ELSEVIER

Experimental Gerontology 37 (2002) 615–627

Experimental Gerontology

www.elsevier.com/locate/expgero

Hypothesis

The reserve-capacity hypothesis: evolutionary origins and modern implications of the trade-off between tumor-suppression and tissue-repair

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Received 17 August 2001; accepted 29 January 2002

Abstract

Antagonistic pleiotropy, the evolutionary theory of senescence, posits that age related somatic decline is the inevitable late-life by-product of adaptations that increase fitness in early life. That concept, coupled with recent findings in oncology and gerontology, provides the foundation for an integrative theory of vertebrate senescence that reconciles aspects of the 'accumulated damage' 'metabolic rate', and 'oxidative stress' models. We hypothesize that (1) in vertebrates, a telomeric fail-safe inhibits tumor formation by limiting cellular proliferation. (2) The same system results in the progressive degradation of tissue function with age. (3) These patterns are manifestations of an evolved antagonistic pleiotropy in which extrinsic causes of mortality favor a species-optimal balance between tumor suppression and tissue repair. (4) With that trade-off as a fundamental constraint, selection adjusts telomere lengths—longer telomeres increasing the capacity for repair, shorter telomeres increasing tumor resistance. (5) In environments where extrinsically induced mortality is frequent, selection against senescence is comparatively weak as few individuals live long enough to suffer a substantial phenotypic decline. The weaker the selection against senescence, the further the optimal balance point moves toward shorter telomeres and increased tumor suppression. The stronger the selection against senescence, the farther the optimal balance point moves toward longer telomeres, increasing the capacity for tissue repair, slowing senescence and elevating tumor risks. (6) In iteroparous organisms selection tends to co-ordinate rates of senescence between tissues, such that no one organ generally limits life-span. A subsidiary hypothesis argues that senescent decline is the combined effect of (1) uncompensated cellular attrition and (2) increasing histological entropy. Entropy increases due to a loss of the intra-tissue positional information that normally regulates cell fate and function. Informational loss is subject to positive feedback, producing the ever-accelerating pattern of senescence characteristic of iteroparous vertebrates. Though telomere erosion begins early in development, the onset of senescence should, on average, be deferred to the species-typical age of first reproduction, the balance point at which selection on this trade-off should allow exhaustion of replicative capacity to overtake some cell lines. We observe that captive-rodent breeding protocols, designed to increase reproductive output, simultaneously exert strong selection against reproductive senescence and virtually eliminate selection that would otherwise favor tumor suppression. This appears to have greatly elongated the telomeres of laboratory mice. With their telomeric failsafe effectively disabled, these animals are unreliable models of normal senescence and tumor formation. Safety tests employing these animals likely overestimate cancer risks and underestimate tissue damage and consequent accelerated senescence.

Keywords: Aging; Cancer; Teleomere; Trade-off; Senescence

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0531-5565/02/$ - see front matter
PII: S0531-5565(02)00012-8

1. Introduction

Why do we get tumors and *Why do we grow old*? These questions are part of a larger puzzle: How can a highly differentiated, self-repairing organism composed of millions, billions or trillions of cells live long enough, in a mutagenic environment, to reproduce, without a single cell escaping the developmental program and producing a deadly tumor? Mechanisms that allow for extensive tissue repair while inhibiting the production of tumors are major evolutionary innovations—prerequisites to the evolution of most vertebrate life history strategies.

2. Synthesizing two views of the aging process

2.1. Senescence: the evolutionary approach

All else being equal, longer lives provide more reproductive opportunities than shorter lives, therefore natural selection opposes senescence. Compared to the immense challenge of building a self-assembling, ten trillion cell organism (such as a human), maintenance should be relatively simple (Williams, 1957). Yet senescence is pervasive among vertebrates. Elaborating on Medawar (1952), Williams (1957) explained the evolution and persistence of senescence as follows: absent senescence, all lives would still be finite due to accident, starvation, predation and disease. As individuals are always at risk of death, selection favors early reproductive opportunities over the potential for later ones. Accordingly, selection is never more efficient than at the age of commencement of reproduction (when potential is greatest), declining thereafter. Thus, traits that have beneficial effects in early life will tend to spread, even if inseparably coupled to deleterious later effects. Selection adjusts pleiotropic balances between longevity and youthful vigor: the greater the risk of death between reproductive opportunities, the stronger the bias toward youth, the faster the rate of senescence becomes.

Outside evolutionary biology Williams' argument has been persistently misunderstood to suggest that 'unselected effects' are the evolutionary cause of senescence (e.g. Campisi, 2001; Harley, 1997). Though the force of natural selection declines from the onset of reproduction, selection remains strong throughout the normal reproductive life-span, even as the effects of senescence are becoming increasingly evident. Further, adaptive variation among adult vertebrate forms leaves no doubt that selection retains substantial power *during* the process of senescence. Logical appeals to 'unselected' effects should be restricted to stages of life that were rarely if ever reached in the species' ancestral environment.

