Fifty Years of Skin Aging

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In developed countries, interest in cutaneous aging is in large part the result of a progressive, dramatic rise over the past century in the absolute number and the proportion of the population who are elderly (Smith *et al*, 2001). The psychosocial as well as physiologic effects of skin aging on older individuals have created a demand for better understanding of the process and particularly for effective interventions.

Skin aging is a complex process determined by the genetic endowment of the individual as well as by environmental factors. The appearance of old skin and the clinical consequences of skin aging have been well known for centuries, but only in the past 50 y have mechanisms and mediators been systematically pursued. Still, within this relatively short time there has been tremendous progress, a progress greatly enhanced by basic gerontologic research employing immunologic, biochemical, and particularly molecular biologic approaches (Figs 1, 2). ***Key words: photoaging/senescence/telomeres. JID Symposium Proceedings 7:51–58, 2002***

WHAT IS AGING?

Aging is a process perhaps best defined as decreased maximal function and reserve capacity in all body organs, resulting in an increased likelihood of disease and death. Aging occurs at the level of individual cells and is regarded by many authorities as a cancer prevention mechanism (Campisi, 1996), impeding the unregulated growth of cells whose DNA has been progressively damaged over the lifespan by internal and external mutagens.

PHOTOAGING *VERSUS* INTRINSIC AGING

Intrinsic skin aging is characterized primarily by functional alterations rather than by gross morphologic changes in the skin. Of equal or greater clinical importance is photoaging, the superposition of chronic sun damage on intrinsic aging. Photoaging is neither universal nor inevitable and is characterized by often striking morphologic and physiologic changes. Briefly, chronologically aged skin appears dry and pale with fine wrinkles, displaying a certain degree of laxity and a variety of benign neoplasms. In contrast, depending on the individual's skin type and degree of damage, photoaged skin may appear not only dry but also irregularly pigmented and (in darker-skinned persons) sallow, often displaying deep furrows in addition to fine wrinkling, or (in fair-skinned persons) severely atrophic, with multiple telangiectases and a variety of premalignant lesions such as actinic keratoses (reviewed in Yaar and Gilchrest, 2001).

Histologically, the most consistent change of intrinsic cutaneous aging is flattening of the dermal–epidermal junction. Also, there is a progressive decrease in melanocyte and Langerhans cell density. The dermis displays loss of extracellular matrix and increased levels of collagen-degrading metalloproteinases (Varani *et al*, 2000), loss of fibroblasts and vascular network, and, in particular, loss of the capillary loops that occupy the dermal papillae (Gilchrest *et al*, 1982b). The characteristic histologic changes of aged skin are associated with decrements in signal transduction pathways that influence protein phosphorylation. Specifically, there is decreased activity of growth factor associated mitogen-activated protein kinases and increased activity of stress-associated kinases (p38 and c-jun-amino-terminal kinase) (Chung *et al*, 2000). The main histologic change in photodamaged skin is dermal elastosis, the deposition of abnormal amorphous elastic material in the papillary dermis. Epidermal changes include variability in thickness accompanied by disorganized maturation and some cytologic atypia. Melanocytes are unevenly distributed in the basal layer, displaying areas of increased number of melanocytes and areas with reduced melanocytes. There is also a significant decrease in the number of Langerhans cells (Gilchrest *et al*, 1982a). Photodamaged skin frequently displays abundant inflammatory cells in the dermis, and collagen and elastic fibers show degenerative changes (Lavker and Kligman, 1988).

The term photoaging, coined by Kligman (1989), was a concept rediscovered in the twentieth century. In the late nineteenth century Unna and Dubreuilh, comparing the skin of farmers and sailors to that of indoor workers, recognized the devastating effects of solar irradiation on the appearance of facial skin. This insight was then lost until 1969, when Kligman published his landmark findings on the structural changes that occur in human skin as a result of sun damage, damage separable from the intrinsic aging process of the skin (Kligman, 1969). Lavker (1979) also described profound structural differences between sun-exposed and sun-protected skin.

