TWO

The Biology of Successful Aging: Watchful Progress at Biogerontology's Known–Unknown Interface

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This chapter is about the biology of aging. It offers a contemporary perspective on the kinds of ideas that biogerontologists wonder about. As investigators, we are particularly keen to highlight individual lines of inquiry that are leading to discoveries about the aging process. We know of no better way of realizing this goal than to situate our subject—the biological underpinnings of healthy longevity—as a moving target, a field in flux. Accordingly, this chapter sets out to sketch in sufficient detail the rich evolution of ideas in this ever-moving field of aging research, underscoring substantial advances and critical gaps in our understanding.

In this chapter, the term *organismal senescence* is used to describe the deteriorative changes that result in decreased viability and ultimately an increase in an organism's risk for mortality. In contrast, the term *aging* refers to any time-dependent changes that occur during the life course of an organism. Thus, aging encompasses changes that are good (e.g., increased wisdom), bad (e.g., arteriosclerosis), or inconsequential (e.g., baldness) in terms of their effect on viability and survival (Finch, 1990). It is an unfortunate reality that scientists and the public often use these terms interchangeably, resorting to using the term *aging* synonymously with deterioration.

Not all organisms undergo senescence at the same tempo (Finch, 1990). Humans exhibit gradual senescence (deterioration over decades), whereas other species, such as bamboo, have very rapid senescence (deterioration over days). There is an eclectic group of

organisms that exhibit negligible senescence; age-related deteriorative changes are virtually imperceptible in these species. Not surprisingly, organisms with negligible senescence—such as rockfish, bristlecone pine, and possibly the naked mole rat—are currently the subject of great scientific interest (Finch, 1998, 2009; Finch & Austad, 2001; Pérez et al., 2009).

Understanding the mechanisms of organismal senescence and how morbidity can be compressed, culminating in extension of a healthy life span, is the goal embraced by most biogerontologists (Fries, 2003; Olshansky, Hayflick, & Carnes, 2002). These aims must be clearly articulated to the public (Binstock, 2004). It is also critical that the public understands what the goals of biogerontology research are not. For example, reversal of aging and achieving immortality are not considered tenable objectives. Including these metrics in the scientific dialogue serves only to compromise the public's understanding of the purpose and value of biogerontology research.

Basic and applied scientists are working diligently to understand the processes of cellular and organismal senescence and to identify the determinants of successful human aging. Some of the domains attracting the deepest inquiry, along with instructive examples of the diverse and complementary scientific approaches at hand, are framed in the sections that follow.

LESSONS FROM EVOLUTIONARY BIOLOGY

It has been argued that longevity, not aging or senescence, per se, is under genetic control (Martin, Austad, & Johnson, 1996). These so-called longevity genes extend longevity by increasing physiological reserve or increasing disease resistance rather than having a direct effect on the intrinsic rate of aging. Evolutionary biologists would concur that genes that regulate late-life deterioration would not be under the influence of natural selection. Instead, natural selection favors genes that influence the likelihood of reproductive success, because organisms rely on the successful completion of development and reproduction to perpetuate the species. During the postreproductive period, however, the force of natural selection quickly diminishes, rendering organisms ill-equipped to protect themselves against the age-related accumulation of molecular damage. Seldom is this an important consequence to animals living in the wild because, in general, they experience relatively brief postreproductive life spans, owing to their vulnerability to predators, accidents, or infectious diseases. In contrast, humans and other highly protected, domesticated animals encounter the manifestations of organismal senescence, including cancer and other age-related degenerative diseases.

When it comes to age-related deterioration and disability, two tenets of evolutionary theory would suggest that humans and other domesticated species are indeed hardwired for postreproductive calamity. First, the *disposal soma hypothesis* (Kirkwood, 1977, 1990) states that after reproduction, the soma (body) can be thrown away. Second, genes that confer an advantage early in life may also exert detrimental effects in the postreproductive period—the theory of *antagonistic pleiotropy* (Williams, 1957). The incidence of several age-related diseases is likely to be under the control of such antagonistically pleiotropic genes. For example, genes that stimulate abundant blood vessel formation within the placenta—favoring successful reproduction early in life—might be considered detrimental later in life if they promote a rich supply of blood vessels to rapidly growing tumors. It is expected that the frequency of alleles that exert these late-life detrimental effects would be maintained or even increased in the population owing to their early-life benefit.

Thus, organismal senescence and accompanying age-related diseases are an inevitable by-product of domestication (see Figure 2.1). An organism is not built with any regard for optimizing aging. Instead, an organism is designed to successfully and advantageously complete its maturation and reproduction. As a consequence, in species attaining a prolonged postreproductive life span, one expects to see an accumulation of molecular disorder (Hayflick, 1998). One such contributor to this molecular disorder is the accumulation of misfolded proteins resulting from the

