Review article

Twenty years of progress in biogerontology research

Huber R. Warner

National Institute on Aging, National Institutes of Health, Bethesda, MD 20892, USA; College of Biological Sciences, University of Minnesota, 123 Snyder Hall, St. Paul, MN 55108, USA; Author for correspondence (e-mail: warne033@umn.edu)

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Abstract

The first 10 years of NIA's existence were characterized by funding for descriptive and discovery research, as the field had not yet come of age. As Couzin expressed it in the July 1, 2005 issue of *Science*, "Just 2 or 3 decades ago, research on aging was a backwater" (Couzin J 2005 How much can human life span be extended. *Science* 309: 83). With the isolation of long-lived animal mutants and the application of the tools of molecular biology and transgenic technology to biogerontology research, the situation has changed dramatically since then, and aging research has become increasingly mechanistic and respectable. This transition has been aided by some well-thought out research initiatives by the NIA, and the purpose of this article is to provide a brief summary of the progress made in the past 20 years, and describe the part that NIA initiatives and funding have played in this transition.

Introduction

This article is adapted from a presentation I made at the 34th annual meeting of the American Aging Association in Oakland, CA on June 5, 2005. From July, 1984 until May 30, 2005 I was a staff member of the National Institute on Aging (NIA), serving the last five years as an Associate Director with responsibility for the Biology of Aging Program. On June 1, 2005 I assumed the position of Associate Dean for Research in the College of Biological Sciences at the University of Minnesota, but while at the NIA I witnessed, and was directly involved in, the transition of biogerontology from a very descriptive science to an increasingly mechanistic science. I will briefly discuss below a few of the areas where real progress has occurred in understanding the molecular basis of aging and age-related disease since I joined the NIA staff in 1984.

The NIA began operation in 1975, splitting off from the National Institute of Child Health and Human Development, and I joined the Molecular and Cell Biology Branch of the NIA nine years later in

July, 1984. At that time biogerontology research was mostly focused on testing theories of aging, and on characterizing aging in humans and various animal models, especially mice and rats. Through a series of reorganizations, this Branch eventually evolved into the Biology of Aging Program whose mandate is to fund research on the molecular, genetic, cellular and physiological changes that accompany aging and underlie the development of adverse non-neurological age-related pathology.

The state of the art in 1984 depended on three salient observations: 1) restricting caloric intake increases longevity and delays the onset of age-related disease in rodents (McCay et al. 1935); 2) oxygen radicals are produced *in vivo* and continuously damage cellular macromolecular components (Harman 1956); and 3) human fibroblasts grown in culture have a finite lifespan (Hayflick 1965). While these three ideas still provide much of the basis for biogerontology research today, so much progress has been made in fleshing out details that the journal *Cell* devoted its entire February 25, 2005 issue to reviews of aging research. What follows is a very

brief summary of some of the critical research milestones that have occurred in the last 20 years in biogerontology, mostly with the help of NIA funding.

Caloric restriction

In 1988, Weindruch and Walford reviewed the status of knowledge about the effect of caloric restriction (CR) on aging and longevity in rodents (Weindruch and Walford 1988). CR extends both mean and maximum life span, and delays the onset of age-related diseases such as cancer, diabetes, kidney failure, auto-immune disease, etc., but the underlying mechanisms for these effects are poorly understood. To address this knowledge gap, the NIA issued a Request for Applications (RFA) ["Molecular and neural mechanisms underlying the effects of caloric restriction on health and longevity"] in 2001 and 13 applications were funded. At the time this RFA was issued it was known that CR delays many age-related changes in gene expression, reduces oxidative stress, and increases the resistance of the animal to a variety of stresses, including heat, UV light and free radical generators. It was also known that caloric restriction can be mimicked by genetically suppressing appetite in mice (Mishkin and Masos 1997).

One of the new findings to come out of the applications funded in response to the RFA is that activators of sirtuins (a family of protein deacetylases) may be mimicking CR in yeast, and Guarente and Picard (2005) have suggested that protein deacetylation by sirtuins may play roles both in "sensing low calories and triggering changes linked to health and longevity." One of these activators is resveratrol, a natural compound found in some foods and in red wine (Howitz et al. 2003). Overexpressing the *sir-2* gene in nematodes also increases life span (Tissenbaum and Guarente 2001), but it remains to be shown whether sirtuins are involved in CR in mammals.