Williams (1957) clearly argued that selection continually minimizes deleterious effects that manifest during the period of reproduction and offspring-rearing. If we mistakenly believe that senescence is the product of unselected effects, then we may harbor unwarranted hopes for therapeutic reduction of senescence. Conversely, if we view senescence as the unavoidable costs that remain after selection has acted to minimize harmful effects, then we will correctly view senescence as the same daunting challenge for medical science that it has apparently been for natural selection. Even in the extreme cases of senescent failures that occur so late that they are likely inaccessible to selection (such as Alzheimer's disease), the effects are only out of selective reach *because* senescence has already evolved. Ricklefs and Finch (1995), extrapolating from mortality rates at the cusp of maturity, concluded that "…if not for aging, 95% of us would celebrate our centenaries and 50% would reach the seemingly astonishing age of 1200 years." It is therefore tautological to claim that senescence results from genetic effects out of selection's reach.

2.2. Telomeres and senescence: the experimental approach

Normal somatic cells, in vitro, undergo a limited number of divisions (Hayflick and Moorhead, 1961). The number of population doublings before the 'Hayflick limit' co-varies (between taxa) with life-span (Rohme, 1981) and may decrease in humans with age (Allsopp et al., 1992; but see Cristofalo et al., 1998). The ends of eukaryotic chromosomes consist of non-coding, repetitive sequences known as telomeres, which shorten slightly with each cell division. Telomere loss may explain the mortality of somatic cell lines, as the erosion of telomeres below a critical length appears to trigger the shutdown of

replicative machinery (Griffith et al., 1999). The reverse transcriptase *telomerase* elongates telomeres (Blackburn, 1992) acting in concert with telomere-binding proteins. Telomerase is active in gametogenesis (allowing germlines to avoid mortality) and undetectable in the vast majority of adult somatic tissues (Kim et al., 1994).

Several lines of evidence support the telomere-erosion hypothesis for Hayflick limits. (1) Telomere length diminishes with cell-line age in vitro (Harley et al., 1990). (2) Most *immortal* somatic cell lines (from tumors) lack Hayflick limits and express telomerase (Kim et al., 1994). (3) Somatic tissues from patients with Hutchinson–Gilford (H–G) and Werner's syndromes (diseases of apparently accelerated aging) have reduced proliferative capacities in vitro. H–G patients have short telomeres at birth (Allsopp et al., 1992). Werner's patients experience rapid erosion of initially normal telomeres (Faragher et al., 1993), and this erosion can, in vitro, be prevented with telomerase (Wyllie et al., 2000). The association of aberrant telomeres with apparently accelerated aging suggests that Hayflick limits may underlie a general mechanism of body-wide senescence, though causal links between 'cellular' and 'organismal' senescence remain to be established.

2.3. Telomeres and cancer

The potential significance of telomere regulation goes beyond senescence. It also appears central to the development of cancer, telomerase activation being prerequisite, in most cases, to the transformation from normal tissue to 'immortal' tumor (Kim et al., 1994). The apparent association of cancer and senescence with the same mechanism is not serendipity, it suggests a fundamental trade-off, the balance of which is unlikely to be medically improved.

2.4. The reserve capacity hypothesis

Juxtaposing an evolutionary perspective on senescence with the gerontological and oncological view of telomeres, we propose that proliferative limits of somatic cells are an antagonistic pleiotropy, one that evolved as a tumor suppressor that reins in runaway proliferation, but that unavoidably precludes indefinite somatic maintenance. We use the term *reserve capacity* to refer to the remaining proliferation that a cell or cell-line can undergo. Absent telomerase, reserve capacity decreases with each cell division.

When a cell is damaged such that it proliferates uncontrollably, the cell-lineage created ultimately reaches a fail-safe (the Hayflick limit) and proliferation ceases. The greater the reserve capacity of the progenitor cell, the larger the resultant mass of growth-arrested daughter cells. We regard this mass of cells as a *proto-tumor*, each constituent cell possessing the first of *several* mutations necessary for tumorigenesis.

If cells tend to retain more proliferative potential early in an organism's life, overgrowth-mutations should on average produce larger proto-tumors in younger individuals than in older individuals. Since each cell in a proto-tumor presents an equivalent opportunity for the acquisition of future, telomerase-activating mutations (the second step in tumor formation), we predict that a given mutagenic exposure in youth is more likely to initiate an eventual tumor than the same exposure late in life. Unfortunately, the mechanistic effect may be obscured by the fact that proto-tumors formed at an early age will also tend to have more time in which to accumulate further genetic changes. The risk from any particular proto-tumor should diminish with time, as growth-arrested proto-tumor cells are lost through normal cellular attrition. Risk reduction will be accelerated if apoptosis is triggered in some or all proto-tumor cells, an effect that would also accelerate the exhaustion of the neighboring lineages that replace the lost cells.