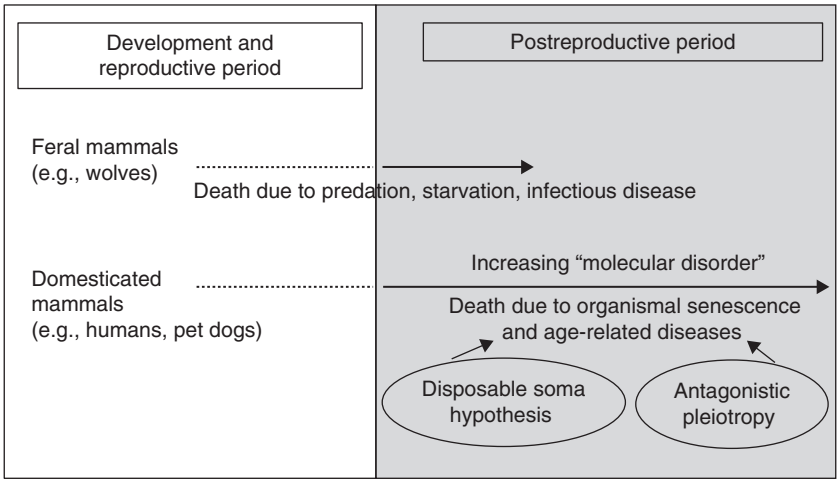

FIGURE 2.1 Domestication's curse: organismal senescence and the incidence of age-related diseases.

age-associated erosion in protein quality control—termed *proteotoxicity* (Koga, Kaushik, & Cuervo, 2011). Mechanistically, both dysregulation at the level of the proteasome and autophagy have been implicated in this disordered physiology (Cuervo, 2010; Powers, Morimoto, Dillin, Kelly, & Balch, 2009; Wong & Cuervo, 2010).

LESSONS FROM THE CELL CULTURE LABORATORY

Fifty years ago, Hayflick and Moorhead (1961) advanced the notion that normal somatic cells have a finite replicative life span in cell culture. Before this, it was believed that normal cells in culture, if cared for properly, could divide indefinitely. Subsequent studies have provided solid support for the concept of *replicative senescence*, whereby normal diploid cells have a limited number of population doublings (known as the Hayflick limit).

The process of in vitro replicative senescence has been studied extensively in normal fibroblasts and other cell types in cell culture (Campisi, 1996; Cristofalo & Pignolo, 1993). Not only do senescent cells suffer irreversible loss of their ability to replicate, they undergo other phenotypic changes. For example, senescent cells increase their production of matrix metalloproteinase enzymes, which might be expected to alter the function of tissues by favoring the turnover of extracellular matrix and tissue remodeling. It has been proposed that the senescence-associated secretory phenotype (SASP) may promote age-related pathologies, such as cancer and inflammation (Davalos, Coppe, Campisi, & Desprez, 2010; Rodier & Campisi, 2011). Importantly, *senescent cells do not die*; in fact, they retain their viability and are actually resistant to apoptotic cell death.

The in vitro replicative life span of normal cells is based on the number of population doublings rather than elapsed time in culture. The number of potential population doublings that a cell can undergo is determined by the length of *telomeres*, the structures that cap the ends of chromosomes (Kim, Kaminker, & Campisi, 2002). When normal cells undergo cell division, each replication witnesses a shortening of the telomere. When telomeric shortening reaches a critical threshold, the ability to replicate is lost; this signal is presumably mediated by a cellular DNA damage response (Dimri, 2005). In contrast, stem cells and most tumor cells possess the enzyme telomerase, which effectively maintains telomere length and enables these cells to escape the rules of replicative senescence (Granger, Wright, & Shay, 2002). Because cellular senescence is linked to telomere shortening, telomere length is actively being investigated as a biomarker to predict successful human aging (Bakaysa et al., 2007; Epel et al., 2004; Mather, Jorm, Parslow, & Christensen, 2011; Song et al., 2010).

There are at least two lines of evidence that make it attractive to consider that aging in the cell culture laboratory is indeed relevant to organismal senescence. First, when one looks across different species, the population doubling potential for cultured normal fibroblasts is proportional to the maximum life span potential of animals (Hayflick, 1976). Second, in studies with human fibroblasts, there is an inverse correlation between the age of the donor and replicative life span of cultured cells (Martin, Sprague, & Epstein, 1970). However, a study by Cristofalo and colleagues (Cristofalo, Allen, Pignolo, Martin, & Beck, 1998) challenged this notion by showing no significant relationship between donor age and the replicative potential of human fibroblasts. Moreover, the persistence of high replicative capacity in skin fibroblasts from 90-year-old people casts further doubt on the inverse association between donor age and growth characteristics of cultured fibroblasts (Maier et al., 2007). One possible explanation for these contradictory results is that in vivo aging does not result in a global, uniform, age-related loss of proliferative capacity. Instead, in vivo aging might be characterized by *mosaicism*—with old tissues representing a mosaic mixture of senescent and "youthful" cells. If true, then even old hosts have populations of youthful fibroblasts with a replicative life span comparable to that of cells retrieved from young hosts.

However, it is still not clear whether senescent cells actually accumulate in vivo in older individuals. Using the enzyme senescence-associated beta-galactosidase (Saβgal) as a marker of cellular senescence, Dimri and colleagues (1995) found more senescent cells in the skin of elderly people than in younger subjects. Although the species and tissue specificity of Saβgal activity are yet to be fully characterized, work with Saβgal is addressing the critical need for biomarkers of senescence so that we can determine the extent to which the accumulation of senescent cells causes the functional decline seen in old tissues and old organs (Debacq-Chainiaux, Erusalimsky, Campisi, & Toussaint, 2009; Lee et al., 2006).