FUNCTIONAL CHANGES

Early studies attempting to define physiologic changes that accompany aging skin date back to the 1950s and perhaps earlier. Similar to studies examining morphologic and histologic changes in aged skin, however, these studies were confounded by not

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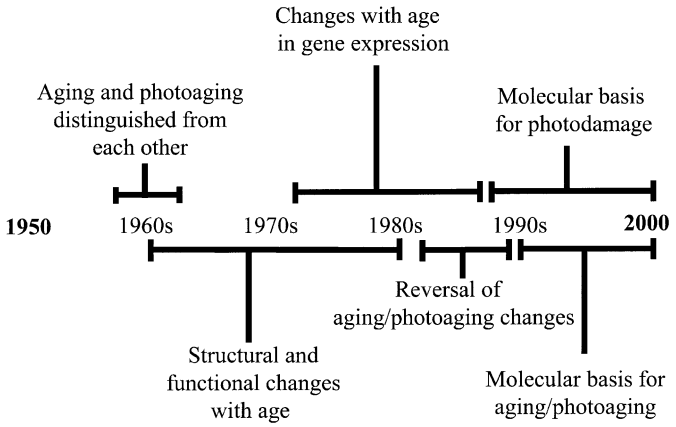

Figure 1. Timeline for skin aging research. In the 1960s Kligman published his findings on structural changes in human skin as a result of sun damage, distinguishing between chronologic aging and photoaging. A succession of reports have later described and detailed the structural and functional changes that accompany chronologic aging and contrasted them with photoaging. In the mid-1960s and through the mid-1980s, the structural and functional changes that accompany chronologic aging and photoaging were described. With the advent of molecular biology methodologies in the 1970s, numerous studies investigated changes in gene expression that are the result of aging, with interesting observations on age-associated alterations in the expression of genes encoding extracellular matrix proteins and enzymes involved in their degradation. Also, changes in the expression of genes whose protein products participate in cell cycle regulation were well documented. In the 1980s and 1990s, with the projection for a dramatic increase in the number of elderly people in the population, numerous studies focused on possible interventions in the aging process. Caloric restriction is probably the most convincing approach to date to slow aging in general, and topical retinoic acid the innovative approach to reverse cutaneous photoaging. Later in the 1990s, Voorhees and colleagues reported the molecular basis of acute photodamage responses. The pathways by which retinoids antagonize the response were demonstrated as well. During this period molecular mechanisms that underlie the aging/photoaging process were described as well.

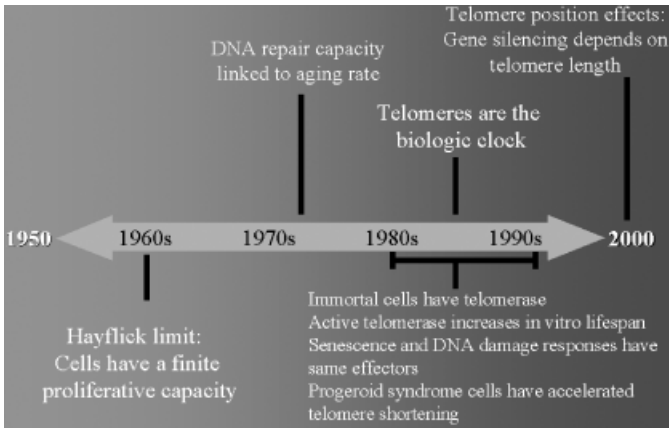

Figure 2. Timeline for cellular aging research. In the early 1960s Hayflick was the first to show that normal human diploid fibroblasts derived from fetal lung have a finite proliferative capacity. His studies were later confirmed and expanded by many investigators who demonstrated that all normal cells have limited proliferative lifespan. In the late 1970s correlations have been demonstrated between species lifespan, which is relatively constant among individuals of the same species, and the ability to repair DNA damage. Although first described in the mid-1980s, major interest in telomeres as the molecular basis for aging began in the 1990s with the observation that telomeres shorten during cellular lifespan, that immortal cells that have telomerase activity have increased *in vitro* lifespan, and that cells derived from individuals with progeroid syndromes display accelerated telomere shortening, suggesting that telomeres represent a biologic "clock" that determines the lifespan of the cell. Very recently, it was demonstrated that genes positioned near a telomere could be silenced as telomeres become shorter. Although the story is still evolving, it is possible that in the future studies will identify those pathways that are activated or silenced by critically short telomeres to provide a better understanding of the complex universal process of aging.

distinguishing between extrinsic and intrinsic aging processes. More interpretable studies measuring functional decrements that occur in non-sun-exposed skin (**Table 1**) date from the 1960s and 1970s.