2.5. Uncompensated cellular attrition and increasing histological entropy: An explicit mechanism linking Hayflick limits to the phenomenon of vertebrate aging

Development continually increases histological differentiation and specialization, which are maximal at reproductive maturity. Throughout life, damage and programmed cellular turnover result in cells being lost from the soma and replaced. When cells provide their own replacements, positional information is not diminished in the tissue, and developmental order can be maintained. But proliferative limits prevent perpetual self-replacement. We propose that the uncompensated loss of some cellular lineages coupled with the replacement of other lineages by neighboring cell-lines (adapted to slightly different roles) or by

developmentally naive (pluripotent) stem cells, diminishes the optimal arrangement of cell types within aging tissues. As the percentage of post-development replacement-lineages increases, the positional information that newly placed cells derive from their neighbors (information that dictates cellular phenotype) becomes increasingly inaccurate, producing a progressive disordering in the arrangement of cells. By our model, body-wide senescence results from the combined effects of: (a) uncompensated cellular attrition and (b) increases in what might be called *histological entropy*, both of which will diminish a tissue's efficiency at accomplishing the tasks that cellular differentiation has evolved to address. Senescence of this type should accelerate with age as positional errors compound, and fewer cellular lineages maintain and repair an ever larger proportion of the body. Aging human skin appears to behave as we predict. Skin thickness decreases approximately 25% between the fourth and eighth decade of life (Black, 1969), and entropy increases:

> The epidermis of older individuals exhibits a marked variation in thickness (often in the same histologic section) and a disparity in the size, shape and staining quality of the basal cell nuclei under light microscopy. There is also a loss of the orderly alignment of cells along the basement membrane and a disruption of the gradual upward uniform differentiation present in the epidermis of younger individuals… Electron microscopic studies show that the basal cells of the flattened epidermis of old individuals lack villi… Deletion and derangement of small blood vessels is found in aged skin, with sun-damaged skin being the most severely affected (Balin, 1994).

There is disagreement regarding evidence that average telomere lengths decrease with donor age. Our model predicts at least some age-correlated reduction, but that pattern may prove difficult to measure because the replacement of expired lineages with unexpired lineages will produce sampling bias throughout the soma favoring cells with longer telomeres. Regardless, the senescence mechanism presented above does not depend on a significant reduction in *average* telomere lengths. Cell lines that expire and are lost without being replaced (uncompensated attrition) will contribute to a decrease in cell number, but not a reduction in average telomere length. Also, increasing histological entropy is, in principle, capable of generating symptoms of senescence (through informational loss) without an average decrease in telomere lengths.

Cardiovascular disease provides an example of what may be negative *consequences* of cellular attrition and histological entropy. Cells in portions of the vascular system that sustain relatively high levels of wear and tear have short telomeres, implying a history of cellular replacement (Chang and Harley, 1995) and likely attrition of cellular lineages. These areas fail to produce a protective layer of cells characteristic of younger tissue, and consequently have an increased propensity to develop atherosclerotic plaques (Chang and Harley, 1995).

2.6. One source, three sinks

In our model, vertebrates use reserve capacity in growth, maintenance, and repair. Each process erodes telomeres, reducing proliferative potential. Though the hypotheses of *antagonistic pleiotropy*, *accumulated damage* and *oxidative stress* have traditionally been viewed as alternative explanations for senescence, the reserve capacity approach integrates them. Damage, even if functionally repaired, will accelerate aging by reducing the capacity for future maintenance and repair. Any factor that damages tissue, including mutagens, pathogens and mechanical wear or trauma, will locally accelerate senescence. Even metabolic rate, long minimized by evolutionists as a factor in senescence, may play an important role. Creatures that employ chemical combustion to maintain an elevated body temperature are likely to have consistently elevated requirements for tissue replacement, as the byproducts of combustion are inherently destructive. Thus birds and mammals are likely to exhibit particularly rapid senescence compared to ectotherms that are otherwise similar. Consistent with this analysis, naked mole-rats (Bathyergidae) have extreme longevity relative to other small mammals and are also unique among mammals in that their body temperature is maintained only about 1 °C above ambient (Buffenstein and Yahav, 1991), reducing caloric requirements and, presumably,

decreasing exposure to the byproducts of caloric combustion.