Now, biogerontologists working in the cell culture laboratory are moving beyond replicative senescence, extending their research techniques in important ways. They are beginning to carefully catalog the resistance of cells to ex vivo challenge with lethal and sublethal stresses as a new approach to getting a cellular handle on what it takes to achieve extended longevity. Testing the resilience of skin fibroblasts cultured from an array of beasts and bipeds is yielding a collection of provocative, insult-specific responses that, once deciphered, will undoubtedly point to new leads, fresh hypotheses to pursue (Dekker et al., 2009; Finch, Morgan, Longo, & de Magalhaes, 2010; Harper, Salmon, Leiser, Galecki, & Miller, 2007; Miller, Williams, Kiklevich, Austad, & Harper, 2011).

DETERMINANTS OF LIFE SPAN: GENES, ENVIRONMENT, AND CHANCE

Evolutionary theory teaches us that there are no genes whose purpose is to cause aging or accelerate the rate of aging. In that sense, senescence is not programmed, but rather a passive by-product of an organism's genetic hardwiring. That genes influence life span is not subject to debate—no amount of environmental protection or enrichment can produce a rat that lives as long as the average human. Specific genes have been identified that regulate life span in worms (*Caenorhabditis elegans*) (Kimura, Tissenbaum, Liu, & Ruvkun, 1997; Morris, Tissenbaum, & Ruvkun, 1996), flies (*Drosophila*) (Clancy et al., 2001; Lin, Seroude, & Benzer, 1998), and mice (Migliaccio et al., 1999). What remains enigmatic is whether any of these genes actually prolongs life span by slowing down the rate of aging.

Data from twin studies (Ljungquist, Berg, Lanke, McClearn, & Pedersen, 1998; McGue, Vaupel, Holm, & Harvald, 1993) suggest that 25% to 30% of the variation in life span among individuals can be attributed to genetic factors. In other words, *there is considerable plasticity to life span*. This means lifestyle is important—both the external environment (e.g., diet, exposure to chemicals) and the internal environment (e.g., hormones, oxidative stress) significantly influence an individual's life span.

Recently, the widely accepted idea that gene–environment interactions are the major determinant of all complex traits, including longevity, has made room for a third element: chance. A complex choreography of genes, environment, and chance orchestrates a vast array of biological outcomes (reviewed in Finch & Kirkwood, 2000). That chance has an important impact on life span is supported by observations that genetically identical organisms raised under identical conditions have differences in life span—all of the individuals do not drop dead on the same day. An elegant study in worms underscores the impact of intrinsic chance in determining longevity (Rea, Wu, Cypser, Vaupel, & Johnson, 2005).

Although several organismal models suggest that life span is indeed under genetic control, there are differing views about how many genes regulate longevity and how those genes should be classified. Martin (1978) estimates as many as 7,000 genes may regulate life span, suggesting that longevity is under polygenic control. However, some scientists favor the idea of oligogenic control—a few critical genes may account for the lion's share of the 25% to 30% portion of human life span that is heritable. This view is supported by a considerable body of evidence from *C. elegans* in which single gene mutations can significantly prolong life span (Kimura et al., 1997; Morris et al., 1996).

Today, the search continues for genes that can persist within natural populations and increase life span by decreasing the rate of aging. Although unproven, the existence of such genes is supported by the profound within-species difference in life span observed in island- versus mainland-dwelling opossums (Austad, 1993), selective breeding experiments in flies (Arking, 1987), and the natural evolution of dog breeds (Deeb & Wolf, 1994; Patronek, Waters, & Glickman, 1997; Waters & Wildasin, 2006). These so-called longevity assurance genes (Butler et al., 2003; Hodes, McCormick, & Pruzan, 1996) are assumed to influence the rate of aging because they are beneficial to the organism, for example, by regulating antioxidant defenses or DNA repair. However, scientists in hot pursuit of candidate longevity assurance genes are bound to uncover some unexpected results, because these genes will likely have tissue-specific effects that manifest themselves differently during different "windows" of the life course. Furthermore, nonlinear U-shaped dose responses and threshold effects, in which further increases in gene action do not enhance the organism's viability, may befuddle investigators (Chiang et al., 2010; Kizer et al., 2011; Munhoz, Sorrells, Caso, Scavone, & Sapolsky, 2010; Wu, Cypser, Yashin, & Johnson, 2008).

To better understand the genetic basis of health, investigators are now looking beyond genetic variants to the impact of *epigenetic changes*. Epigenetic changes are transmissible alterations in gene expression caused by mechanisms other than variations in DNA sequence (Aguilera, Fernández, Muñoz, & Fraga, 2010). Differences in epigenetic programming—the capacity of an organism to turn on or off genes in response to environmental signals such as maternal care—provide a mechanistic basis to explain, in part, striking interindividual differences in resilience and healthy longevity (Weaver et al., 2004). Discovering which aging-related processes are regulated by epigenetic mechanisms is considered a high priority, because each individual's epigenetic state is potentially reversible—not hardwired like genomic DNA sequences (Gravina & Vijg, 2010). Results from rodent studies showing the ability to experimentally manipulate epigenetic changes—using drugs in adulthood to reverse adverse stress behaviors caused by low-quality maternal care earlier in life—open the door to almost limitless possibilities to investigators thinking epigenetically (Weaver, Meaney, & Szyf, 2006).

