxidative damage. Organisms have evolved mechanisms to prevent the production of free radicals and the oxidative damage they create on a number of levels.

(i) Changing the proton-motive force across the mitochondrial membrane can alter free radical generation in the mitochondria. Increasing the proton leak through the inner mitochondrial membrane via uncoupling proteins results in a net reduction in free radical production (Hulbert et al., 2007).

(ii) Enzymatic antioxidants located inside of the cell terminate the chain-reaction of free radicals before it begins. For example, superoxide dismutase converts

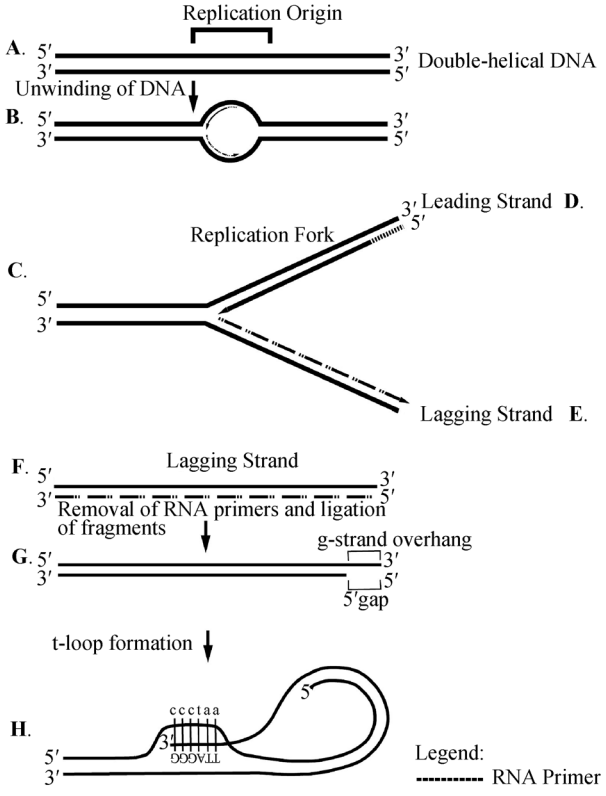

Fig. 1 The DNA end replication problem

A. DNA replication begins at the origin of replication where the double stranded helix is unwound by enzymes. **B**. As the strands separate, two replication forks form in opposing directions. **C**. One half of the replication fork is shown. **D**. The leading strand demonstrates continuous replication because DNA polymerase can only synthesize new DNA in the direction 5′→3′. **E**. The lagging strand carries out discontinuous replication producing small fragments and requiring multiple RNA primers. **F**. After DNA polymerase synthesizes the new fragments, exonuclease must remove the RNA primers and the fragments are ligated together. **G**. DNA polymerase is unable to fill in the 5′ gap. H. The g-strand overhang is tucked into a protective t-loop structure.

superoxide, one of the first formed and most potent of the free radicals, into hydrogen peroxide. Hydrogen peroxide is also hazardous, but can be converted to water by the action of the enzyme catalase (Finkel et al., 2000).

(iii) Chain-breaking antioxidants neutralize the spread of free radicals without passing on their reactivity. This class of antioxidants can be made endogenously, but many important molecules are acquired through the diet.

(iv) Despite the aforementioned protection mechanisms some oxidative damage still occurs. A host of mechanisms in the cell can either repair or destroy and replace molecules damaged by free radicals (Halliwell et al., 2007).

With age, however, unrepaired damage accumulates resulting in disease and increased mortality (Finkel et al.,

2000). Oxidative stress, then, can be defined as the balance between free radicals and antioxidant defense mechanisms. If free radicals and antioxidants are in homeostatic balance oxidative stress is low, but if free radical generation exceeds antioxidant defense, oxidative stress is high resulting in oxidative damage (Monaghan et al., 2009).