In late 2001 the NIA also issued an RFA ["Caloric restriction and aging in NIA non-human primates"] for applications proposing to use the NIA calorically restricted rhesus monkey colony for limited non-invasive studies in cooperation with NIA intramural staff. Too few animals have died in the studies going on at the University of Wisconsin and the NIA Intramural Program to yet determine whether the CR monkeys have an extended life span, but the phys-

iological changes seen so far do parallel those seen in calorically restricted rodents (Mattison et al. 2003).

Oxidative stress

Since Denham Harman proposed that oxygen free radicals are a major factor in aging (Harman 1956), considerable effort has been expended by the biogerontology research community to 'prove' this theory of aging. The NIA sponsored a workshop in Berkeley, CA in 1992 to discuss promising avenues for research on aging and oxidative stress, and issued a Program Announcement (PA) later that year to encourage submission of proposals in this area of research. This initiative was partly responsible for the fact that in Fiscal Year 1999 (the only year for which this analysis was done), the NIA committed the second-highest amount of extramural research funding to support research on oxidative stress than did any other institute in the NIH, and the highest amount based on the percentage of its total grant budget. There is an ongoing commitment at the NIA to fund research clarifying the relationship between free radical damage and aging processes.

There is wide acceptance that mitochondria generate oxygen free radicals in the course of normal metabolism, and that these free radicals damage nucleic acids, proteins and lipids, but it remains unclear whether these free radicals actually "cause or merely correlate with aging" changes (Balaban et al. 2005). Nevertheless, caloric restriction clearly reduces oxidative stress (Yu 1996), and in at least one example, overexpression of antioxidant enzymes does correlate with genetically induced life span extension (Larsen 1993). Therefore it was a surprise that mice heterozygous for the gene for the mitochondrial manganese-containing superoxide dismutase activity (Mn-SOD) exhibit more oxidation of DNA and higher cancer incidence, but do not die prematurely (Van Remmen et al. 2003). Furthermore, Kujoth et al. (2005) found that mice with a proof reading-deficient mitochondrial DNA polymerase age rapidly, but without a concomitant increase in oxidative damage in liver total DNA. These findings do not support the idea that nuclear DNA damage is a critical factor in aging. In contrast, transgenic expression of catalase targeted to the mitochondria, where it is not usually expressed, does lengthen life span slightly (Schriner

et al. 2005), suggesting that oxidation of mitochondrial DNA may be a more critical factor, as proposed by Barja (2004) and others.

The oxidation of specific proteins such as mitochondrial *cis*-aconitase (Yan et al. 1997), adenine nucleotide translocase (Yan and Sohal 1998) and/or lipids, may be a critical factor in aging, but more research is needed to establish this. Peroxidation of unsaturated fatty acids in complex lipids produces many toxic products, including 4-hydroxy-2-nonenal (4-HNE). These compounds can crosslink proteins and inhibit the degradation of altered proteins by the proteasome (Friguet and Szewda 1997), thus compromising the ability of the cell to rid itself of damaged proteins.

Cell death

While it has long been known that programmed cell death (apoptosis) occurs routinely in many tissues during development, and is necessary for proper development, the pathway by which cell death is genetically programmed was not known until Robert Horvitz's isolation of nematode mutations in cell-death genes, known as *ced* genes (Yuan and Horvitz 1990). Prior to this work, the NIA had issued a PA in 1989 ["Mechanisms of cell death in aging"] soliciting proposals to elucidate mechanisms of cell death during aging. One of the first applicants funded in response to this PA was Junying Yuan, who shortly thereafter demonstrated that the *Caenorhabditis elegans* (*C. elegans*) *ced-3* gene codes for a protease similar to interleukin-1 β -converting enzyme, also known as ICE (Yuan et al. 1993). This eventually led to the identification of a series of proteases, now called 'caspases', that play various roles in programmed cell death. This work was summarized recently by Zhang et al. (2003).

While the general outlines of the execution pathways in apoptosis have now been worked out (Slee et al. 1999), much remains to be learned about how programmed cell death is triggered, how the stimulus is transduced to the execution machinery, the tissue specificity of the various pathways, and the mechanisms used to retain tissue structure and function when cell death does occur. It is of utmost therapeutic importance to determine the molecular basis for the neuronal tissue specificity observed in various neurodegenerative diseases such as Parkinson dis-

ease, Alzheimer disease, amyotrophic lateral sclerosis, etc., so that specific treatments can be devised.