HORMONES AND AGING: ALMOST NOTHING IS RAGING

The endocrine system, a major contributor to the host's internal environment, is profoundly and predictably affected by aging. An age-related decline in hormone production results in lower circulating

levels of growth hormone/insulin-like growth factor-1 (IGF-1) (somatopause), estrogen (menopause), testosterone (andropause), and DHEA (adrenopause) (Lamberts, van den Beld, & van der Lely, 1997). The age-associated decline in these regulators of homeostasis has several important biological and clinical implications.

Although unproven, the idea that alterations in hormone levels cause organismal senescence, including the physiological deterioration seen in older adults, is supported by clinical observations. For example, loss of estrogen action in postmenopausal women has been associated with an increased risk for cardiovascular disease, accelerated loss of bone mass, and a decline in cognitive function (Love et al., 1992). In addition, individuals suffering from inherited growth hormone deficiency show many signs that mimic normal aging, such as thinning skin and decreased bone and muscle mass (Rudman et al., 1990). It follows from these observations that hormone supplementation might prove a useful intervention to significantly decrease the rate of age-associated physiological decline.

From a biological standpoint, there are several issues that transform successful hormonal replacement from a straightforward proposition to a formidable task. First, one must decide whether to use a *replacement* dose (i.e., restoring circulating hormone concentrations in older adults to normal youthful levels) or *supraphysiologic* dose (i.e., achieving high-circulating hormone concentrations that exceed normal levels). Other issues, such as timing of hormone administration during the life course (Bartke et al., 1998), use of a single hormone or combination (Shumaker et al., 2003; Tang et al., 1996), and sex-specific effects (Blackman et al., 2002) add another layer of complexity.

Second, hormones have *tissue-specific actions*—some desirable, some undesirable. For example, testosterone supplementation may decrease frailty in older men by increasing muscle mass and muscle strength (Krause, Mueller, & Mazur, 2005). However, an expected downside of testosterone supplementation is an increased incidence of prostatic disease. Because of potentially troubling trade-offs, scientists are developing hormonal agents with tissue-selective actions. For example, selective estrogen receptor modulators (SERMs), such as tamoxifen and raloxifene, exert beneficial effects on particular target organs (e.g., brain, blood vessels, bone) without detrimental stimulatory effects on cancer cells in the breast or uterus (Jordan, 2004).

Finally, it will be important to develop reliable biomarkers that can identify those individuals who will benefit most from hormone supplementation. By accomplishing this, it will be possible to finger hormone supplementation regimens that have the best risk–benefit profile (Bhasin et al., 2010).

It is not clear, however, whether an age-related decline in circulating hormones is in actuality a bad thing. Take growth hormone for example. If decreased growth hormone production contributes significantly to the age-related decline in muscle mass and bone density, then a decline in circulating growth hormone levels would be seen as maladaptive. On the other hand, the age-associated drop in growth hormone might serve as a favorable anticancer mechanism. Decreased growth hormone translates into lower levels of circulating IGF-1, a peptide that stimulates the survival and proliferation of most human breast, prostate, and colon cancers (Chan et al., 1998; Giovannucci et al., 2000; Schernhammer, Holly, Pollak, & Hankinson, 2005). Experimentally, carcinogen-treated mice with growth hormone deficiency are cancer resistant; growth hormone infusions render these mice more vulnerable to cancer development (Ramsey et al., 2002). Studies across several species have linked *low somatotropic tone*—a relative reduction in GH and IGF-1 action—with increased longevity (Bartke, 2008; Piper, Selman, McElwee, & Partridge, 2008). The association between exceptional human longevity and loss-of-function mutations in the IGF receptor, leading to diminished IGF-1 signaling, is consistent with this notion (Suh et al., 2008).

CALORIC RESTRICTION: IMPRACTICAL INTERVENTION, INVALUABLE RESEARCH TOOL

In 1935, McCay and colleagues reported that restricting food intake delayed the onset of age-related diseases and extended the life span of rodents (McCay, Crowell, & Maynard, 1935). Seventy-five years later, caloric restriction (CR, also called undernutrition without malnutrition or dietary restriction) is regarded as the most robust experimental intervention for extending life span in rodents (Masoro, 2003). The beneficial effects of CR on life span extension and cancer suppression are observed when total energy intake is curtailed to 20% to 40% less than the caloric intake of ad libitum fed animals. The experimental paradigm of CR is distinct from starvation: CR animals receive fewer calories but nutritionally adequate levels of all essential nutrients.

Most experiments in rodents employ lifelong CR initiated soon after weaning. However, life span extension in mice has been documented when CR was initiated at middle age, that is, 12 months old (Pugh, Oberley, & Weindruch, 1999). Both CR rodents and CR monkeys show significant improvements in several physiological parameters, including decreased fasting blood glucose and insulin levels (Roberts et al., 2001). Recent results showing that CR reduces age-related mortality

and disease incidence in nonhuman primates have fueled further inquiry (Colman et al., 2009).

However, a study of 41 different recombinant inbred strains of mice has refocused attention on a glaring deficiency in our understanding of just who will benefit from CR (Liao, Rikke, Johnson, Diaz, & Nelson, 2010). The surprising result: *The majority of mouse strains showed no life extension with CR*. In fact, CR *shortened* the life span of more strains than it lengthened. It is safe to say that the previously held notion that life span extension by CR is a universal outcome has been thrown under the bus (Harper, Leathers, & Austad, 2006; Liao et al., 2010). Now investigators are turning these genetically heterogeneous mouse strains into powerful tools of discovery—deciphering which genes are linked to differences in the longevity-extending effects of CR (Liao et al., 2011).