In human cells, telomeres shorten by 50–300 base pairs per cell division (Harley et al., 1990; Aubert et al., 2008), but only approximately ten base pairs of this reduction is thought to be due to the end-replication problem (von Zglinicki, 2002). Much of the remaining loss is caused by oxidative stress. Compared with other regions of DNA, telomeres are particularly vulnerable to oxidative damage (Rubio et al., 2004; Houben et al., 2008). This apparently is due both to a relatively high guanine content (Henle et al., 1999; Oikawa et al., 2001) and also reduced DNA repair, possibly because shelterin proteins block DNA repair enzymes (Houben et al., 2008). Furthermore, oxidative stress exacerbates telomere loss – the amount of unrepaired oxidative damage to the telomeres influences the magnitude of telomere loss at the next cell division (von Zglinicki, 2002). Given that the vast majority of telomere shortening is a consequence of oxidative stress, these two proximate cellular aging mechanisms should no longer be viewed individually but as integral pieces of the larger aging puzzle (Houben et al., 2008). Telomeres may act as sentinels of the general level of DNA damage occurring in the cell. High levels of damage to the telomeres would be indicative of high levels of damage to the coding sequences. In this way, telomeres provide a mechanism to ensure that cells with high levels of DNA damage soon cease division (von Zglinicki, 2003).

2.2.3 Telomere restoration Different taxa possess different telomere restoration mechanisms. Some immortalized mammalian cell lines and tumors are able to maintain telomere length through recombination in a process termed ALT (Alternative Lengthening of Telomeres; Dunham et al., 2000; Aubert et al., 2008). Certain insects, such as Drosophila, utilize transposable elements to maintain telomere length (Cenci, 2009). However, the most common form of telomere restoration is through the enzyme telomerase. Telomerase is a ribonucleoprotein capable of rebuilding and maintaining telomeres (Greider et al., 1985). The telomerase enzyme consists of a reverse transcriptase protein (TERT) and a RNA template component (TERC). Telomerase activity is regulated at multiple levels including transcription, alternative splicing, assembly, localization, and post-translational modification (Hug et al., 2006). Telomerase activity in most cell lines is not sufficient to prevent telomere loss (Engelhardt et al., 1997; Lansdorp, 2005), and thus telomeres in tissues like blood cells shorten with age. Interestingly, oxidative stress also dramatically decreases TERT activity (Borras et al., 2004; Kurz et al., 2004) and therefore oxidative stress not only hastens telomere shortening by direct damage to telomeres, but also by inhibiting telomere restoration. While telomerase activity appears to be essential for telomere maintenance, it is repressed in most normal adult somatic tissues, probably as a mechanism to prevent tumor growth (Taylor et al., 2000; Parwaresch et al., 2002).

3 Telomeres and Life History

The concept of trade-offs is central to our understanding of the evolution of life histories. Differences within and among species in life history strategies are generally framed in terms of differences in the optimal allocation of resources among growth, reproduction, and self-maintenance. Identifying mechanisms that underlie variation in survivorship should provide insight into the evolution of life history strategies and phenotypic variation in longevity. Two candidate mechanisms that may link molecular and cellular mechanisms with organismal processes such as growth and survival are oxidative stress (Costantini, 2008; Monaghan et al., 2009) and telomere dynamics (Monaghan et al., 2006). As discussed above these two cellular aging mechanisms are closely connected, and while we focus on telomere dynamics below it is important to note that telomeres can be used as a biomarker for chronic oxidative stress (Houben et al., 2008). Therefore many of the conclusions we draw about telomeres role in mediating life history trade-offs is likely to be shared by oxidative stress. In the following section we explore how telomere biology may shed light on some of the different processes that are traded-off against one another from a life history perspective.

3.1 Telomeres and survival

The relationship between telomere loss and advancing age is well established *in vitro* and *in vivo*. Detectable telomere shortening has been shown in humans (Harley et al., 1990; Aubert et al., 2008), non-primate mammals (Coviello-McLaughlin et al., 1997; Nasir et al., 2001), birds (Haussmann et al., 2002; Haussmann et al., 2003b; Haussmann et al., 2008a), reptiles (Scott et al., 2006), and fish (Hatakeyama et al., 2008; Hartmann et al., 2009). Most of these studies are cross-sectional in

nature, making them subject to cohort effects or selective mortality (Haussmann et al., 2008b), but data from longitudinal studies are beginning to accumulate and confirm the age-related declines in telomere length. Longitudinal studies also demonstrate that telomere loss is much faster early in life (Hall et al., 2004; Baerlocher et al., 2007; Aviv et al., 2009; Salomons et al., 2009), probably because growth and cell division is most rapid at this time. Recent work in free-living jackdaws *Corvus monedula* showed that within individuals, long telomeres shorten more rapidly than short telomeres regardless of subject age (Salomons et al., 2009). Concurrently, an unrelated study in humans also showed that telomere attrition was greatest in individuals with long telomeres (Nordfjall et al., 2009). Taken together, the results of these studies suggest that a telomere maintenance mechanism exists *in vivo* that preferentially protects the shortest telomeres from further degradation. Even though telomere loss is variable at different ages and in different tissues, the gradual loss of telomeres with advancing age allows for the possibility of telomere-based age estimation (Haussmann et al., 2008a). While this method will never provide completely precise age estimation, it can provide some information on age structure in natural populations where longitudinal data are limited (Haussmann et al., 2003a; but see Juola et al., 2006).

