Workshop Report

Longevity Genes: From Primitive Organisms to Humans

# Longevity Genes: From Primitive Organisms to Humans


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*An Affiliate of*
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*An Interdisciplinary Workshop of the*
INTERNATIONAL LONGEVITY CENTER–USA


*Sponsored by*
International Longevity Center-USA
American Federation for Aging Research
Ellison Medical Foundation
Glenn Foundation for Medical Research
Institute for the Study of Aging
Canyon Ranch Health Resort

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# Dedication

WHEREAS THIS WORKSHOP DEALS WITH POSSIBILITIES FOR LONGEVITY GENES,
AND WHEREAS
THE MOST IMPORTANT FORCE IN THE FOUNDING OF THE NATIONAL INSTITUTE
ON AGING IN THE 1970S IS NOW A CENTENARIAN,

WE DEDICATE THIS WORKSHOP TO FLORENCE MAHONEY.

# Participants

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# Preface

Census figures indicate that there are some 50,000 centenarians in the United States and that there could be more than 800,000 by mid-century. One startling projection is that there could be five million by the end of the century. There are even those in the so-called anti-aging movement who suggest that we can one day achieve decades to hundreds of years of additional life.

Longevity genes have been identified in yeast, worms, fruit flies, and mice, in some instances with possible homology with humans; however the term "longevity gene" itself is subject to question. Perhaps it refers to genes that extend life beyond what is presently considered the maximum life span. Or perhaps longevity genes only refer, in the most general sense, to biological vigor over time, starting at the very beginning of life. These genes might direct resistance to internal and external damage, such as those that result from free radicals and glycation. Perhaps they control rapid and effective repair.

The International Longevity Center and its sponsors assembled a group of leading scientists who have contributed to the growing field of what may best be called "longevity science" (as it is in Japan).The purpose of this workshop was to reach a consensus on what we currently know about longevity. The participating scientists were asked to recommend a research agenda, suggest what should be responsibly told to the public, and delineate policy implications for business, foundations, and government. This report discusses their conclusions.

Robert N. Butler, M.D.
President and CEO
International Longevity Center-USA

# Executive Summary

Robert N. Butler, M.D.
*President and CEO, International Longevity Center*

A variety of lines of evidence indicate that aging and longevity are subject to genetic regulation, but until fairly recently the identity of these genes was unknown.This has changed dramatically over the last 10 years as investigators have taken advantage of both invertebrate and vertebrate model systems to study longevity genes. Invertebrates include nematodes (roundworms) and fruit flies. Early success with nematodes has been quickly followed up in fruit flies, and even studies in mice are beginning to identify "longevity genes." The ultimate purpose of this research is to determine whether similar genes are factors in regulating longevity and timing development of aging phenotypes in humans.

The strongest rationale for believing that genes as well as environmental and behavioral factors can influence the rate of aging within a species was provided by the demonstration that it is possible to select for long-lived strains of fruit flies. With regard to fruit flies, scientists have identified a variety of single gene mutations that either increase their life expectancy or modify the extension of life expectancy by other mutations.

Mice provide a very useful model system for a bridge between invertebrates and humans because of their more complex anatomy and our knowledge of their physiology and metabolism. At least four distinct genes have been identified in which loss-of-function mutations lead to dwarfism and increased mean and maximum longevity.

Experimentally, longevity genes are usually identified by showing that full or partial ablation of the gene, or overexpression of the gene, alters life expectancy. Longevity genes can be sorted into a variety of theoretical categories for the purpose of better understanding what role(s) they play in human aging.

**Genes that cause aging.** Most gerontologists believe there are no such genes because a gene that promotes aging would most likely decrease reproductive fitness and, therefore, be subject to negative selection.

**Genes that affect what kind of old individual you are.** Thousands of these genes are present in mice and humans. The polymorphic differences (alleles) help to determine which and how soon individuals will become gray or bald or develop specific pathologies such as osteoporosis, macular degeneration, or cognitive impairment. However, this is not the same as saying that these genes actually regulate the rate of aging.

**Genes that extend life expectancy or maximum life span.** These genes affect processes such as response to growth hormone, insulin-like signaling, and response to stress; in doing so they influence life expectancy in a wide range of organisms. Such genes were first found in model systems, especially in nematodes and fruit flies, but examples in mice are starting to appear. Alleles of this sort probably exist in humans, but they are harder to demonstrate in this long-lived species than in short-lived animal models.

**Naturally occurring alleles and allele combinations that alter life expectancy because they affect aging.** If there are any polymorphic genetic loci that influence aging rate within a species, they probably include a large number with very small effects and at least a few with detectably large effects. At least five such loci have already been detected in mice. It is hoped that mice such as these will be particularly useful for understanding genetic factors affecting the aging process.