Despite relatively little change in gross and histologic cutaneous appearance, maximal function and reserve capacity deteriorates with aging. This compromised function is particularly evident after injury. Briefly, functional decrements that are the result of intrinsic aging include slow wound healing due to decreased keratinocyte and fibroblast proliferative capacity (Gerstein *et al*, 1993), reduced cytokine production (Sunderkotter *et al*, 1997), decreased fiber synthesis, and delayed recovery of barrier function after damage (Elias and Ghadially, 2002). Also, the barrier to water loss is more easily disturbed in the elderly compared to young adults, and the time required to reconstitute competent stratum corneum is more than double in the elderly, in part because of decreased lipid synthetic capacity (Ghadially *et al*, 1995). Interestingly, female hormones were reported to significantly influence stratum corneum sphingolipid composition, suggesting that age-associated decreases in hormone levels may impact barrier function in the elderly (Denda *et al*, 1993). Moreover, in women, both bone mass and skin collagen decline rapidly in the immediate postmenopausal years, suggesting that estrogens influence collagen synthesis and degradation in both sites (Castelo-Branco *et al*, 1994).

In combination with age-associated changes in B and T lymphocyte function (Miller, 1996), decreased production of keratinocyte immune cytokines and decreased density of Langerhans cells lead to decreased cutaneous immune responsiveness with aging (Sauder, 1986). The decreased number of melanocytes is presumed to contribute to reduced protection against ultraviolet (UV) irradiation and to render the elderly more susceptible to UV-induced epidermal DNA damage (Gilchrest, 1984). Furthermore, DNA repair rate is decreased in the elderly, a loss shown to correlate inversely with mutation risk and skin cancer susceptibility (Moriwaki *et al*, 1996; Goukassian *et al*, 2002). Indeed, skin cancer incidence increases exponentially with aging independent of insolation (reviewed in Gilchrest, 1984).

An important endocrinologic function of human epidermis that declines with aging is vitamin D3 production. UV irradiation (< 320 nm) converts the epidermal precursor of vitamin D3, 7-dehydrocholesterol, to previtamin D3, which subsequently isomerizes to form vitamin D3. With aging, the levels of the epidermal precursor of vitamin D3 decrease, contributing to decreased vitamin D3 production in the elderly (MacLaughlin and Holick, 1985) and rendering them susceptible to vitamin D3 deficiency in the absence of regular sun exposure (Gloth *et al*, 1995).

Changes in vessel wall architecture contribute to vascular fragility, a common clinical occurrence in the elderly. In addition, changes in dermal vascular bed and vascular responsiveness lead to compromised thermoregulation, predisposing the elderly to heat stroke or hypothermia (Wagner *et al*, 1972; Bruck, 1974). Also, the reduction of vascular network around the hair bulbs and eccrine, apocrine, and sebaceous glands probably contributes to their gradual age-associated atrophy and as a result decreased sweat and sebum production. Decrements in vascular network are probably also responsible for decreased integumental reactivity and attenuated inflammatory response to a variety of stimuli

including injurious chemicals and UV irradiation. Changes in microvasculature may also diminish the rate at which substances are cleared from the dermis.

Finally, decreased perception of light touch, vibratory and corneal sensation, ability to discriminate two points, and spatial acuity have been documented in the elderly (Shimokata and Kuzuya, 1995; Stevens and Patterson, 1995). Available data do not allow differentiation between aging changes in skin innervation and altered central perception of stimuli, and both phenomena probably contribute to the age-associated decline in processing outside stimuli.

INSIGHTS INTO CELLULAR GERONTOLOGY (1950–2000)

Early tissue culture systems Studies by Carrel *et al* at the beginning of the twentieth century appeared to suggest that cells, once separated from the organism, could be maintained for an unlimited length of time (Carrel and Burrows, 1910), although this immortality was eventually found to be an artifact of the then-crude cell culture methodologies. Half a century later, Hayflick (Hayflick and Moorehead, 1961; Hayflick, 1965; 1977) showed that normal human diploid fibroblasts derived from fetal lung proliferated in culture only for finite periods of time, laying the foundation for modern cellular gerontology. He convincingly demonstrated that human fibroblasts eventually enter a permanent state of proliferative refractoriness and that the number of divisions before attaining proliferative senescence is inversely proportional to donor age.

Hayflick's seminal work was later expanded by other investigators throughout the world who demonstrated in a variety of cell types derived from different species, different tissues, and donors of different ages, that normal cells have limited proliferative lifespan. The exception to this rule appeared to be embryonic stem cells and germline cells (Suda *et al*, 1987). It was proposed that before differentiation into somatic cells, stem cells or germ cells possess the machinery required for continuous proliferation, a machinery that is subsequently lost upon their differentiation.