The big question in the context of life-history tradeoffs is how changes to telomeres at the cellular level influence organismal survival. As mentioned previously, telomere loss and restoration have costs and benefits that need to be balanced. For example, reducing telomerase activity can serve as a tumor protective mechanism, but it also hastens cellular senescence. While the replicative potential of cells in culture is positively correlated to the longevity of the species they came from (Rohme, 1981), data at the organismal level is less clear (Hornsby, 2002; Hornsby, 2006). In humans studied over a 20 year period, individuals > 60 years of age with shorter than average blood cell telomeres had lower survival, due to higher mortality from heart and infectious disease, than did individuals of the same age with longer than average blood cell telomeres (Cawthon et al., 2003). While similar patterns of telomere length and survival were found in other human studies (Honig et al., 2006; Bakaysa et al., 2007; Kimura et al., 2007), there is additional human work that shows no clear association between telomere length and survival (Martin-Ruiz et al., 2005; Bischoff et al., 2006; Njajou et al., 2009).

Studies of telomere length and survival are also accumulating in wild populations. Yearling female tree swallows *Tachycineta bicolor* with shorter than average telomere lengths were less likely to return to the breeding site in subsequent years than those with longer than average telomere lengths (Haussmann et al., 2005). In comparison to human studies, this suggests that telomere maintenance is associated with early survival, and not just late-life mortality. In other avian studies, individuals with the highest telomere loss rate also have the lowest likelihood to survive (Pauliny et al., 2006; Bize et al., 2009; Salomons et al., 2009). A study of alpine swifts *Tachymarptis melba* reported that telomere dynamics were better predictors of survival than age (Bize et al., 2009). In addition, work done in free-living jackdaws *Corvus monedula* demonstrated that telomere shortening rate predicted survival, and that rate of telomere shortening was greatly accelerated during an individuals last year in the colony (Salomons et al., 2009).

Taken together, the studies on human populations and wild avian populations suggest that the relationship between telomeres and survival may depend on the age of the individuals studied. The discrepancy in the human data may be because these studies focus on individuals nearing the end of their species maximum lifespan. In contrast, the avian studies sampled either very young individuals or individuals across their lifespan. Work in an extremely long-lived bird, the Leach's storm-petrel *Oceanodroma leucorhoa* showed that there is age-specific selection based on telomere length; only those young birds with the longest telomeres survive to old age (Haussmann et al., 2008b). Given these findings, variation in telomere length likely decreases with advancing age in a population as those individuals with the shortest telomeres die. In studies focusing only on the oldest subset of individuals there may not be enough variability in telomere length to see a clear pattern with survival or no relationship may exist between telomere length and survival in this biased subset of the population.

3.2 Telomeres and lifespan

We know very little about physiological constraints on the evolution of life-history traits in general, and, in particular, about physiological and molecular adjustments that accompany the evolution of variation in lifespan (Ricklefs et al., 2002). Elucidating factors that influence lifespan in wild populations, especially those that may mediate life history trade-offs, is a major focus of evolutionary ecology. One such factor may be telomeres, and one might expect that particularly long-lived species would have relatively long telomeres. To date, there is only one phylogenetically-controlled

study exploring telomere length and lifespan. In a comparison of 15 rodent species, no relationship was found between telomere length and lifespan (Seluanov et al., 2007). In addition, laboratory mice vary widely in their telomere lengths (10 kb–200 kb; Kipling et al., 1990; Hemann et al., 2000), although this variation does not correlate with lifespan differences. Jemielity et al., (2007) explored the relationship between telomere length and lifespan in ants *Lasius niger*, a species with markedly different lifespan in different castes. While long-lived queens (up to 28 years) have longer telomeres then short-lived males (2–3 months), there is no difference in telomere length between queens and workers (1–3 years).