The exact mechanism by which CR extends life span is under intense investigation. Newer research is even beginning to explore the radical notion that CR is a form of dietary imbalance whose effects may be more closely linked to amino acid balance than calories (Grandison, Piper, & Partridge, 2009; Piper, Partridge, Raubenheimer, & Simpson, 2011). By identifying key targets modulated by CR, scientists hope to identify critical regulatory points that control senescence and longevity. One thing is certain: CR exerts pleiotropic effects, significantly altering numerous biochemical, immunologic, and hormonal networks.

The dogged search for the mechanistic basis for the longevity-enhancing effects of CR is more than just an academic exercise. Scientists are avidly seeking to develop CR mimetics because, long-term, 20% to 40% CR is an impractical intervention strategy to implement in humans (Hass et al., 1996). The discovery of effective CR mimetics would allow individuals to reap the benefits of CR without the undesirable effects of severe food restriction. However, because of our rudimentary understanding of how CR really works, there is considerable scientific debate as to what kind of agents would be the most effective CR mimetics.

Finally, as an alternative to sustained CR, *intermittent* CR might provide a more practical lifestyle modification. Currently, it is unclear whether intermittent CR would effectively mimic the beneficial effects of CR. Several lines of evidence indicate that alternate day fasting has different effects on both hippocampal and gonadal gene expression compared with an isocaloric CR regimen enforced every day (Martin et al., 2008; Martin et al., 2009). Alternate-day fasting exerts beneficial effects on blood glucose regulation and insulin sensitivity in mice without the reduction in body weight that typically accompanies CR (Anson et al., 2003). Moreover, intermittent fasting has been

linked to cardioprotection and cancer preventive effects in rodents (Varady & Hellerstein, 2007). In the future, we can expect further studies designed to establish the potential for intermittent CR to reduce disease risk and promote healthy longevity in humans.

RUSTING OUT: THE OXIDATIVE STRESS HYPOTHESIS

In 1956, Denham Harman proposed that endogenous products of aerobic metabolism are a major determinant of organismal senescence (Harman, 1956). Harman's free radical theory of aging predicted that organisms would inevitably suffer age-related accumulation of macromolecular damage induced by reactive oxygen species (ROS), leading to physiological decline and increased risk of mortality. Over the ensuing half century, substantial correlative data have been collected from animal studies that support this popular hypothesis, although definitive evidence that oxidant stress causes organismal senescence is lacking. Elucidating the precise role of oxidative stress in the aging process is complicated by the fact that the impact of oxidative stress on an organism's well-being depends on: (a) the amount of ROS production; (b) the vulnerability of macromolecules to ROS-induced damage, which reflects cellular antioxidant defenses; and (c) the capacity to repair oxidative damage (Beckman & Ames, 1998). Moreover, emerging data suggest that conceptualizing oxidative stress as "good" or "bad" is an ill-advised oversimplification—one that could ultimately mislead our assumptions about oxidative stress and aging.

Sources of Reactive Oxygen Species

The mitochondria represent the most important cellular source of ROS. The electron transport chain within mitochondria generates the adenosine triphosphate (ATP) necessary to power the cell, but is an imperfect system, resulting in the generation of ROS, especially hydroxyl radical. Oxidative damage to mitochondrial DNA leads to the production of defective mitochondrial proteins, which favors impaired electron transfer and a resultant increase in ROS production. According to the mitochondrial theory of aging (Miquel, 1992), the age-related accumulation of mitochondrial DNA mutations contributes significantly to organismal senescence by driving a vicious cycle of mitochondrial dysfunction and cellular energetic collapse. There are also important non-mitochondrial sources of oxidants, including beta oxidation of fatty acids, cytochrome p450 enzyme, and the oxidative burst used by phagocytic cells to destroy pathogens.

Macromolecular Targets and Measures of Oxidative Stress

Lipids, proteins, and nucleic acids are vulnerable to damage by oxidative stress. Lipid peroxidation leads to changes in membrane fluidity that can corrupt the cell's signal transduction—that is, how a cell processes information from its environment and interacts with other cells (Monteiro & Stern, 1996). The level of F2 isoprostanes or malondialdehyde is commonly used to assess the extent of lipid peroxidation. Protein oxidation disrupts homeostasis by inactivating essential enzymes and altering the cell's ability to recognize and dispose of worn-out proteins. Scientists measure the level of protein carbonyls or advanced glycation end products to assess the amount of oxidative protein damage. Oxidative damage to nucleic acids can lead to deleterious mutations that may diminish cellular function or contribute to cancer development. The extent of oxidative lesions in mitochondrial DNA exceeds those seen in nuclear DNA by 10 to 1, suggesting that oxidative stress significantly contributes to disruption of mitochondrial function. The oxidative DNA adduct, 8-hydroxyguanosine, is the biochemical marker most widely used to measure the extent of oxidative DNA damage (Wu, Chiou, Chang, & Wu, 2004).