**Candidate genes assumed to influence the rate of aging because of the function of the proteins coded by these genes.** Examples of these candidate genes include genes coded for proteins that either repair or prevent damage to cellular components. Naturally occurring alleles of such genes could alter the rate of aging under at least some conditions. Examples of such genes are found in mice, and it is expected that similar genes will usually influence longevity in humans. Human examples might include genes for DNA repair and antioxidant defense systems.

**Genes that influence life expectancy differences among species.** These are the real longevity genes of interest and should explain the wide differences in the rate of aging among diverse species such as nematodes, fruit flies, mice, and humans. Although no genes in this category have yet been unequivocally identified, it has been posited that these genes will regulate the pace of multiple developmental and degenerative processes, much as caloric restriction appears to do but to a much greater extent.

## RELEVANCE TO HUMAN AGING

Major questions arising from these studies with animal models are:

1. Can these results be extrapolated to humans?

2. If so, will this information lead to the development of safe and effective nongenetic interventions to delay some of the adverse aspects of aging in humans or at least provide clues about modulating mechanisms of aging in humans?

3. What other genes can be mutated or over-expressed to produce delayed aging, and does natural variation at these loci influence the rate of aging and risk of developing aging phenotypes?

4. Most importantly, do any of the loci where null mutations greatly extend longevity in model systems provide leads to the genetic differences responsible for the much greater aging rate differences among species?

## RESEARCH AGENDA

The ultimate payoff for this genetic research is to learn enough about aging to identify promising avenues for nongenetic interventions to delay, prevent, or even reverse the adverse age-related changes leading to disease and disability in humans. Workshop participants particularly emphasized the need to develop approaches to identify human longevity genes.This will require the recruitment of centenarians and their long-lived sibs and long-lived multigenerational families for genetic analysis. Other areas include research to identify the molecular basis for increased life expectancy induced by caloric restriction; identify reliable biomarkers of aging; expand understanding of why some species either escape aging or appear to be longer-lived than expected; develop new mammalian models to facilitate discovery of genetic factors that influence the rate of aging and life expectancy, as well as the risk for age-related disease; identify the environmental and behavioral factors that account for up to an estimated 70 percent of the risk factors for aging.

# Introduction

The strongest rationale for believing that not only environmental and behavioral factors but also genes can influence the rate of aging of individuals within a species was provided by the demonstration that it is possible to select for long-lived strains of fruit flies (Luckinbill et al. 1984; Rose 1984). Such genes have been variously called longevity-assurance genes, longevity-enabling genes, longevity-associated genes, longevity genes, or gerontogenes. For the purpose of the workshop and this report, the term "longevity genes" is used. Because longevity genes may mean different things to different people, it is important to sort out the nuances of different kinds of genes at the beginning. Longevity genes may manifest themselves in a variety of ways, including:

- Full or partial ablation may increase or decrease life expectancy, or
- Overexpression of the gene or a particular allele may increase or decrease life expectancy.

Whereas the overexpression or ablation of almost any gene may decrease life expectancy, the intention here is to focus on genetic changes that increase life expectancy, or shorten life expectancy if they do so with indications of premature aging. Examples of such genes associated with alterations of life expectancy are found in Tables 1, 2, and 3. Workshop participants suggested that these genes be sorted into distinct theoretical categories. Seven categories are described below with comments, where appropriate, about the nature and quantity of human genes that might be found in these categories.

Although the genes of primary interest for understanding aging are the ones that increase life expectancy when either overexpressed or mutated, other genes may be informative about a variety of age-related changes that influence life expectancy in other ways. It is important to recognize that there are only a few traits in the invertebrate systems that can be used as a measure of organismic aging. Thus, the best measure of retarded aging in invertebrate systems is usually increased life expectancy. In contrast, age-related changes in disease risk can be evaluated in mammals, so it might be somewhat easier to distinguish between genes that influence mammalian life expectancy by altering the rate of aging globally and those that only regulate some aspects of aging and may or may not also influence life expectancy.



### GENES THAT "CAUSE" AGING

This category includes genes that evolved to bring about the aging process. Most gerontologists believe there are no such genes in most species, including humans, because a gene that promotes aging would most likely decrease reproductive fitness and therefore would be subject to negative selection.

### GENES THAT ALTER LONGEVITY BECAUSE THEY MODULATE THE RISK OF EARLY-LIFE PATHOLOGY AND DISEASE

Genes that cause or increase the risk of pathology or disease may lead to a dramatic diminution of life span but are unlikely to provide important insights into aging. Therefore, while there may

be a large number of such genes, they should only be considered to be longevity genes if it can be shown that mutant alleles accelerate multiple aspects of aging. This may not be an easy task. For example, the complete absence of superoxide dismutase 2 (SOD2), the SOD form found in mitochondria, causes mice to die of dilated cardiomyopathy less than one month after birth (Li et al. 1995). Because these animals die very young with atypical pathology, the Sod2 gene would only be considered a true longevity gene if it could be shown that reduced levels of SOD2 accelerate the usual aging-induced adverse changes. Knock-out mice heterozygous for Sod2 do show altered mitochondrial function and increased sensitivity of cardiomyocytes to apoptosis-inducing agents, but this is not necessarily accelerated aging, and the life expectancy of these mice is normal (van Remmen et al. 2001).