Although absolute telomere length might not explain differences among species in longevity, the rate at which telomere erosion occurs might be more important. One study explored how the rate of telomere shortening is related to interspecific variation in lifespan. In a comparison of 5 avian species, the rate of telomere shortening is inversely related to maximum lifespan; shorter-lived species show greater telomere loss per year than longer-lived species (Haussmann et al., 2003b). Subsequent work has been consistent with the pattern as long-lived great frigatebirds *Fregeta minor*; (Juola et al., 2006) and northern fulmars *Fulmarus glacialis* (Haussmann et al., 2008a), have much slower loss rates than short-lived species. A survey of the mammalian literature also demonstrates a relationship between the rate of telomere shortening and lifespan (Haussmann et al., 2003b). Further comparative work is needed to determine whether this pattern holds in other taxa, but given the variable length of the g-strand overhang and size of the t-loop in different species (Aubert et al., 2008), it seems that both absolute length of telomeres and the rate at which they shorten is important in the accumulation of critically short telomeres and cellular senescence (Haussmann et al., 2008a).

Comparative work exploring the relationship between telomerase and lifespan among species is also increasing. Because telomerase activity allows for unlimited cellular proliferation, long-lived organisms are thought to down-regulate telomerase at early developmental stages as a tumor-protective mechanism (Wright et al., 2001; Djojosubroto et al., 2003). A comparison between mice and humans does in fact show that humans down-regulate telomerase in most tissues whereas telomerase activity is high in many rodent tissues (Forsyth et al., 2002). However, telomerase activity in other domesticated animals has shown no clear pattern with lifespan, with some species showing human-like patterns (horses - Argyle et al., 2003; sheep - Cui et al., 2003) (domestic cats - McKevitt et al., 2003) (domestic dogs - Nasir et al., 2001), and other species have telomerase profiles more similar to laboratory mice (pigs - Fradiani et al., 2004).

A phylogenetically controlled comparison of telomerase activity in 15 rodent species showed that telomerase activity does not coevolve with lifespan but instead coevolves with body mass; larger rodents appear to repress telomerase activity in somatic cells (Seluanov et al., 2007). In mammals, this suggests that body mass, and not lifespan presents a greater cancer risk, and large mammals evolve repression of telomerase activity to mitigate that risk. Alternatively, in four species of birds, telomerase was measured in a variety of tissues at three ages and the longest-lived species tended to have the highest telomerase activities regardless of body mass (Haussmann et al., 2007). This suggests that telomerase activity in bone marrow may be associated with the rate of telomere loss in birds; birds with lower rates of telomere loss and longer lifespans have higher bone marrow telomerase activity throughout life. More comparative work in broader taxonomic groups that control for phylogeny is needed to examine the pattern between telomerase, body mass, and lifespan; and whether differences in telomerase activity impacts telomere loss and survival.

4 Physiological Stress: The Glucocorticoid Stress Response

Environments are unpredictable, and the ability to acclimate to an ever-changing environment to maintain homeostatic balance is advantageous. For organisms in natural populations, environmental stressors such as temperature change, food scarcity, and predators are particularly important. The vertebrate “stress response” is a suite of integrated physiological response mechanisms regulated primarily by the endocrine system, which allows organisms to cope with stressors. The cascade of hormones released during a stress response prompt a reallocation of resources to physiological processes and behaviors that maximize chances of survival. Two of the major hormone classes involved are catecholamines, including epinephrine and norepinephrine, and steroid glucocorticoid hormones (GCs), such as cortisol and corticosterone. Secretion of these hormones is regulated in part by a negative feedback system. Both physical and psychological conditions at the time of activation of the stress response impact an organism’s re-

sponse; thus the physiological state of the organism must be taken into account when evaluating the levels of stress (McEwen et al., 2003; Romero 2004).