Cellular and molecular studies of skin aging Until the mid-1970s virtually all *in vitro* aging studies were performed using the system popularized by Hayflick (1965; 1977). In this system, fetal lung fibroblasts are serially passaged under well-defined culture conditions. Early passage "young" cells are compared to late passage "old" cells. Work by Schneider and Mitsui (1976), however, identified inconsistencies between the Hayflick model for cell aging and a newer model comparing early passage dermal fibroblasts from young adults *versus* old adults. In this newer model, cells are permitted to age in the organism and hence better reflect the *in vivo* aging process. Furthermore, this newer system allowed comparison between chronologic cutaneous aging and photoaging simply by comparing the behavior of cells obtained from photodamaged skin to that of cells obtained from sun-protected skin of the same individual (Gilchrest, 1979; 1980). Moreover, it allowed studies of cell types other than fibroblasts, such as keratinocytes and melanocytes, allowing for generalization of the conclusions and more confidence in the results (Rheinwald and Green, 1975; Gilchrest, 1983; Gilchrest *et al*, 1984; Medrano *et al*, 1994).

As in the Hayflick system, it was found that there is an inverse relationship between donor age and the number of population doublings achieved by cultured cells (Martin *et al*, 1970; Schneider and Mitsui, 1976). Subsequent publications established that dermal fibroblasts derived from patients with diseases of premature aging, such as progeria, Werner's syndrome, and diabetes, have shorter culture lifespans (fewer cumulative population doublings, CPD) than do cells derived from age-matched controls (reviewed in Stanulis-Praeger, 1989). Additional evidence of the relevance of culture lifespan to physiologic age was the observation that both keratinocytes and fibroblasts derived from habitually sun-exposed (photoaged) skin undergo fewer CPD prior to senescence than do the same cells derived from sun-protected sites of the same individuals (Gilchrest, 1979; 1980). The mechanism(s) of cellular aging and irreversible growth arrest remained unknown, however.

Keratinocytes, fibroblasts, and melanocytes also display a decreased short-term response to mitogenic stimuli with increasing donor age (Gilchrest, 1983; Plisko and Gilchrest, 1983). At least in the case of normal human fibroblasts there is age-associated decreased responsiveness to epidermal growth factor that can be ascribed to decreased number and density of receptors, as well as to decreased ligand binding, receptor autophosphorylation, and internalization (Reenstra *et al*, 1993; 1996). Keratinocytes derived from adult *versus* newborn donors also produce significantly less of the immunomodulatory cytokine now termed interleukin-1 (Sauder *et al*, 1988). Such findings suggest several mechanisms by which aging can compromise skin function.

Gene expression and aging With the advent of recombinant DNA technology, there has been an explosion of data regarding specific mRNA transcripts whose expression changes with age, both in early *versus* late passage cells and in cells derived from young *versus* old donors. Although the expression of many genes does not change, some transcript levels decrease with age, and some even increase.

Of relevance to cutaneous biology, the expression of extracellular matrix proteins and enzymes involved in their degradation appear to be affected with aging. Both in the early/late passage senescent model and the young/old donor model, fibronectin expression increases with age (Smith and Pereira-Smith, 1989; Kumazaki *et al*, 1993). Also, the expression of certain metalloproteinases, collagenase and stromelysin, increases as a function of serial passage (Millis *et al*, 1992; Zeng and Millis, 1996; Khorramizadeh *et al*, 1999) and donor age (Burke *et al*, 1994), whereas the level of the tissue inhibitor of metalloproteinases (TIMP-1) decreases (Burke *et al*, 1994; Khorramizadeh *et al*, 1999). These changes are consistent with the age-associated decrease in dermal thickness (reviewed in Yaar and Gilchrest, 1999), presumed to reflect a shift in the balance between synthesis and degradation of collagen.

With regard to genes that participate in cell cycle progression, a coordinated expression of genes that encode nuclear transcription factors, cyclins, and cyclin-dependent kinases (Cdk) takes place during the G1 phase of the cell cycle, and this program is dysregulated during aging and senescence (reviewed in Stein and Dulic, 1998). In response to serum stimulation, senescent cells fail to induce some of the genes that are induced in early G1 (immediate early genes), including c-fos that encodes a component of the AP-1 transcription factor (Seshadri and Campisi, 1990), and Id1H and Id2H, encoding helix–loop–helix proteins that are thought to act as inhibitors of differentiation (Hara *et al*, 1994). Senescent cells fail to express many genes that are expressed in late G1, including cyclin A, p34 kinase, and the E2F family of transcription factors that induce the expression of genes required for cell proliferation (Stein *et al*, 1991; Dimri *et al*, 1994). Senescent cells also fail to express several genes whose protein products are required for DNA synthesis, including dihydrofolate reductase, PCNA, and histones. Senescence does not affect the expression of genes encoding Cdk4/6 and Cdk2, c-jun, the other component of the AP-1 transcription factor, c-myc, and c-H-ras. Furthermore, certain genes like cyclin D1 and cyclin E are over-expressed in serum-stimulated senescent cells. These studies suggest that coordinated expression of an entire array of genes is required to enable the cell to progress through the G1 phase of the cell cycle, and that this coordination is compromised during aging.