A possibly more appropriate example is the Klotho gene in mice (Kuro-o et al. 1997). A mutation in this gene does lead to various pathologies associated with aging in humans, such as atherosclerosis and osteoporosis, and therefore studying Klotho mutants may be informative about human aging.

### Human genes

There are probably a good many genes that affect the risk of early-life pathology and disease. Examples include tumor-suppressor genes RB and BRCA1, the Hutchinson-Gilford gene, and a whole host of mutated genes responsible for the so-called inborn errors of metabolism. Few, if any, of these should be considered to be true longevity genes. For example, the growth and development of children with Hutchinson-Gilford syndrome is usually affected by the end of the first year of life, and these children have premature cardiovascular disease and often die of heart attacks in their midteens. While studying such a gene might provide important clues about aging, it seems clear that this is not premature aging per se (Mills and Weiss 1990); for example, many aspects of aging, such as neurodegeneration, are not seen in these children. Human DNA does contain a homologue of the mouse Klotho gene, but it is unknown whether it is associated with human aging.

## GENES THAT AFFECT WHAT KIND OF OLD INDIVIDUAL YOU ARE

There are thousands, perhaps tens of thousands, of these genes in mice and humans. Such genes might be more difficult to identify in invertebrates because death is not currently associated with well-characterized pathology.

### Human genes

The differences in these genes among humans help to determine which and how soon individuals will become gray or bald or develop specific pathologies such as osteoporosis, macular degeneration, Type II diabetes, sarcopenia, cognitive impairment, immune senescence, or a raft of other complaints. George Martin has described these as loci where mutations produce one or more changes in old people and are thus age-related (Martin 1978). This is not the same as saying that these loci regulate the aging process. A few examples of these are listed in Table 4. Single nucleotide polymorphisms are proving informative in detecting these genes. An excellent example is the APOE locus where the alleles have a strong influence on the risk for early onset of Alzheimer's disease (Schachter et al. 1994), cardiovascular disease, and perhaps other diseases (Makley and Rall 2000).

## GENES THAT EXTEND LIFE EXPECTANCY OR MAXIMUM LIFE SPAN

Such genes are being increasingly found in model systems, especially nematodes (Table 1) and fruit flies (Table 2), and examples in mice are also starting to appear (Table 3). These genes affect processes such as response to growth hormone, insulin-like signaling, and response to stress; in so doing, they influence life expectancy in a wide range of organisms (Table 5). These longevity genes may also influence aging per se, by regulating pathways that influence the rate of aging, but data showing this are scarce. The best documented example is the Snell ($Pit1^{dw}$) dwarf mutant mouse (Flurkey et al. 2001), which is not only long-lived but also shows delayed development of both age-sensitive immunological changes (cellular aging) and collagen cross-linking (extracellular aging), as well as deceleration of both lethal illness and incidental pathology such as arthritic changes. The value of studying these genes and their alleles lies in the hope that they will provide revealing glimpses of the control panel by which aging can be regulated.The specific pathways, both biochemical and cellular, altered by mutations in these genes may be the ones nature uses to produce long-lived species and/or individuals.

### Human genes

Alleles of this sort exist in humans, but they are harder to demonstrate in this long-lived species than in short-lived animal models. It is reasonable to assume they will modulate the processes listed in Table 5; examples include the PROP1 and GHR genes. The best way to identify these genes may be to first identify them in invertebrate and mammalian models.

## NATURALLY OCCURRING ALLELES AND ALLELE COMBINATIONS THAT ALTER LIFE EXPECTANCY BECAUSE THEY AFFECT AGING

If there are any polymorphic genetic loci that influence aging rate within a species, they probably include a large number with very small effects and at least a few with detectibly large effects. At least five such loci have already been detected in mice, segregating in a four-way cross derived from four common mouse stocks: BALB/c, C57BL/6, DBA/2 and C3H/He, although the gene products corresponding to these loci are not known (Miller et al. 2000; Jackson et al. 2002). It is hoped that mice such as these will be particularly useful for asking questions about the genetics of aging rather than the genetics of longevity, but this will depend on whether or not the alleles in question influence longevity by means of a general effect on multiple forms of late-life vulnerability. Although the relevant genes have not been identified, the differences in life expectancy between mainland and island opossums (Austad 1993) may be explained by these genes.