The Hypothalamic-Pituitary-Adrenal (HPA) axis is responsible for the secretion of glucocorticoids. In ecological studies, the release of glucocorticoids is the most common metric used to measure the stress response. Glucocorticoids act to mobilize energy stores and also to inhibit other physiological systems (e.g reproduction, immune function, growth) in order to conserve energy during the stress response. Glucocorticoids also act on the brain to increase appetite and to increase locomotor activity and food-seeking behavior, thus regulating behaviors that control energy intake and expenditure (McEwen et al., 2003). To maintain normal physiological function, glucocorticoids are secreted at a baseline level, although there is currently controversy whether baseline levels of glucocorticoids are a reliable indicator of organismal fitness (Bonier et al., 2009). During stress, glucocorticoid secretion increases in part to mobilize more metabolic fuel to cope with the stressor, and once the stress is overcome glucocorticoids return to a baseline level. While the immediate stress response provides significant benefits in the short-term, the stress response may be detrimental and even fatal if activated for the long-term (Sapolsky et al., 2000). Long-term oversecretion of glucocorticoids is referred to as chronic stress (McEwen et al., 2003; Romero, 2004). Repetitive challenges to homeostatic balance, for example in the form of environmental irritants, poor health, social status, unpredictable environments, or work-induced anxiety in humans result in chronic stress, although the degree to which animals experience chronic stress in the wild is unclear (Goymann et al., 2004).

One idea that has permeated gerontology for a century is that physiological stress accelerates the aging process. Organismal aging is broadly defined as a set of cumulative, progressive, intrinsic, deleterious changes that result in damage to cells and tissues. Over time this "wear and tear" results in increased mortality. In this way, aging accommodates theories of stress physiology, which hypothesizes that risk of disease can be increased and exacerbated by prolonged exposure to psychological or physical challenges (Sapolsky, 2004). To better elucidate the link between physiological stress and oxidative stress, Epel et al. (2004) connected data on chronically stressed individuals with measures of oxidative stress and telomere shortening. They found that women with higher levels of stress, by both an objective and subjective measure, had shorter telomeres, lower telomerase activity, and higher oxidative stress compared with women with lower levels of stress (Fig. 2). This suggested physiological stress may directly influence premature cellular senescence as the lymphocytes of the stressed woman had aged an equivalent of 9–17 more years based on telomeres loss in comparison to the low stress woman (Epel et al., 2004).

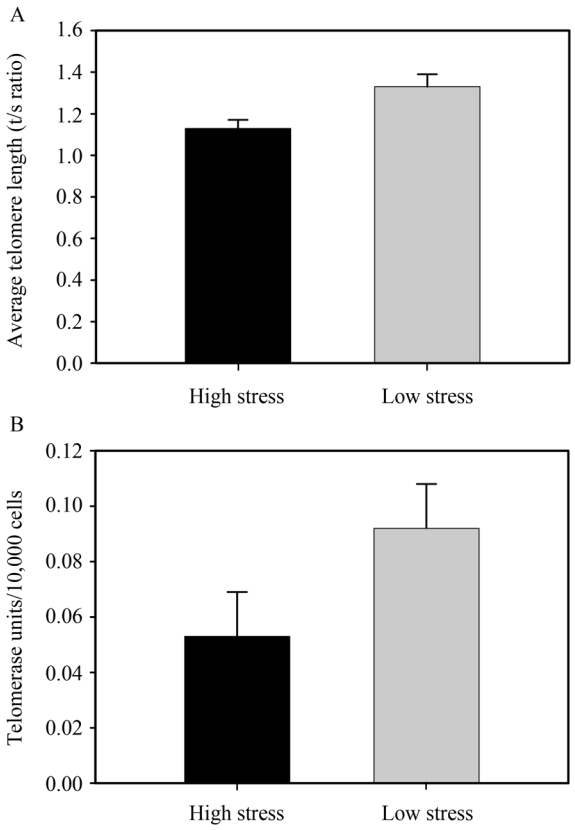

Fig. 2 Telomere length and telomerase activity of mother's with either a healthy child or a chronically ill child

Mother's of chronically ill children were more likely to have high perceived stress scores while mothers of healthy children were more likely to have low perceived stress scores. A. Average telomere length and SE. B. Average telomerase activity and SE. The high-stress group had shorter telomeres and lower telomerase activity even after controlling for age and BMI. (Reproduced from Epel et al., (2004) with permission of Copyright (2004) National Academy of Sciences, U.S.A.)

5 Linking Stress Hormones with Oxidative Stress and Telomeres

Identifying the mechanisms underlying variation in survival provides important insight into the evolution of life history strategies and phenotypic variation in longevity. Glucocorticoids are often employed as indi-

ces of physiological condition or individual fitness (Wikelski et al., 2006; Bonier et al., 2009), although there is conflicting evidence connecting organismal survival as a consequence of either an acute stress response (Breuner et al., 2008) or baseline glucocorticoid levels (Bonier et al., 2009). While glucocorticoids are sure to impact organismal survival in a number of ways, the recent biomedical evidence linking elevations in stress hormones with elevated oxidative stress presents a new model of one way that glucocorticoids may impact survival. Here, we present a conceptual model that explores the links among the glucocorticoid stress response, oxidative stress, telomere dynamics, and survival (Fig. 3).