The activities of other nuclear proteins that play a crucial role in cell cycle progression are not regulated at the transcription level but rather by the phosphorylated state of the protein. For example, phosphorylation of the retinoblastoma protein (Rb) is

a critical event required for cell cycle progression through G1, as phosphorylated Rb releases factors like E2F that are otherwise bound to the hypophosphorylated form of Rb and hence lack transcriptional activity (reviewed in Yaar, 2001). In contrast with quiescent cells, senescent cells fail to phosphorylate Rb despite serum stimulation, consistent with their inability to progress through G1 into S (Stein *et al*, 1990).

Direct inhibition of cell cycle progression proteins offers another mechanism of regulation. Notably, p21 ubiquitously inhibits cyclin–Cdk complexes (Xiong *et al*, 1993; Zhang *et al*, 1994). In fibroblasts, p21 transcripts increase gradually with increasing CPD and reach their highest level in early senescence (Noda *et al*, 1994). The levels of p21 transcripts and protein decrease to normal levels, however, within 2 mo of attaining senescence. In contrast, the expression of p16, another Cdk inhibitor, does not change with fibroblast CPD level but increases sharply during senescence and appears to remain elevated (Alcorta *et al*, 1996; Hara *et al*, 1996). These studies suggest that the persistent elevation of p21 is essential for entry into senescence, whereas p16 elevation is required to maintain the senescent phenotype.

Oxidative stress and aging Oxidative stress is considered to be a major contributor to the process of aging (reviewed in Harman, 2001). Oxygen, required for survival of aerobic organisms, readily accepts single electron transfers, generating reactive oxygen species (ROS) such as $\cdot O^{-2}$, H_2O_2, and $\cdot OH$ that can damage biologic molecules (Davies, 1995). Such damage continuously occurs throughout the lifespan of the organism and contributes to membrane peroxidation, DNA base alterations, single-strand DNA breaks, sister chromatin exchange, DNA–protein cross-links, carbonyl modifications, and loss of sulfhydryl groups in proteins (Stadtman, 1992). Oxidative stress also hastens replicative senescence of cultured fibroblasts (Chen *et al*, 2000; Dumont *et al*, 2000). Although cellular and mitochondrial defense mechanisms including the antioxidant enzymes glutathione peroxidase, glutathione reductase, and superoxide dismutases have evolved to quench ROS, these antioxidant defense systems are not fully efficient, and hence throughout life cells accumulate molecular oxidative damage, sometimes leading to apoptotic cell death (Stadtman, 1992; Ames *et al*, 1993). Several studies show that there is an age-associated increase in both ROS generation (Sohal and Brunk, 1992) and the level of oxidatively damaged proteins (Stadtman, 1992) and DNA (Ames *et al*, 1993; Agarwal and Sohal, 1994). Caloric restriction extends the lifespan in all species examined, including fish, spiders, rats, mice, and primates (Sohal and Weindruch, 1996). It was speculated that caloric restriction decreases the metabolic rate of the organism, reduces oxidative stress, and hence contributes to lifespan extension. Recent findings in *Saccharomyces cerevisiae*, however, show that caloric restriction increased the yeast's respiration rate implying that, as ROS are a by-product of respiration, lifespan extension is not the result of decreased ROS but may be the outcome of improved ability to detoxify these free radicals (Lin *et al*, 2002).

The role of telomeres in cellular aging The Hayflick limit, the finite "lifespan" or number of CPD for cultured human cells (Hayflick and Moorehead, 1961; Hayflick, 1965; 1977), implies that aging occurs at the cellular level and is the consequence at least in part of a genetic program. Subsequently, strong correlations were noted between DNA repair capacity and average lifespan in a large number of diverse species (Hart and Setlow, 1974) and between metabolic rate and average lifespan (Sohal and Allen, 1985). These findings suggested that aging was also in part the consequence of "wear and tear" from both internal and external environmental insults, with aging rate determined by the balance between incurred damage and its repair.

The first major advance in understanding the mechanism of cellular aging was the demonstration that critical shortening of telomeres underlies proliferative senescence of cultured human cells. First described in eukaryotic cells by Greider and Blackburn (1985), telomeres are tandem repeats of short base sequences, …TTAGGG… in mammals, at the end of each chromosome, that are required for chromosome stability.