Selection should tend to optimize reserve capacities integrating (1) age at reproduction, (2) normal rate of cellular repair and turnover, and (3) extrinsic risk of mortality. Although telomere erosion begins at whatever point in ontogeny telomerase is inactivated in the soma, selection should adjust reserve capacities so the loss of cellular lineages does not typically begin before the usual age of first reproduction. Further, in iteroparous species, selection should tend to coordinate reserve capacities among tissues so that senescence is synchronized throughout the body, minimizing the fitness cost of early senescence in any particular organ (Hamilton, 1966; Williams, 1957). But, because of the stochastic nature of environmental insults, the evolutionary coordination of tissue reserve capacities will not fully synchronize senescence within many individuals. An otherwise healthy individual may die from the premature senescence of a particular tissue, despite the synchronizing force of selection, if the tissue has had an unusual damage history. Stochasticity in damage exposure also potentially accounts for wide divergence in rates of senescence between genotypically similar individuals.

Selection for synchronization may be superceded in certain tissues where proto-tumors would be particularly costly. The circulatory system, which has very limited capacity for self-repair, is both prone to premature senescent failures, and highly resistant to tumor formation. We suggest that this is an evolutionary response to disproportionate harm that is likely caused when small growths occur in the heart and blood vessels.

Telomere lengths optimized for average species' parameters will be suboptimal for many individuals. The telomere length on a chromosome passed from a 170 cm tall father to his 185 cm tall son, for example, will likely be longer than optimal for the father and shorter than optimal for the son. This constraint may explain why the positive inter-specific correlation between body size and longevity (addressed in Williams, 1957) is reversed within species. Controlling for obesity, larger humans (Samaras and Elrick, 1999) and dogs (Li et al., 1996) tend to be comparatively short lived. The extra cell divisions required to become larger and, perhaps more significantly, to repair and maintain a larger body, are expected to diminish reserve capacity and thereby decrease longevity. We expect smaller individuals to suffer a greater per-cell risk of developing tumors due to longer-than-optimal telomeres at maturity. They should also show increased resistance to senescent effects. Since smaller individuals are composed of fewer cells, we do not expect their increased per-cell tumor risk to outweigh their decreased rate of senescence. Therefore, within a species, smaller individuals should be less prone to intrinsic sources of mortality than larger individuals. Gender bias in longevity might be at least partially accounted for by such an effect. This question could be addressed by comparing maximum longevity in species with larger males to that in species where male-male competition has favored a *reduction* in male size.

3. Reinterpreting experimental results

3.1. Senescent cellular phenotypes: misregulation or adaptive response?

At proliferative exhaustion, many cell types begin expressing genes that were previously untranscribed, and cease expression of previously active genes. Several authors have conjectured that organismal senescence results from the accumulation of cells with 'senescent phenotypes' that result from increases in genetic 'misregulation' due to selection's diminished power to regulate genes to the continuing benefit of the organism (Campisi et al., 1996; Ly et al., 2000). We propose a contrary interpretation: Williams (1957) argued that late negative effects would spread if pleiotropically associated with early benefits. He went on to argue that selection would then produce modifiers that would minimize the harm caused by these late effects. We suggest that 'senescent cellular phenotypes' are actually adaptations that *limit* the harm caused by the expiration of cellular lineages.

We propose that selection has produced a system that locally breaks down the extra-cellular matrix (ECM) as cells reach or approach Hayflick limits, thereby facilitating replacement by adjacent (or circulating) cells. Early in life, the ECM maintains the developmentally optimal placement of cells. But this system may impede cell movement. Selection may

have programmed senescent cells to locally dismantle the ECM, paving the way for their eventual replacement.

3.2. Lab mice and cloned sheep: life on strange islands

If individuals disperse from a high risk environment to a low risk environment (e.g. a remote island) the resultant increase in longevity will enhance the potency of selection on late-life effects, eventually slowing the rate of senescence (Williams, 1957; Austad, 1993; Reznick, 1997). We expect that, in such circumstances, selection increases telomere lengths. This adjustment would come at some cost, such as increased risk of tumors and/or an increased burden from larger proto-tumors.

In the early part of this century, a small number of *Mus musculus* dispersed into a novel environment: the laboratory. In breeding colonies there is no predation, no resource limitation and the spread of pathogens and contaminants is controlled. Perhaps most significantly, breeders are retired at 8 months (National Research Council, 1981) so the mice that contribute most to future generations are those that begin reproduction early, and sustain a high rate of reproduction until the cut-off age. Such conditions are dramatically different from those in the environment mice originally evolved to exploit, likely favoring a different pattern of senescence.

The telomere systems of laboratory mice are hard to reconcile with the notion of Hayflick limits as tumor suppressors, or as the cause of senescence. Compared to humans, lab mice have 'ultra-long' telomeres, exceeding human telomeres by an order of magnitude (Kipling and Cooke, 1990). Further, somatic tissues of lab mice produce telomerase, and can 'spontaneously immortalize' in culture.

One of us (BSW) predicted to Greider that long telomeres in laboratory mice would be atypical for mice in general. Hemann and Greider (2000) tested this prediction with a survey of telomere lengths in a number of mouse strains with shorter histories of captivity than typical lab strains. All strains tested had dramatically shorter telomeres, approximately one tenth the length of telomeres in common lab mice.