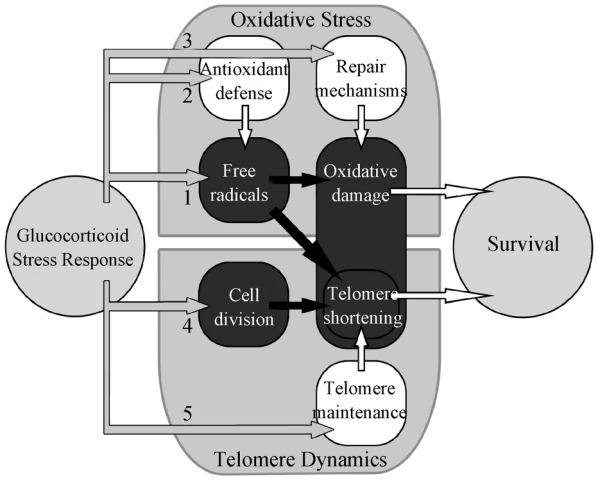

Fig. 3 Proposed connections among the glucocorticoid stress response, oxidative stress, telomere dynamics, and survival

At the center of the model are two gray rectangles representing oxidative stress and telomere dynamics. Oxidative stress is the balance between free radical generation, antioxidant defense and oxidative damage repair. These three mechanisms determine the current level of oxidative damage (dark arrows and boxes show a positive effect while white arrows and boxes show a negative effect). Telomere dynamics is the sum of telomere loss mechanisms, the end-replication problem and free radical damage, and telomere maintenance mechanisms, like the enzyme telomerase. These mechanisms determine the current telomere length (dark arrows and boxes show a positive effect while white arrows and boxes show a negative effect). Both high levels of oxidative damage and short telomeres are thought to decrease survival (white arrows moving out to the right of the gray rectangles showing a negative effect on survival). Recent evidence suggests glucocorticoids modulate telomere dynamics and oxidative stress (arrows moving into the left of the gray rectangles – gray arrows represent unknown or tentative relationships). Glucocorticoids may impact oxidative stress by altering free radical generation (arrow 1), antioxidant defense (arrow 2), or oxidative damage repair (arrow 3). Glucocorticoids may impact telomere length by altering cell division (arrow 4) or telomere maintenance (arrow 5).

There is increasing evidence that oxidative stress and telomere dynamics are important in mediating life history trade-offs. The underlying regulation of both of these processes has been well studied over the past few decades and remains a hotbed of current research. This work was briefly reviewed in the first part of this contribution and is summarized by the central gray rectangles in Fig. 3. Notice that while telomere dynamics and oxidative stress are separate processes, some mediators of oxidative damage also impact telomere shortening, and thus, telomere length can be viewed as an integrative measure of both telomere dynamics and oxidative stress. Both oxidative stress and telomere dynamics are thought to influence survival (Fig. 3, arrows leaving the right side of the central gray rectangles), and in this review we concentrated specifically on how telomere dynamics relate to aging and survival in natural populations. Other recent reviews have concentrated on how oxidative stress relates to survival in natural populations (Costantini, 2008; Monaghan et al., 2009).

Perhaps the most intriguing and certainly the most recent connection has been the relationship between physiological stress and cellular aging, but we know relatively little about underlying mechanisms of this connection (Fig. 3, arrows entering into the left side of the central gray rectangles). Here we synthesize what is currently known about how glucocorticoids impact oxidative stress and telomere dynamics. We highlight recent advances that have uncovered important connections and also point to areas where more data is needed to determine either the existence or direction of those connections.

5.1 Glucocorticoids and oxidative stress

Glucocorticoids may have diverse effect on oxidative stress, and these effects can be summarized in three main categories: effects on free radical generation (Fig. 3, arrow 1), effects on antioxidant defense (Figure 3, arrow 2), and effects on oxidative damage repair systems (Fig. 3, arrow 3).