Cellular Antioxidant Defenses

Cellular protection from oxidative stress relies on enzymes that speed up ROS detoxification and on the activity of free radical scavengers. The antioxidant enzyme superoxide dismutase (SOD) catalyzes the conversion of superoxide anion to hydrogen peroxide; then hydrogen peroxide is converted to water by the enzymes catalase or glutathione peroxidase to complete the detoxification process. There are two distinct forms of SOD: (a) CuZnSOD, which is cytoplasmic and constitutively expressed and (b) MnSOD, which is mitochondrial and inducible. SOD activity is highly conserved across species, and interspecies comparisons show a strong positive correlation between SOD activity and maximum life span potential (Sohal, Sohal, & Brunk, 1990). Free radical scavengers that are hydrophilic, such as ascorbate, urate, and glutathione, operate within the cell cytoplasm. Lipophilic scavengers, such as carotenoids (e.g., beta carotene, lycopene) and tocopherols (e.g., vitamin E), provide valuable protection against peroxidation of membrane lipids.

Defining the precise role that a particular antioxidant defense enzyme plays in the aging process can be difficult for several reasons. First, some enzymes have sequential activity—SOD alone cannot drive the complete detoxification of superoxide anion to water. In addition, there is functional redundancy built into the system. Two different

enzymes, catalase and glutathione peroxidase, can complete the detoxification process of hydrogen peroxide to water. Furthermore, the inducibility of enzyme systems renders ambiguous the interpretation of "low" versus "high" measured enzyme activities. Should high basal SOD values be considered "good" or "bad"? The answer is a bit tricky. High activity might reflect innately strong defenses or, instead, induced activity suggesting the organism is under siege by overwhelming oxidative stress. Clearly, more studies are needed that not only measure baseline antioxidant enzyme activities but also assess cells *after oxidative challenge.*

Age-Related Changes in Oxidative Damage

In general, older hosts appear to be more susceptible to oxidative injury. For example, old mice exposed to low-dose (2Gy) irradiation had a twofold greater increase in oxidative DNA damage in the liver, brain, and heart than young mice receiving the same radiation exposure (Hamilton et al., 2001). In that study, evaluation of repair kinetics showed no difference in the capacity for young or old mice to repair oxidative DNA lesions (Hamilton et al., 2001). Studies probing whether age-related increases in oxidative damage reflect increased ROS production, diminished antioxidant defenses, or decreased repair of oxidative damage have yielded inconsistent results (Blumberg, 2000).

Interventional Studies

The evidence is mixed regarding whether manipulation of antioxidant defenses influences longevity. Transgenic flies that overexpress both CuZnSOD and catalase show increased maximum life span (Orr & Sohal, 1994). Similarly, selective overexpression of CuZnSOD in motor neurons also increases the maximum life span of flies (Parkes et al., 1998). However, upregulation of CuZnSOD activity in transgenic mice to levels up to five times greater than those seen in normal mice failed to increase life span (Huang, Carlson, Gillespie, Shi, & Epstein, 2000). Results from a more extensive catalog of studies in transgenic and knockout animals, in which a single gene or multiple genes have been overexpressed or deleted, have likewise failed to draw strong linkage between antioxidant defenses and life span in mammals (Salmon, Richardson, & Pérez, 2010).

Perhaps the most convincing evidence to support the oxidative stress hypothesis comes from the work of Melov and colleagues (2000) in *C. elegans*. Experimentally, the antioxidant defenses of worms were boosted by exposing them to a synthetic SOD mimetic in their culture

media, while control worms were exposed to vehicle alone. Worms exposed to the SOD mimetic had a significant 44% increase in mean life span. Furthermore, when genetically altered short-lived worms were exposed to the SOD mimetic, normal life span was restored in these short-lived mutants.

Unfortunately, direct evidence is lacking to support the hypothesis that increasing the dietary intake of antioxidants can increase life span in mammals. A synthetic free radical spin trap compound called PBN (N-tert-butyl alphaphenylnitrone) decreased brain protein oxidation (Carney et al., 1991) and increased life span of mice in some experiments (Floyd, Hensley, Forster, Kelleher-Andersson, & Wood, 2002). However, in studies of genetically heterogeneous mice, dietary supplementation with the PBN metabolite 4-hydroxy-PBN did not extend longevity (Strong et al., 2008). In humans, the dietary antioxidants vitamin C and vitamin E blocked the beneficial effects of exercise on blood glucose control, consistent with the notion that indiscriminant use of antioxidant supplements has the capacity to exert detrimental effects on human health (Ristow et al., 2009). Undoubtedly, new research will continue to challenge old thinking. And nowhere is this more clearly apparent than in the field of oxidative stress and aging.

LESSONS FROM THE OLDEST-OLD

Centenarians provide a potentially valuable natural experiment of highly successful aging. To date, the search for specific environmental exposures or lifestyle factors (e.g., diet, education, physical activity) that confer an increased likelihood of living to be 100 has not been fruitful (Perls, Levenson, Regan, & Puca, 2002). Among centenarians, females outnumber males by about four to one (Terry, Sebastiani, Anderson, & Perls, 2008). Among women, successful late reproduction—live childbirth at age 40 years or more—is a strong predictor of exceptional longevity (Perls, Alpert, & Frett, 1997). Larger particle size of high- and low-density lipoproteins (Barzilai et al., 2003) and higher plasma antioxidant capacity (Hyland et al., 2002) have also been associated with exceptional longevity.