The unusual telomere system of lab mice may be an unintended consequence of captive breeding. Retirement of breeders after 8 months eliminates selection on late-life effects. Tumor-forming mutations take time to occur, tumors take time to become lethal, and the likelihood of tumor initiation is presumably a function of the number of cells in the body, so in small bodied animals like mice, tumors may be rare and inflict minimal cost in the first eight months of life, even absent a telomeric fail-safe. Further, selection for sustained high reproductive output (beginning early and maintained for 8 months) should strongly favor a reduction in senescent effects occurring in that window. Selection acting to eliminate senescent effects and increase early reproductive output may tend to elongate telomeres. Because of the inextricable connection between tumor suppression and somatic maintenance, telomere elongation should dramatically increase the risk of eventual tumor formation, but any effects manifesting after the breeding cut-off will be selectively irrelevant. By our model, selection for early high rates of reproduction in the absence of selection for longevity or tumor suppression should produce long telomeres and a strong propensity for eventual tumor incidence. Despite diminished senescence, we expect these mice to have reduced maximum longevity compared to wild conspecifics. At all ages, lab mice (with elongated telomeres) should be more likely to die of tumors than wild mice. These mice should also be unusually resilient to somatic damage and show few signs of aging other than tumor formation. Alexander (1966) presents evidence consistent with this pattern:

> The most striking fact is that even very old [lab] mice (e.g. more than 2.5 years) when killed while still fit have remarkably few pathologies and are almost indistinguishable from young animals.

The hypothesis that an 8 month breeding cut-off should select for non-senescent, tumor prone mice seems paradoxical. One might expect the elimination of selection on late life effects to *accelerate* senescence, not retard it. But in lab mice, selection for high, sustained rates of breeding appears to be the dominant factor. The tumor fail-safe has effectively been turned off, condemning these animals to form tumors, but leaving an early-life window of reproduction within which there is minimal senescent decline. This would likely not occur in much larger mammals,

which are slower to mature and composed of more cells. Absent a fail-safe, we predict the early production of tumors would not allow any reproductive window in such animals.

It has been widely assumed and asserted that 'ultra-long' telomeres are characteristic of 'mice' or even 'rodents' leading de Lange (1998) to argue:

> …it seems very unlikely that mice use telomeres as a tumor suppressor system and perhaps with good reason. Since the telomere barrier to proliferation does not manifest itself until many cell divisions have passed, this mechanism may not be useful for a small animal in which a 2 cm mass of misplaced cells could be life-threatening.

We agree that the telomere system of small animals would need to arrest very small growths to serve as a useful tumor suppressor, but the conjecture that 'mice' do not use this system is premature. The tissues of wild mice might have very limited reserve capacities, thus protecting them from lethal growths and limiting their life-spans.

To test the hypothesis that telomeric limits on the proliferative capacity of somatic cells underlie body-wide senescence, a strain of laboratory mice with two disabled copies of a gene necessary for telomerase activity was produced (Blasco et al., 1997). This telomerase-negative strain did exhibit apparently accelerated aging, but only after six generations and only in some tissues. These results strengthened the argument that telomere erosion is involved in somatic senescence, but suggested that the role of telomeres in the phenomenon of senescence might be limited to those few somatic tissues with high endogenous rates of turnover (Lee et al., 1998). The six generation delay was taken to imply that normal senescence, of the type that occurs in a single generation, must involve important undiscovered factors (Rudolph et al., 1999).

Telomerase-negative mice were created from stock with ultra-long telomeres. If they had been produced from stock with normal telomeres we predict that accelerated senescence would have been observed in the first generation. Even in such an experiment we expect that the gross acceleration of senescent effects would have been limited to high-turnover tissues because other tissues, which typically use reserve capacity to repair damage, will tend to senesce minimally in a protected environment.

Care must also be taken in interpreting the equivocal findings regarding the pattern of aging in animals produced through nuclear transfer cloning. It appears telomeres were essentially reset to a normal length, via reprogramming of telomerase activity during the blastocyst stage of development, in a series of calves cloned from cultured fetal and adult cell lines (Lanza et al., 2000). However, the sheep Dolly, cloned from an adult nucleus (Campbell et al., 1996), had shorter telomeres than a normal sheep zygote, though as yet Dolly does not appear to be senescing abnormally (Shiels et al., 1999). Like lab mice, Dolly lives in a controlled environment, protected from the traumas, illnesses and impurities of a wild or even a typical farm habitat. We expect Dolly to senesce earliest in tissues with high endogenous turnover rates (because her need for damage repair is likely to be minimal), and to display early senescence compared to sexually produced controls reared in the same protected environment. But compared to farm sheep, her senescence may not appear accelerated, as it is likely being slowed by her isolation from environmental insults. (note: as this paper was being revised an unpublished report was released by Campbell revealing abnormal arthritis in Dolly).