Harley *et al* (1990) observed that increasing CPD of normal human fibroblasts is associated with shortening of their telomeres. Further work established that telomere length of cells obtained directly from tissues *in vivo* is inversely related to the individual's physiologic age, being shorter in cells derived from older *versus* younger adults (Harley *et al*, 1990; Allsopp *et al*, 1992) and in cells from patients with premature aging syndromes like Werner's and progeria *versus* age-matched controls (Allsopp *et al*, 1992; Schulz *et al*, 1996).

Interest in the biologic role of telomeres was heightened by the observation (reviewed in Harley *et al*, 1994; Harley and Kim, 1996) that almost all human malignant cells express the reverse transcriptase component of telomerase (hTERT) responsible for maintaining telomere length in germline cells but not normally considered to be expressed in somatic cells (reviewed in Harley, 1991). This finding suggested that prevention of progressive telomere shortening with each round of cell division was necessary for dysregulated cells to divide indefinitely and hence become sufficiently numerous to harm the host. This finding was expanded by the report that transfecting normal human cells (fibroblasts and retinal pigment epithelial cells) with hTERT appeared to immortalize them, increasing their *in vitro* lifespan at least several fold (Jiang *et al*, 1999). Further studies determined that in other cell types, such as keratinocytes, immortalization requires ablation of the Rb/p16 protein pathway, in addition to expression of telomerase activity (Kiyono *et al*, 1998). Importantly, hTERT expression did not alter the cells' behavior, only their proliferative capacity (Jiang *et al*, 1999). Transfected cells remained appropriately responsive to environmental signals and did not display characteristics of transformed cells, confirming the prediction that unlimited growth potential is necessary but not sufficient for malignant conversion.

Interestingly, although the current dogma states that normal somatic cells do not express telomerase, telomerase activity is found *in vitro* in normal epidermal keratinocytes (Yasumoto *et al*, 1996). Telomerase activity is also detected *in vivo* in normal human epidermis, primarily in the proliferative basal layer, but not in the dermal compartment of the skin or in cultured fibroblasts (Harle-Bachor and Boukamp, 1996). During the growth phase of human hair follicles, telomerase activity is detected in the bulb, the portion of the hair follicle that contains rapidly multiplying cells, and its activity in this part of the hair follicle is higher than its activity in hair follicle stem cells located in the bulge area (Ramirez *et al*, 1997). These findings suggest that telomerase expression may not be restricted to stem cells, germline cells, and malignant cells although its role in normal tissues remains to be elucidated.

In tandem with work elucidating the role of telomeres and telomerase in aging and malignancy, Yu *et al* (1996) identified the gene defective in Werner's syndrome, a recessively inherited classic premature aging syndrome in which development is relatively normal but many features of aging, including malignancies, become apparent in early to mid-adulthood and death usually occurs in the 40s or 50s. Although the precise *in vivo* function of the Werner's syndrome gene product is largely unknown, it is a DNA helicase/exonuclease and is thought to play a role in DNA replication and recombination during cell division and DNA repair (Gray *et al*, 1997; Huang *et al*, 1998; Shen *et al*, 1998; Fry and Loeb, 1999). The Werner's syndrome protein in particular unwinds G-rich DNA structures (Wyllie *et al*, 2000; Brosh *et al*, 2001) and has recently been implicated in telomere maintenance (Hisama *et al*, 2000; Ouellette *et al*, 2000). Recently, it was reported that patients with dyskeratosis congenita, a second condition characterized by premature graying of hair and cancer predisposition, have markedly shorter telomeres than normal individuals (Vulliamy *et al*, 2001). Finally,

in human skin specimens derived from donors of different ages, an inverse relationship was found between telomere length and donor age (Friedrich *et al*, 2000).