Siblings of centenarians are 15 times more likely to live to be 100 compared with the general population (Perls, Wilmoth, et al., 2002). This longevity advantage is sustained throughout the life course, suggesting that their good fortune may be more strongly influenced by genetics than by environmental factors (Perls, Wilmoth, et al., 2002). A few genes have already been implicated as candidate longevity-enabling genes because the bad polymorphic variants of these genes are underrepresented in centenarians. These sinister variants

include the apolipoprotein E∈4 allele, and the angiotensin-converting enzyme D allele (Deelen et al., 2011; Schächter et al., 1994). Genetic linkage analysis has provided evidence that a gene or several genes on human chromosome 4 significantly contribute to exceptional longevity (Puca et al., 2001), while candidate gene studies point to two other longevity foci—FOXO3A and AKT1 (Christensen, Johnson, & Vaupel, 2006; Pawlikowska et al., 2009). It should be noted that the genetic factors that contribute to exceptional longevity may be different from the genetic determinants of average life span. In addition, men and women may follow different trajectories to achieve exceptional longevity; male centenarians are less heterogeneous and healthier than female centenarians (Franceschi, Motta, et al., 2000).

Centenarians outlive most of us, but just how healthy are centenarians? When it comes to vulnerability to major age-related diseases, a study of lifetime medical histories suggests there are three types of centenarians: (a) survivors (38%)—individuals with onset of at least one major disease prior to 80 years of age; (b) delayers (43%)—individuals who are free of all major diseases until after 80 years of age; and (c) escapers (19%)—individuals who are free of all major diseases until after 100 years of age (Evert, Lawler, Bogan, & Perls, 2003). This important study confirmed that centenarians are indeed a highly heterogeneous population. It is apparent that strong disease resistance, however, is a characteristic shared by the majority of centenarians; almost two-thirds of centenarians remained free of all major diseases until after the median age at death for their birth cohort. An analysis of exceptional longevity in pet dogs reached a similar conclusion: 76% of oldest-old dogs delayed the onset of major diseases, and more than one-half of extreme-aged dogs had profound disease resistance (Cooley, Schlittler, Glickman, Hayek, & Waters, 2003). In humans, mortality from cancer, a disease strongly associated with aging, actually *declines* in the tenth decade of life—a puzzling paradox (Harding, Pompei, & Wilson, 2011; Smith, 1996; Stanta, Campagner, Cavallieri, & Giarelli, 1997). Like the oldest-old humans, extreme-aged pet dogs also experience a significant decline in cancer-related mortality (Cooley et al., 2003; Perls & Hutter-Silver, 1999).

Several aspects of studying human centenarians present problematic hurdles for researchers: age validation, stochastic influences, concurrent disease, selection of adequate controls for comparison, and long-term recall of diet and other lifestyle factors. Recognizing the big payoff that could come from deciphering some of the secrets behind highly successful aging, biogerontologists have been on the lookout for fresh, new approaches that would be complementary to studies of human centenarians. In 2005, we established the Center for Exceptional Longevity Studies at the Gerald P. Murphy Cancer

Foundation. The aim was to test a new idea: The secrets to successful human aging might be revealed by studying exceptional longevity in pet dogs. To accomplish this, we set out to conduct the first, systematic study of the oldest-old dogs, starting with Rottweilers. We created ELDABA (Exceptional Longevity DataBase), the world's first collection of medical history and lifestyle information on canine centenarians (for Rottweilers, this means still kicking at the age of 13). This work has already led to important discoveries. By carefully studying the association between the number of years of lifetime ovary exposure and longevity in female Rottweilers, we discovered that keeping ovaries longer is associated with living longer (Waters et al., 2009). This work in dogs, together with findings on the longevity-promoting effects of ovaries in women and mice point to a new line of thinking: Ovaries are part of a system that promotes longevity (Mason, Cargill, Anderson, & Carey, 2009; Parker et al., 2009; Rocca, Grossardt, de Andrade, Malkasian, & Melton, 2006; Waters et al., 2009).

APPLYING A LIFE COURSE PERSPECTIVE TO BIOGERONTOLOGICAL DISCOVERY

Biogerontologists should not confine themselves to the study of old cells, old dogs, or old people. Instead, it makes better sense to apply a life course perspective to studying the determinants of healthy longevity, because the origins of many adult health outcomes are shaped significantly by early-life events. For example, the risk of older people to suffer a pathologic bone fracture secondary to osteoporosis strongly depends on the peak bone mass established in the second and third decades of life.

The discovery of biomarkers that can accurately identify those individuals in a population that are aging at different rates is a high research priority. Finding reliable biomarkers of aging, however, has been a frustrating failure, forcing investigators to fall back on longevity as the end point for their studies testing antiaging interventions. Could paying closer attention to life course perspective go far to fit together some of the pieces of the biomarker puzzle? We think so. It turns out that the relationship between certain physiologic parameters and health outcomes in people apparently "flip-flops" during the life course. For example, high serum cholesterol at 50 years of age is associated with an increased risk of developing Alzheimer's disease 20 years later (Kivipelto et al., 2001). In contrast, in people who are 70 years of age, high cholesterol seems to be *protective* against Alzheimer's disease (van Vliet, van de Water, de Craen, & Westendorp, 2009; Zuliani et al., 2010). Human studies likewise suggest that obesity in young

and middle-aged populations is associated with diminished life expectancy, whereas in old age (beyond 60 years, especially in men), fatness loses its sting as a harbinger of earlier death (Freedman, Ron, Ballard-Barbash, Doody, & Linet, 2006; Thinggaard, Jacobsen, Jeune, Martinussen, & Christensen, 2010). In advanced age, more favorable survival may be more closely related to increasing adiposity than muscle mass (Auyeung et al., 2010).