4. Selective inactivation of the telomeric tumor suppressor

4.1. The counterintuitive nature of early development

If finite reserve capacity is an evolved fail-safe against runaway cellular lineages, we must give special consideration to those times and places where selection has disabled this mechanism. In humans the majority of prenatal cell divisions occur before the end of the fifth month of gestation, while telomerase is active. The period of telomere maintenance ends, on a tissue-by-tissue basis, beginning in the fourth month and continuing through the fifth month (Ulaner and Giudice, 1997). In contrast, the vast majority of prenatal weight is gained after this point, as body fat is accrued. This pattern may have evolved to minimize the resources placed at risk by developmental telomerase activity. Further, maternal

aversion to chemically complex foods in early pregnancy may have evolved to isolate the fetus from mutagens during telomerase activity, when runaway cellular proliferation would necessarily result in abortion. Though fetal telomerase activity carries risks, a lack of telomerase during the period of rapid cellular doublings would result in a substantial erosion of the telomeres, accelerating the onset and rate of senescence later in life. Selection could counter this problem by lengthening germline telomeres, thus adding reserve capacity soma-wide. The fact that selection has favored early telomerase activity (and its associated risks) over a simple lengthening of telomeres, suggests that telomerase activity has a significant benefit. The benefit may relate to Williams' (1957) argument that selection should tend to synchronize senescence across the soma. If finite proliferative capacities determine the senescence rates of different tissues, and if those rates are to be synchronized by selection, telomere lengths must be adjusted according to the typical rates of cellular turnover expected in different parts of the soma. Simply lengthening germline telomeres could not produce this synchronization. Absent telomerase activity, the reserve capacity of a particular tissue would simply be an inverse function of the number of cell divisions that produced it from the zygote. In contrast, tissue-specific regulation of developmental telomerase activity timing can establish the inter-tissue synchronization of eventual senescence, at some added risk. This is least costly in early development when (1) the investment placed at risk is minimal, (2) the fetus is insulated from mutagens, and (3) the number of potential runaway cells is relatively small. In this model, the reserve capacity of mature tissues is adjusted through developmental modification of the number of progenitor cells in each tissue before telomere maintenance ceases. The demonstration that organ senescence is prenatally synchronized would unequivocally indicate that patterns of senescence are products of natural selection rather than unselected effects.

After fetal telomerase is shut down, our model predicts developmental cell divisions reduce adult reserve capacity. Wistar rats that were growth-retarded prenatally (i.e. during telomere maintenance), but grew to normal size after birth, had shorter telomeres in their kidneys and shorter life-spans than control rats (Jennings et al., 1999).

4.2. Cellular over-proliferation in early and late life: tumors of two natures

If the shortening of telomeres is part of an adaptive tumor suppressor mechanism, why are tumors most common late in life, when telomeres are likely to be shortest? Tumors may be divided into two classes: (1) tumors that arise when telomere lengths are exceedingly long or are being maintained by telomerase (these could occur at any point in the life-span); and (2) tumors arising after telomeres have become critically short (late in life or following tissue damage). Reserve capacity limitation appears to counter early life tumors so successfully that we may fail to realize that a serious threat would otherwise exist. The few systems in which telomere lengths are maintained provide a window into life without the telomeric fail-safe.

Most of the tumors common in the elderly are essentially unknown in young people. The most common childhood tumors, leukemias and lymphomas, arise from cells that must retain the capacity for hyper-proliferation in an immune response (e.g. B- and T-cells and their progenitors). Telomerase activity in such cells appears to greatly diminish the effectiveness of the telomeric failsafe, resulting in a disproportionate childhood risk of developing leukemias and lymphomas.

Testicular cancer is very rare in boys, and peaks between ages 20 and 34. Spermatogenic cells necessarily express telomerase during gametogenesis (Kim et al., 1994). The lack of a telomeric fail-safe beginning in puberty likely explains the disproportionate occurrence of testicular cancer in young men. In contrast, female mammal gametogenesis occurs in utero, and as might be expected, there is no increase in risk of germ cell tumors at puberty. Indeed, minimization of fitness costs associated with germline tumors may account for the evolutionary shift of female gametogenesis to fetal development.

Late-life tumors can arise by at least two pathways. A proto-tumor cell (descended form a progenitor that was genetically damaged such that it became insensitive to signals halting growth) may gain a second mutation that activates telomerase. This is statistically

unlikely in any individual cell, but since the many cells in a proto-tumor will all carry the initial over-growth mutation(s), the risk that one will gain an additional mutation increases with the proto-tumor's size. Independently, neither the over-growth mutation nor the telomerase activating mutation is sufficient to produce a tumor; both are required.