Thus, apoptosis is a two-edged sword: it is required both for normal development and to remove damaged cells that could become transformed into cancerous cells, but the loss of cells in non-proliferative tissues *via* apoptosis can have severe pathological consequences (Warner et al. 1997; Campisi 2002). The role of proteins involved in transducing damage signals to the cell death machinery, such as p66^{shc} (Migliaccio et al. 1999), in the overall aging of the organism remains unclear. In contrast to the role of apoptosis in specific diseases, a generalized role for apoptosis in aging is not yet well developed, but evidence for this role is mounting (Campisi 2002).

Control of cell proliferation

When cells are lost through apoptosis or necrosis, maintaining adequate tissue function requires that these cells be replaced either through proliferation of a neighboring cell, or through recruitment of cells from an appropriate stem cell or progenitor cell pool. By 1985 cell senescence was an established *in vitro* phenomenon, but little was known about the mechanisms by which it occurs, whether senescent cells occur in the living organism, or about their potential impact if they did. Hence, the NIA organized a series of workshops on the mechanisms of cell senescence in 1986, 1989 and 1992, and published a PA in 1986 ["Control of cell proliferation in senescent cells"]. The latter two workshops were held in Montreal, Quebec with Eugenia Wang as the local host. This has been a very productive initiative, and has led to a number of important discoveries about how the cell cycle is regulated.

An early breakthrough was made by Seshadri and Campisi (1990), who showed that senescent fibroblasts cannot induce the expression of the *c-fos* gene; the *c-fos* protein is a transcription factor required for fibroblast proliferation. This was followed by the important discovery that senescent cells produce an inhibitor of the cell cycle called p21 (Harper et al. 1993; Noda et al. 1994). A huge breakthrough then occurred when Harley et al. (1990) showed that telomere shortening occurs with increasing passage number, and that once telomeres reach some minimum length, replication stops. Some time later Bodnar et al. (1998) demonstrated a strong linkage between

proliferative potential and the presence of telomerase activity in human fibroblasts, and that transgenically expressed telomerase could overcome the senescence-induced block to proliferation. In the meantime an increasing number of proteins have been implicated in telomere binding and regulation of the structure and function of the telomere (van Steensel et al. 1998; Griffith et al. 1999). Mice deficient in telomerase activity are initially viable, but by the fifth generation such mice begin to show aging-like phenotypes (Rudolph et al. 1999), and eventually fail to reproduce. Furthermore, humans with dyskeratosis congenita are deficient in telomerase activity and proliferative potential, and develop numerous pathologies (Mitchell et al. 1999). Thus, while telomerase seems to be a factor in maintaining health and function during aging, presumably *via* processes necessary for stem cell, germ cell and lymphocyte proliferation, the initial hope that telomerase would also be an target for anti-cancer drug therapy remains unfulfilled.

An important and persistent question in the field of aging has been whether cells senesce in humans, and if so, what impact does this have? Using the senescence-associated β -galactosidase assay, Dimri et al. (1995) showed that some cells do senesce *in vivo*. This could potentially create a tissue environment that synergizes with oncogenic mutations to drive the rise in cancer incidence with age, because senescent cells secrete factors that disrupt tissue architecture and/or stimulate nearby cells to proliferate (Krtolica et al. 2001; Krtolica and Campisi 2002). Thus, “the senescence response may be antagonistically pleiotropic, promoting early-life survival by curtailing the development of cancer but eventually limiting longevity as dysfunctional senescent cells accumulate” (Campisi 2005).

Longevity genes

For the purpose of this brief discussion, a longevity gene will be simply considered to be any gene that can be manipulated to increase life span. In 1984, Tom Johnson's *C. elegans* long-lived *age-1* mutant had already been isolated and partially characterized (Friedman and Johnson 1988). The idea that a mutation would actually increase life span seemed counterintuitive to me then, and therefore unlikely to be a

general result, even for an organism as uncomplicated as a nematode. However, work of Rose (1984) and Luckinbill et al. (1984), showing that long-lived fruit flies could be obtained by selecting for mothers able to reproduce late in life, indicated that alleles or combinations of alleles with regulatory effects on longevity exist, and have the potential be identified and characterized. Thus, the NIA began an initiative to identify longevity assurance genes (LAGS) by issuing an RFA in 1992 [“Genetic and molecular basis of aging”].