5.1.1 Glucocorticoid effects on free radicals Early work by McIntosh and Sapolsky (1996b; 1996a) showed that the presence of glucocorticoids in rat neuronal cell culture exacerbated the generation of free radicals. Since that time, there have been a growing number of studies both in rodents (Liu et al., 1999; Kotrschal et al., 2007) and humans (Cernak et al., 2000; Irie et al., 2001; Irie et al., 2003; Epel et al., 2006; Simon et al., 2006; Damjanovic et al., 2007) that find a connection between glucocorticoids and oxidative

stress. Other taxa have received less attention, although domestic chickens (*Gallus gallus domesticus*, Lin et al., 2004) and captive kestrels (*Falco tinnunculus*, Costantini et al., 2008) both show an increase in oxidative damage markers after chronic exposure to glucocorticoids.

The majority of studies infer glucocorticoids effects on oxidative stress by measuring oxidative damage, and this probably reflects the inherent difficulty in the direct measurement of free radicals because of their intrinsic reactivity and short half-lives (Monaghan et al., 2009). However, recent *in vitro* work has shown that blocking the glucocorticoid receptors via RU486, a glucocorticoid receptor antagonist, also blocks the glucocorticoid-mediated rise in free radical production suggesting that glucocorticoids regulate genes involved in free radical generation (You et al., 2009). We do not know the nature of these genes however, and more effort is needed to determine whether they alter free radical generation through increasing mitochondrial respiration rate or changing the proton-motive force across the membrane.

5.1.2 Glucocorticoids effects on antioxidant defense Glucocorticoids may promote oxidative stress by disabling either enzymatic antioxidants or dietary antioxidants. The effects of glucocorticoids on enzymatic antioxidants have been the best studied, and in many cases glucocorticoids decreases enzymatic antioxidant activity (Liu et al., 1999). However, other work has highlighted a more complex relationship between stress hormones and antioxidants then first imagined. Long-term *in vivo* supplementation of glucocorticoids in rats caused a decrease in Cu/Zn superoxide dismutase in the brain but an increase in the liver, while catalase was unaffected in the brain, but decreased in the liver (McIntosh et al., 1998). In another case, activation of the glucocorticoid receptor by hydrogen peroxide in vitro results in overexpression of the antioxidant thioredoxin (Makino et al., 1996), reiterating that antioxidant activity isn't only decreased in the presence of glucocorticoids. The effect of glucocorticoids on dietary antioxidants has received less study, and it is not known whether glucocorticoids affect the absorption of these important molecules. Even so, administration of either enzymatic or dietary antioxidants with glucocorticoids appears to have a protective effect on glucocorticoid-induced oxidative damage (Liu et al., 1999; Herrera et al., 2010). For example, while glucocorticoid treatment in newborn rats had detrimental effects on survival, the coadministration of glucocorticoids with either vitamin C or vitamin E improved survival, possibly by alleviating enhanced free radical production by glucocorticoids. Thus, the overall effect of glucocorticoids on antioxidant activity appears to be both situation and tissue dependent. Some of this variation is likely not only due to differences in the types and doses of glucocorticoids used, but also because glucocorticoids themselves serve different functions in different tissues or at different doses and durations of exposure. We also need a better understanding of whether upregulation of enzymatic antioxidants is a result of the glucocorticoids themselves or a response to glucocorticoid-mediated increases in free radical generation.

5.1.3 Glucocorticoids effects on oxidative damage repair systems There have been far fewer studies exploring the link between glucocorticoids and oxidative damage repair mechanisms (Fig. 3, arrow 3). While some studies report that psychological stress impairs the repair of oxidative damage, very few have specifically examined the effects of glucocorticoids (Gidron et al., 2006). Recently, in vitro work showed that short-term exposure to glucocorticoids induced a five-fold increase in DNA damage and pre-treatment with RU486 eliminated this increase. Interestingly, the glucocorticoids specifically interfered with DNA repair mechanisms in the cell (Flint et al., 2007). Taken together, research exploring the effects of glucocorticoids on antioxidants and repair suggests that a critical part of glucocorticoid-induced oxidative damage is through inhibition of these defense pathways. More work in these relatively understudied areas promises to uncover new insight into how physiological stress impacts cellular aging.