Throughout the 1990s, data implying interrelatedness of telomeres, aging, cancer, DNA damage, and DNA damage responses continued to accumulate. Most compelling were a series of reports demonstrating substantial overlap in the proteins mediating proliferative senescence, reversible cell cycle arrest, apoptosis, and DNA repair. For example, the p53 tumor suppressor protein and transcription factor, first identified in the late 1970s as a cellular protein that binds the SV40 large T antigen (Lane and Crawford, 1979; 1980; Crawford *et al*, 1980), was found to be upregulated immediately before cells entered the senescent state (reviewed in Stein and Dulic, 1998). The p53-regulated protein p21, centrally involved in DNA damage responses (Stein and Dulic, 1998), was independently identified as playing a major role in proliferative senescence in the absence of DNA damage (Noda *et al*, 1994), leading to its alternative designation as the senescent cell-derived (growth) inhibitor or SDI-1. Furthermore, it was noted that over-expression of proto-oncogenes such as ras and raf may induce proliferative senescence rather than increase cellular proliferation (Serrano *et al*, 1997; Zhu *et al*, 1998). Interestingly, E2F1, a transcription factor that regulates cell cycle progression, can induce senescence in human fibroblasts when over-expressed in the presence of p53 (Dimri *et al*, 2000). In addition, E2F1 together with p53 can induce apoptosis (Wu and Levine, 1994; DeGregori *et al*, 1995), suggesting that, depending on the cell type and specific experimental conditions, certain proteins may induce apparently the antithecal cellular behaviors of growth *versus* proliferative senescence or programmed cell death. These combined observations have led to the concept that aging, or at least proliferative senescence, is a fundamental cancer prevention mechanism in multicellular organisms, employing many of the same gene products as DNA repair (Campisi, 1996). Interesting *in vivo* observation consistent with this concept is the report that mice with amplified p53 activity as a result of a mutated p53 gene display signs of premature aging including decreased lifespan, premature hair graying, and cutaneous atrophy, as well as osteoporosis and internal organ atrophy (Tyner er al., 2002), in addition to the anticipated decrease in cancer incidence.

How or why telomere shortening might induce genes also involved in DNA damage responses is at present the subject of intense investigation. The de Lange group has reported that disrupting the telomere loop structure by ectopic expression of a dominant negative form of the telomeric repeat binding factor 2 ($TRF2^{DN}$) induces p53 and, in at least one cell type, the classic DNA damage response of apoptosis (Karlseder *et al*, 1999), and in another cell type a senescent phenotype (Van Steensel *et al*, 1998). Eller has observed that supplementing cultured cells with oligonucleotides substantially or completely homologous to the telomere 3′ overhang sequence TTAGGG also induces and activates p53 and generally results in cell behaviors observed after DNA damage (Eller *et al*, 2002a; 2002b). In combination, these findings suggest that an as yet unidentified nuclear sensor of this single stranded DNA sequence, which is normally concealed in the telomere loop, may be responsible for initiating these molecular pathways. Telomere loop disruption may occur after acute DNA damage, $TRF2^{DN}$ expression, or critical telomere shortening, all leading to exposure of the 3′ overhang (Li *et al*, in press) (**Fig 3**). Furthermore, this model provides a molecular connection between photoaging (UV-induced DNA damage) and chronologic aging (telomere shortening through replication), both pathways leading to telomere destabilization and exposure of the 3′ overhang. Of interest, in fibroblasts these oligomers and telomere loop disruption also induce the same gene products as serial passage and critical telomere shortening, leading to a senescent phenotype, allowing for the speculation that critically short telomeres may be stochastically unstable and hence prone to disruption of the loop structure with consequent exposure of the TTAGGG signal (Eller *et al*, 2002a; 2002b)

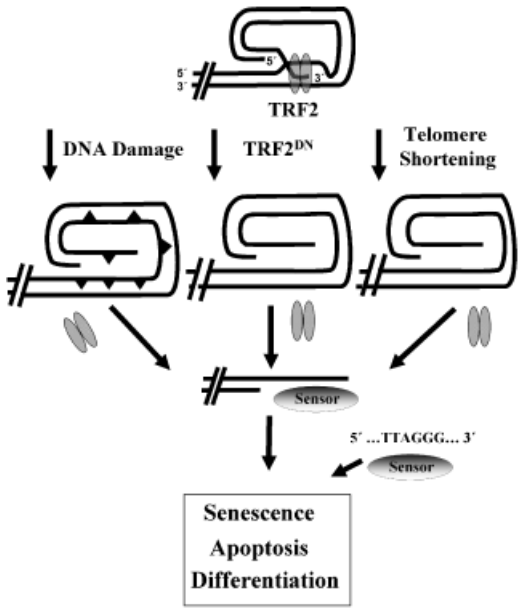

Figure 3. Proposed mechanism for induction of senescence, cell cycle arrest, differentiation, or apoptosis by exposure of the single-stranded telomere DNA sequence. The 3′ telomere overhang is normally sequestered within a loop structure stabilized by TRF2. Destabilization of this loop structure by DNA damage due to UV irradiation or chemical adducts, expression of $TRF2^{DN}$, or gradual erosion during aging is hypothesized to expose this single-stranded DNA (repeats of TTAGGG). Displacement of the TRF2 protein as shown in the figure might or might not accompany loop disruption under physiologic conditions. This single-stranded DNA is then detected by an as yet unidentified sensing mechanism. Interaction of this sensor with the 3′ overhang initiates a cascade of events. Depending on cell type and/or intensity and duration of the signal, these events might lead to cell cycle arrest, the eventual induction of senescence, differentiation, or apoptosis. In the present experiments, we hypothesize that DNA oligonucleotides homologous to the overhang sequence are recognized by the same sensing mechanism, triggering the cascade in the absence of telomere disruption.