The initiative was renewed with RFAs in 1998 and 2003, and once the human genome sequence became available, it was possible to show that the *C. elegans* *age-1* gene codes for a PI-3-kinase activity (Morris et al. 1996). Many *daf-2* mutations also promoted longevity, and the *daf-2* gene was identified as an insulin-like receptor (Kimura et al. 1997). This placed two nematode ‘longevity genes’ in the insulin-signaling pathway, suggesting that this pathway can play a role in regulating longevity. This stimulated research to determine whether a similar situation obtains in fruit flies, and it does (Tatar et al. 2001; Clancy et al. 2001). Furthermore, the observation that dwarf mice unable to produce growth hormone in the pituitary gland (Brown-Borg et al. 1996), or to respond to it (Coschigano et al. 2000), are also long-lived, indicates that IGF-I levels also regulate longevity in rodents. The relevance of this to humans and other primates is an important unanswered question.

Numerous genetic and a few pharmacological interventions that increase longevity in these various model organisms have now been identified, and much of this information has recently been summarized elsewhere (Warner 2005). These interventions are usually associated with one of the following processes: insulin signaling, stress resistance or caloric deprivation, and a program to take advantage of this knowledge by rigorously testing promising compounds in genetically heterogeneous mice has been underway for over two years (Warner et al. 2000). By now at least eight compounds are currently in ongoing trials.

Premature aging syndromes

The gold standard for identifying longevity genes has been to find genes that can be manipulated to increase

longevity, and many such genes have now been identified, as discussed above. However, it is not so clear what role these genes play in aging *per se*. An alternative approach is to identify genes that can be manipulated to either delay or hasten the appearance of aging-related phenotypes such as osteoporosis, loss of muscle mass, loss of subcutaneous adipose tissue, cardiovascular disease, loss or graying of hair, etc. Genetically induced extension of longevity in animal models is often accompanied by a delay in appearance of age-related phenotypes (Herndon et al. 2002; Ikeno et al. 2002), but the question remains whether the human homologs of such genes play any role in development of these adverse phenotypes during human aging.

Research with nematodes and fruit flies is not as likely to be informative about causes of age-related human pathology as that using mice would be; hence mutant mice that age prematurely might be useful in evaluating the relevance of these same genes in human aging (Warner and Sierra 2003). In addition to the two best-known human progeroid syndromes, Hutchinson–Gilford syndrome and Werner syndrome, there is now a growing list of mouse mutants defective in some aspect of DNA metabolism that are short-lived and prematurely develop a variety of age-related phenotypes. These include mice with mutations in the genes for telomerase (Blasco et al. 1997), ku86 (Vogel et al. 1999), p53 (Tyner et al. 2002), XPD (De Boer et al. 2002), lamin A (Mounkes et al. 2003) and mitochondrial DNA polymerase (Trifunovic et al. 2004; Kujoth et al. 2005). The most obvious common element among these mutants is that all of their resulting deficiencies have the potential to increase the vulnerability of the cells to apoptosis due to double-strand breaks in DNA, stalled transcription or replication complexes, or dysfunctional mitochondria or nuclei (see Table 1).

If in fact the cells in these mutants are subject to excessive apoptosis, how does this relate to the premature development of adverse aging phenotypes? One possibility is a lack of balance between cell loss and cell replacement either due to declining ability to recruit cells from stem cell or progenitor cell pools, or to dysfunction in the differentiation pathways. For example, Nishimura et al. (2005) showed that graying of hair can be caused by “incomplete maintenance of melanocyte stem cells” and suggested that stem cell apoptosis and/or ectopic differentiation “may contribute to stem-cell loss in other aging organ systems.” Age-dependent depletion of stem cell pools could provide an explanation for the interval of time between birth and the onset of observable pathology, and the small body size and eventual growth failure of these mutants, and Kujoth et al. (2005) proposed that their “findings suggest that apoptosis and loss of irreplaceable cells may be an important mechanism of aging in mammals.” Chien and Karsenty (2005) have recently reviewed evidence for how “aging influences tissue-specific progenitors and differentiated cell lineages” and the role that these influences may play in the development of cardiovascular disease, muscle wasting and osteoporosis.