5.2 Glucocorticoids and telomere dynamics

While the majority of the studies on the relationship between glucocorticoids and oxidative stress have focused on measuring oxidative damage, in a similar way, the majority of the studies on the relationship between glucocorticoids and telomeres have focused on measuring telomere length. But like oxidative stress, glucocorticoids may impact telomere in diverse ways. These can be summarized in three main categories: effects on telomere length through increasing free radical generation (Fig. 3, central gray rectangles), effects on cell division and the end-replication problem (Fig. 3, arrow 4), effects on telomere maintenance (Fig. 3, arrow 5). The first of these three categories has been covered above, and the other effects will be summarized below.

The pioneering work of Epel and colleagues (2004) established that chronic stress resulted in an increased rate of telomere shortening and decreased telomerase activity (Fig. 2). Further work showed that elevated glucocorticoids were related to the negative effects on telomeres, suggesting that stress hormones mediate the destructive effect of stress on telomere maintenance (Epel et al., 2006). This work has been correlative and studies exploring the mechanistic links between glucocorticoids and telomere regulation have been limited. One intriguing possibility is that glucocorticoids shorten telomeres by increasing cell proliferation and the resultant end-replication problem. While glucocorticoids have been linked to apoptosis in certain tissues their role as a mitogen is less clear (Clark et al., 2003) and the link between glucocorticoids and cell division awaits further study. Recently, however, one potential mechanism linking glucocorticoids and telomere loss was proposed. Chronic exposure to cortisol *in vitro* down-regulates telomerase activity in activated human T lymphocytes. Specifically, this effect is caused by a reduction in the transcription of TERT, the catalytic component of telomerase (Choi et al., 2008), and it may be that elevated glucocorticoids hasten telomere loss through this mechanism.

6 Conclusions

As the number of studies increase on the connection between glucocorticoids, oxidative stress and telomere dynamics the relationships become more complex. That complexity serves as an interesting puzzle that invites more study. Rodent and human studies connecting stress to cellular aging are steadily increasing, but the taxonomic breadth of these types of relationships is currently unknown. For example, only one study to date has explored physiological stress and telomeres in non-human animals. Male and female wild-caught mice *Mus musculus* that were exposed to overcrowding stress had shorter telomeres than mice that were not stressed (Kotrschal et al., 2007). While these studies have helped to establish an interesting pattern, they often don't measure stress hormones or explore underlying mechanisms (Epel et al., 2004; Kotrschal et al., 2007; Tyrka et al., 2010). As we move forward in this fascinating field we need to continue to probe the causal link between glucocorticoids and cellular aging, and we need much more data on non-human animals and rodents, particularly those from natural populations.

The literature on the glucocorticoid stress response in natural populations is vast (Breuner et al., 2008; Bonier et al., 2009), while work on oxidative stress and telomeres in natural populations are just now beginning to accumulate (Haussmann et al., 2005; Monaghan et al., 2009; Salomons et al., 2009). We need to better understand how the glucocorticoid stress response is mechanistically linked to increased oxidative stress and telomere dynamics. In natural settings, linking the well-established study of stress hormones to the relatively new study of oxidative stress and telomere dynamics promises to provide answers to many interesting questions:

- Are populations in chronically stressful environments experiencing higher mortality due to an increase in oxidative damage and short telomeres?

- Life history trade-offs in general may be mediated in part by stress's effects on cellular aging. If increased investment into reproduction is partially accomplished through an increase in glucocorticoids, is this paid off in the long-term by decreased survival?

- Maternal allocation of glucocorticoids to the developing fetus or to the yolk in oviparous species may signal a stressful environment and result in offspring with a thrifty phenotype. Are the long-term costs of this thrifty genotype a hyperactive stress response and increased cellular aging?

- Stress has profound effects on immune function. Are some of those effects mediated by oxidative stress and telomere dynamics? For example, does chronic stress result in the rapid loss of telomeres leading to fewer possible cellular divisions of T lymphocytes and eventual immunosenescence?

These questions just begin to scratch the surface of how a better understanding of stress and cellular aging can shed light on important ecological questions. If the link between the glucocorticoid stress response and oxidative stress and telomeres is established in natural populations, it ties together two fields long thought to be important to organismal survival: stress physiology and aging biology. Establishing this integrative link will require continued collaboration between the biomedical community and physiological ecologists. Doing so would examine how physiological trade-offs are explained at the molecular level and shed light on how environmental perturbation impacts life history trajectories.

Acknowledgements We thank Morgan Benowitz-Fredericks for valuable discussion, Robert Mauck for helpful comments, three anonymous reviewers for constructive criticism, and NSF support to MFH.

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