The second pathway does not depend on telomerase or a population of cells at increased risk. Typically cells cease proliferation when telomeres become critically short. But a cell carrying a mutation that prevents such arrest may continue to divide, eroding its telomeres *below* the threshold necessary to stabilize the chromosome ends, leading to instability and fusion into closed structures (Greider, 1999). This has dramatic, unpredictable effects and can lead to uncontrolled growth, even absent telomerase. The erratic telomere shortening and resultant chromosomal aberrations characteristic of Werner's syndrome results in both tumorigenesis and accelerated senescence.

4.3. A senescence 'rescue' mechanism: reactivation of telomerase in failing tissues

Telomerase is believed to be inactive in nearly all healthy somatic tissues of adults, but we suspect this is a significant oversimplification. Selection should balance the risk posed by the early senescence of heavily damaged tissues against the risk of tumorigenesis. If relatively early senescence of a tissue (as opposed to a cell line) threatens the survival of the individual, localized activation of telomerase may be a worthy risk. Evidence suggestive of such a rescue mechanism has recently come from two in vitro studies: Savre-Train et al. (2000) found that cell lines with critically shortened telomeres activate telomerase, and Figueroa et al. (2000) found that aging fibroblasts increase expression of a telomere–telomerase binding protein. If exhaustion of cellular reserve capacities was due to damage or age rather than hyper-proliferation, then telomerase can safely extend the life of the failing tissue. However, if the rescued section includes a proto-tumor, telomerase activation will likely result in tumorigenesis. We predict localized activation of telomerase to increase with age (as the body is increasingly threatened by organ senescence), and only a small subset of telomerase activation to be tumor-associated. Additionally, failure of telomerase reactivation may be relevant to H–G syndrome. H–G progeria is a homozygous recessive condition that we predict results from two inactive copies of a gene necessary for normal telomerase functionality. Without telomerase, the erosion of telomeres during early development would be substantial, and could account for the abnormal ontogeny and early onset of senescence in H–G patients. The inability to rescue senescent tissues by selectively reactivating telomerase may account for the rapid decline of H–G patients compared to normal elderly people. Consistent with our theory, and in contrast to the truly old, H–G patients rarely get cancer.

Several types of basal epithelial cells (which must proliferate extensively for normal functioning) express telomerase (reviewed in Greider, 1998). Yet basal layers are not a common source of tumors in young people. There are at least two reasons: first, the basal layer is protected from superficial contact with environmental mutagens. Second, progeny of the basal cells are sloughed from the body regularly, likely purging hyper-proliferative cells from these tissues before they become a danger (Cairns, 1975).

5. Conclusions

5.1. Antagonistic pleiotropy in retrospect

The above analysis suggests that the evolutionary theory of senescence (Medawar, 1952; Williams, 1957) was remarkably foresighted. Refinement is, however, in order. Tissue-by-tissue adjustment of reserve capacity may have effects across the soma that match the expectation of synchronization without the presumptive requirement of multiple distinct senescence-causing pleiotropies. Further, Williams' (1957) ostensibly falsifying prediction that an individual cannot be both unusually vigorous and unusually long-lived is likely false. We agree that individuals cannot be genetically predisposed to both, but *a propensity toward tumors*, coupled with either (1) *low exposure to mutagens* or (2) *luck regarding mutations* may allow an unusually vigorous, and long, life.

The belief that senescence evolves because the harmful effects of genes are invisible to selection late in life, and thus accumulate by drift, is inadequate to account for senescence as it progresses in

iteroparous organisms. Despite Williams' (1957) elucidation of this point, chronic confusion persists, with important implications for present and future work. A focus on genetic drift (unopposed by selection) as a causal agent may have produced misinterpretations of empirical patterns (e.g. senescent cellular phenotypes) and may have obscured others (e.g. inter-tissue co-ordination of reserve capacities). Most importantly, a failure to understand the active way that environmental hazards selectively adjust patterns of senescence has allowed haphazard breeding strategies to compromise model organisms such as mice. Inadvertent selection has altered model systems in ways that may camouflage the very patterns we seek to understand. Not only are common lab mice unfit for studies of aging and cancer, but because they have extraordinary reserve capacities, their use in the safety testing of drugs, pesticides and other agents is likely to underestimate somatic damage. Toxins prone to hasten cellular-attrition, thereby accelerating organ degeneration in humans, may appear harmless when administered (even in high doses) to mice with telomeres long enough to last six generations. The harmful effects on humans may be difficult to recognize if they manifest after a delay of many years and appear similar to normal effects of aging. We should therefore reconsider the use of substances deemed safe primarily because they proved harmless to 'mice'. At the same time, safety testing with lab mice may tend to overestimate cancer risks, leading to undue caution regarding some potentially valuable substances.

5.2. An optimal window of reproductive opportunity

Slowing senescence and reducing the threat posed by tumors are desirable goals. Shay and Wright (1999) have outlined a research plan to accomplish both:

> The key issue is to find out how to make our cancer cells mortal and our healthy cells immortal, or at least longer lasting. Inhibition of telomerase in cancer cells may be a viable target for anti-cancer therapeutics while expression of telomerase in normal cells may extend lifespan.