One apparent anomaly in the accumulating evidence that aging results in some way from telomere shortening was the fact that the telomeres of mice, a widely used animal model with a relatively short lifespan (approximately 2–4 y), are far longer than human telomeres and do not shorten appreciably (as a percentage of initial length) over the murine lifespan (Kipling and Cooke, 1990; Starling *et al*, 1990). To further investigate this apparent exception to the rule, Greider, DePinho and colleagues created telomerase knock-out mice and observed the animals through multiple in-bred generations (Blasco *et al*, 1997; Rudolph *et al*, 1999). The first and second generation animals (G1, G2) appeared completely normal, but the G3–G6 mice developed gray hair and hair loss as early as 6 mo of age and delayed wound healing or chronic ulcers at 15–18 mo of age (Rudolph *et al*, 1999). G4–G6 mice also developed spontaneous tumors at a younger age and had shortened lifespans, phenomena suggestive of premature aging. Although later generations could not be studied because the G6 mice were infertile, telomerase null mice displayed a 4–5 kb decrease in telomere length per generation. These findings strongly suggest that, whereas lifespan in mice must normally be regulated differently than in man, critical telomere shortening, if achieved experimentally over several generations by inactivating telomerase in germline cells, ultimately has the same consequences as in human cells.

Caloric restriction, defined as reduction by more than half an animal's *ad libitum* food intake, had been observed to increase

lifespan and delay onset of age-associated diseases in many species, including mammals (reviewed in Lane *et al*, 1999). Although the mechanism of this effect is poorly understood, caloric restriction was widely presumed to act through reduction in oxidative damage that is secondary to ROS generated during cellular metabolism (Lass *et al*, 1998; Lee *et al*, 1999; Zainal *et al*, 2000). Employing a yeast model, Lin *et al* (2000) showed that lifespan extension by caloric restriction requires the presence of Sir2, an rDNA silencing protein, and nicotinamide adenine dinucleotide (NAD) that is necessary for Sir2 function. Lifespan extension of the yeast as a result of Sir2 activation is most probably due to decreased rDNA recombination and hence decreased senescence inducing extrachromosomal rDNA circles (Sinclair and Guarente, 1997). Also, as noted above, caloric restriction in this model increased the yeast respiration rate, suggesting that mechanism(s) other than decreased ROS production give rise to extended lifespan in this model (Lin *et al*, 2002). It is possible that Sir2 encodes proteins that have the capacity to quench ROS. Such proteins have not been identified to date, however. Although the extrapolation to higher organisms is not intuitively obvious, this work suggests molecular links between aging, oxidative metabolism, and genomic instability that might well apply to mammalian cells as well as to yeast. Very recently, using human HeLa cells, Wright, Shay, and colleagues provided compelling evidence that genes positioned near a telomere can be silenced, demonstrating a 10-fold variation in expression of a reporter gene depending on both its proximity to the telomere and telomere length (Baur *et al*, 2001). These experiments offer an explanation for the selectively altered gene expression patterns observed in late passage or old donor cells and again tie telomere length to the aging phenotype, with the caveat that aging is a complex process that occurs in nondividing cells as well as in proliferative ones and hence may be incompletely modeled by cultured cells (Rubin, 1997).

SUMMARY AND CONCLUSIONS

Skin aging has been well characterized clinically, histologically, and functionally. As in other organ systems, skin aging has been shown to have an intrinsic component and a variable environmental component termed photoaging. Since Hayflick's *in vitro* aging studies in the early 1960s, there has been an explosion of information regarding molecular mechanisms that regulate these aging processes. Aging is now conceptualized as a cellular safeguard against malignant transformation and aging rate as a balance between the rate at which DNA damage is incurred and repaired, with many of the same gene products mediating both DNA damage responses and proliferative senescence. Telomeres appear to play a central role. These observations suggest that, in skin as in all tissues, a common signaling pathway is activated both by critical telomere shortening after multiple rounds of cell division and by repeated environmentally induced DNA damage to yield the aging phenotype.

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