So how might we reconcile these puzzling observations that seem to be leading us toward a less certain view of how we can best promote healthy longevity? Instead of seeing the aging organism as engaged in a monotonous, downward trajectory, we need to see the organism as a dynamic and changing system, capable of resetting itself during the aging process to remain as resilient as possible amid the challenges and constraints of physiologic decline. With this reality more clearly in view, we should reexamine carefully not only our choices of interventions designed to sidestep the disabilities of aging, but also examine how we might *optimize the timing of those interventions*. It is expected that age at the start of any intervention, such as a CR mimetic, will strongly influence that intervention's longevity-altering effect. In some instances, earlier intervention will be preferred over late. It is likely that some interventions may be specific for promoting or restoring health only later in life. Importantly, we will need to look closely for the tradeoffs that will inevitably occur between beneficial and detrimental effects, so we can intelligently optimize our choices.

THE FRONTIER OF BIOGERONTOLOGICAL DISCOVERY: CRITICAL KNOWLEDGE GAPS, FAR-REACHING OPPORTUNITIES

The next generation of biogerontologists must be transdisciplinarians who excel in their home discipline yet are conversant in related areas of inquiry. Amid the substantial advances in our understanding of the aging process, we see four critical gaps that translate into opportunities:

1. *There is a critical need to identify biomarkers that can accurately identify individuals who are aging at significantly different rates*. Much recent attention has focused on biomarkers of inflammation. A decade ago, Franceschi, Bonafè, and colleagues (2000) coined the term *inflammaging* to describe the apparent pro-inflammatory state of elderly humans. Since then, numerous studies have documented elevated levels of inflammatory cytokines, such as IL-6 and TNF-alpha, in older adults fueling the

hypothesis that aging is innately pro-inflammatory (Danesh et al., 2008). However, other investigators have challenged whether increased inflammation is an obligate phenotype, positing that the pro-inflammatory state of the elderly may reflect an accumulation of lifestyle factors, particularly obesity, or the high incidence of atherosclerosis and other comorbid diseases (Ferrucci et al., 2005). Validation of biomarkers is an essential step toward the rigorous evaluation of interventions to slow the rate of senescence (Nakamura, Lane, Roth, & Ingram, 1998). Without them, investigators will have to rely on life span as an end point—making studies in long-lived species like humans impractical. Moreover, in the absence of validated biomarkers of senescence, the public will continue to be bombarded by unsubstantiated claims from peddlers of antiaging interventions.

2. *There is genuine concern about the relevance of the model systems used to study aging.* At best, animal and cellular models are imitations of human aging. It is imperative that investigators understand and freely communicate the strengths and limitations of the experimental model systems they use. Animal models should never drive the research questions. Instead, the particular research question should determine the selection of the most appropriate animal model (Waters, Bostwick, & Murphy, 1998). There is a recognized need for development of a broader array of model systems, including birds (Holmes & Austad, 1995), marsupials (Austad, 1997), and other mammals (Miller et al., 1999), to test important hypotheses relevant to human aging. A recent issue of *The ILAR Journal* was devoted to benchmarking current thinking about animal models of human health span (Sprott, 2011; Waters, 2011).
3. *There is need for a coherent theoretical framework that can be used to test important hypotheses*. Useful conceptual models should distinguish between senescence and longevity. Aging is complex and the process of senescence is undoubtedly driven by multiple causal mechanisms. It seems rational therefore to apply systems thinking so that we might integrate key concepts and side-step the intellectual paralysis potentially produced by the vast number of competing theories, now numbered in excess of 300 (Kirkwood, 2005; Medvedev, 1990). Development of a consensus framework would undoubtedly help investigators posit hypotheses and propose experiments to answer the most fundamental of all questions: *Why do old tissues have increased vulnerability to disease?* This should also enable the formal testing of the hypothesis that a slower rate of aging is, in fact, an important determinant of exceptional longevity.

4. *It makes good sense to apply a life course perspective to studying the determinants of life span*. Biogerontology should not be confined to the study of old organisms because the origins of many adult health outcomes are shaped significantly by early life events and experiences. A contemporary approach to finding the determinants of successful aging must also integrate how psychosocial factors modify physiologic homeostasis and the trajectory of age-related functional decline. We must begin to analyze the extent to which the timing and sequence, as well as the duration and intensity of experiences and exposures impact aging trajectories. The wisdom of using this kind of integrative approach is illustrated by the groundbreaking work documenting that the number of adverse childhood events has a significant impact on adult health outcomes (Brown et al., 2010; Brown et al., 2009; Felitti et al., 1998). It follows that careful studies of just how epigenetic reprogramming during the life course can be exploited to prevent or reverse environmentally driven, adverse health outcomes should command high priority (Weaver et al., 2006).

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