This illustrates the danger of isolating medical research from evolutionary biology. If one believes that senescence results from a lack of selection, then it seems reasonable to pursue a technological solution to fill in where selection leaves off. But evolutionary theory indicates that gradual senescence results primarily from trade-offs, not from incidental effects or a lack of selection. Longevity and tumor suppression are antagonistic goals. Our first question should be: *How well has selection optimized the balance between them*? It is not clear there is much room for improvement. We suggest that a staggering majority of our proto-tumor cells are already mortal, allowing only a miniscule risk of tumorigenesis in the first four decades of life. And it is likely that selection has already greatly extended our life-spans by modifying telomere lengths and coordinating the reserve capacities among our various tissues. It is a reasonable guess that maximum longevity cannot be greatly extended without a dramatic increase in the rate of tumor formation, and that increasing the effectiveness of telomeric tumor suppression would accelerate aging.

5.3. Medical applications

If a simple modification of telomere-system parameters would extend life without significant costs, it should already have spread due to selection. But this does not imply that medical benefits cannot be derived from technological telomere regulation. In fact, it holds great medical promise. Telomerase treatment, in vitro, may rejuvenate tissues or organs before transplant, extending telomeres in accordance with the amount of cell division expected to occur in the recipient (but see Wang et al., 2000). This may be particularly useful for liver transplants in which fractions of a divided liver grow to normal size in multiple recipients. Further, replacement tissues could be grown from a person's own cells, in the presence of telomerase, to provide a patient threatened by the premature senescence of a tissue with an MHC-matched replacement. This might be useful in treating early stage HIV patients. HIV-reactive T-cells might be removed early in the course of infection, maintained in vitro, and treated with telomerase. When the in vivo T-cell count begins to crash, the invigorated cells could be reintroduced into the patient where they might greatly extend the latent phase of HIV. Finally, given our increasing ability to detect and surgically or chemically eliminate tumors, we *might* one day be willing to accept an increase in

our tumor risk in order to extend youth. The in vitro lengthening of zygote telomeres would likely produce that heritable effect.

Avenues of research likely to lead to viable therapies are those to which natural selection has not had access (e.g. surgery and in vitro methodologies). The idea that medical science will improve the cell-by-cell regulation of telomerase in healthy people, thereby extending youth while at the same time reducing cancer risks, is wishful thinking of the highest order.

5.4. Historical note

The publication of Tyner et al. (2002), on a closely related topic, came as the current version of this paper was in final revisions at Experimental Gerontology. As Tyner et al appear to have been unaware of our theory (Weinstein and Ciszek, 2000, 2001), their serendipitous finding appears to provide strong empirical support for our model. Though their result arose from a presumed enhancement in p53 activity (rather than a change in telomere regulation), their hypothesis to account for their unanticipated result substantially overlaps our reserve-capacity hypothesis. We regard their emphasis on the role of the proliferative limits in stem cells to be a valuable contribution to the model, but would argue that our own analysis of lab-mouse telomere elongation is essential to the interpretation of the pattern they have unearthed. Further, since normal p53 is involved in triggering programmed cell death, enhanced expression likely hastens proliferative exhaustion, much as Tyner et al. suggest. But as such, enhancement of p53 likely acts generically to reveal the underlying tumor/tissue-repair trade off, just as any non-mutagenic agent that accelerates cell turnover would. Though p53 was clearly central to Tyner et al.'s experiment, we regard their analytic focus on p53's role in the trade-off to be somewhat misdirected. Weinstein and Ciszek (2000), which was declined scientific review at the same journal that later published Tyner et al. (2002), contains greater detail and additional references that could not be included here due to limits on space. It is being made available at www.telomere.org.

Acknowledgements

The authors are grateful for the excellent and tireless mentoring of Richard D. Alexander. George C. Williams was both an inspiration and a great help during the production of this work. Eric Weinstein's many skills were invaluable in the ironing out of various logical wrinkles. Useful observations and suggestions were contributed by Jennifer Ast, Phyllis Coley, Richard Dawkins, George Estabrook, Heather Heying, Laura Howard, Lisa Curran, Nat Kurnick, Thomas Kursar, David Lahti, Arnold Marglin, David Marshall, Drude Molbo, Courtney Murren, Randolph Nesse, Wayne Potts, Andrew Richards, Nancy Shefferly, Gerald Smith, Frank Solomon and Karen Weinstein. We are indebted to each of them for struggling with this complex topic so that we might come to understand it more completely. Michael Hemann and Carol Greider's test of the prediction regarding mouse telomeres was particularly valuable to us. This paper also greatly benefited from the insights of the editors, who invited the submission, and were patient and helpful through the process of its refinement.

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