The possibility that aging may be linked to an inability of stem cell pools to sustain the appropriate panoply of progenitor cells for cell replacement, led the NIA to publish RFAs related to this question in 2001 [“Biology of adult stem cells in aging”] and 2004 [“Biology of stem cells in aging”]. The applications funded in response to these two RFAs added to the growing NIA portfolio of grants supporting research on stem cell biology, reaching a total commitment of \$20 million by the end of Fiscal Year 2004. A PA [“Testing stem cell therapy in mouse models of premature aging”] for studies using short-lived mouse mutants to test potential cell replace-

Table 1. Short-lived mutant mice^a with possible relevance in aging research.

Gene	Protein	Defective function	Reference
<i>terc</i>	Telomerase	Telomere maintenance	Blasco et al. (1997)
<i>Ku86</i>	Ku86	Repair ds breaks in DNA	Vogel et al. (1999)
<i>p53</i>	p53	Checkpoint, apoptosis, senescence	Tyner et al. (2002)
<i>XPD</i>	DNA helicase	DNA repair, transcription	De Boer et al. (2002)
<i>LmnA</i>	lamin A	Nuclear envelope	Mounkes et al. (2004)
<i>polA</i>	DNA polymerase γ	Mitochondrial DNA synthesis	Trifunovic et al. (2004)

^a The life span of these mutants varies from 20 to 80% of normal, depending on the gene mutated.

Table 2. Cell replacement therapies in mice and humans.

Tissue/Syndrome	Replacement cells	Reference
Ischemic heart	Bone marrow cells	Orlic et al. (2001)
Damaged skeletal muscle	Bone marrow cells	LaBarge and Blau (2002)
α -sarcoglycan deficiency (a muscular dystrophy)	Mesoangioblasts	Sampaolesi et al. (2003)
Osteogenesis imperfecta	Mesenchymal stem cells	Chamberlain et al. (2004)
Hurler's syndrome (humans)	Cord blood cells	Staba et al. (2004)

ment therapies to provide 'proof of principle' that such therapies could work in humans was published in mid-2005. Several examples of the therapeutic potential of this kind of repair are listed in Table 2.

Treating Hutchinson–Gilford patients?

Hutchinson–Gilford is an early progeroid syndrome that until recently had drawn little more than curious attention from the gerontology community. However, when Leslie Gordon urged the NIA to respond to congressional language about supporting research on this incurable syndrome, the NIA co-funded a workshop with the NIH Office of Rare Diseases to discuss the possibilities. As a result of the workshop the NIA released a PA ["Innovation grants for research on Hutchinson–Gilford progeria syndrome"] to do that, and when the laboratory of Francis Collins at the National Human Genome Research Institute identified the defective gene as the gene for lamin A/C, the NIA quickly followed up with another PA ["The biological basis of Hutchinson–Gilford syndrome: relationship to mutations in the lamin A/C gene (Lmna) and to other known laminopathies"]. The relevance of this syndrome to normal aging remains uncertain, but interest is increasing with the production of the mouse mutants described above.

If lamin A mutations disrupt the nuclear envelope (Eriksson et al. 2003; Goldman et al. 2004) and predispose the affected cells to undergo apoptosis in both mice and humans, stem cell replacement therapy might provide an actual cure for this condition, if applied early enough before overt pathology develops. This would require isolating stem cells from an

appropriate pool in the patient (e.g., bone marrow?), repairing the genetic defect, amplifying the repaired cells, and returning the cells to the patient to repopulate the pools and eventually replace the defective cells altogether through the selective survival advantage these repaired cells would have over the defective cells of the patient. This appears to have been successful in the experiments reported by Sampaolesi et al. (2003) to treat a mouse model for limb-girdle muscular dystrophy. An alternative approach might be to use cord blood cells as was done by Staba et al. (2004) in treating Hurler's syndrome, a mucopolysaccharidosis leading to progressive deterioration of the central nervous system. This apparently worked because cord blood cells are tolerated reasonably well by a heterologous recipient.

Conclusions

Considerable progress has been made in elucidating the genes and pathways involved in longevity regulation in yeast, fruit flies, nematodes and mice. The roles of cell death, cell senescence and oxidative stress in aging are less understood, but major clues are emerging. The development of stem cells for therapeutic use in dealing with the health problems of older people is a tantalizing possibility, but much research remains to be done.

Note: Portions of this article overlap with material published under the title "Developing a research agenda in biogerontology: Basic mechanisms" published in *Sci. Aging Knowl. Environ.*, Vol. 2005, Issue 44, pp. pe33, 2 November 2005.

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