### Human genes

If such genes exist in mice, they will also exist and can be identified in humans. A possible example is the gene that causes Werner's Syndrome (WRN) when mutated (Epstein et al. 1966; Yu et al. 1996; Martin and Oshima 2000).

### CANDIDATE GENES ASSUMED TO INFLUENCE THE RATE OF AGING BECAUSE OF THE FUNCTION OF THE PROTEINS CODED BY THESE GENES

Examples include genes coding for proteins that either repair or prevent damage to cellular components. Naturally occurring alleles of such genes could alter the rate of aging under at least some conditions. A possible specific example for this category is the gene for methionine sulfoxide reductase (Moskovitz et al. 2001). The role of this gene is to convert oxidized methionines on the surface of proteins back to their normal state. Complete ablation of this activity in mice shortens life expectancy by about 40 percent. Because of this, and because oxidative stress is thought to be a risk factor for aging, it is reasonable to assume that partial loss of methionine sulfoxide reductase activity could have small effects on the rate of aging if naturally occurring alleles coding for a protein with decreased activity do exist. A more convincing experiment would be to show that overexpression of the gene increases life expectancy.

#### Human genes

If such genes exist in mice, it is expected that similar genes will usually influence longevity in humans. Human examples might include genes for DNA repair and antioxidant defense systems.

### GENES THAT INFLUENCE LIFE EXPECTANCY DIFFERENCES AMONG SPECIES

These are the real longevity genes of interest and should explain the wide differences in the rate of aging among diverse species such as nematodes, fruit flies, mice, and humans.They should also explain why similarly sized rodents and birds (e.g., rats, pigeons) and different species of rodents or fish (e.g., mouse vs. naked mole rat) sometimes have anomalous life expectancies. No genes in this category have yet been unequivocally identified, but it is posited that these genes will regulate the pace of multiple developmental and degenerative processes much as caloric restriction appears to do but to a much greater extent (Weindruch and Walford 1988). Well-designed comparative studies and perhaps a good deal of luck will be required to unequivocally identify these genes.

# Longevity Genes Identified in the Nematode (C. elegans)

**Table 1**

| GENE | BIOCHEMICAL FUNCTION | COMMENTS | REFERENCES |
|---|---|---|---|
| daf-2 | Insulin/IGF-I-like receptor | First step in insulin-like signaling pathway; mutations increase life expectancy; the mutation need occur only in neurons to achieve extension of life expectancy. | Kenyon et al. 1993<br>Larsen et al. 1995<br>Kimura et al. 1997<br>Wolkow et al. 2000 |
| age-1 (daf-23) | PI-3-kinase activity | Operates in insulin-like signaling pathway; mutations increase life expectancy; activates Akt kinases; increases levels of catalase and superoxide dismutase activities. | Johnson 1990<br>Larsen 1993<br>Morris et al. 1996 |
| sir-2 | NAD+ dependent histone deacetylase | Overexpression increases life expectancy; sir-2 yeast mutants are short-lived. | Tissenbaum and Guarente 2001 |
| eat-2 and other eat mutants | Unknown | Mutations cause defects in pharyngeal function and may mimic caloric restriction; eat-2 further extends the life expectancy of daf-2, but not clk-1, mutants. | Lakowski and Hekimi 1998 |
| daf-16 | Transcription factor | Expression required for life expectancy extension by daf-2, daf-23, and old-1 mutations; overexpression increases life expectancy and resistance to stress. | Lin et al. 1997<br>Lin et al. 2001<br>Murakami and Johnson 2001 |
| old-1 and old-2 | Receptor tyrosine kinases | Overexpression increases life expectancy and positively modulates resistance to stress; formerly known as tkr-1 and tkr-2; not closely related to daf-2. | Murakami and Johnson 1998<br>Rikke et al. 2000<br>Johnson et al. 2000 |
| clk-1 | Mitochondrial polypeptide similar to yeast COQ7 | Mutants are unable to synthesize coenzyme $Q_9$, but are long-lived when fed E. coli that supply coenzyme $Q_8$; overexpression shortens life expectancy. | Lakowski and Hekimi 1996<br>Felkai et al. 1999<br>Jonassen et al. 2001 |
| ctl-1 | Catalase | Activity is required for increased life expectancy induced by daf and clk mutations; ctl-1 mutants are short-lived. | Taub et al. 1999 |
| mev-1 | Cytochrome b in mitochondrial complex II | Increased production of superoxide anion by complex II and shortened life expectancy. | Senoo-Matsuda et al. 2001 |

# Longevity Genes Identified in Fruit Flies (Drosophila melanogaster)

**Table 2**

| GENE | BIOCHEMICAL FUNCTION | COMMENTS | REFERENCES |
|---|---|---|---|
| drop-dead | Unknown | Mutations lead to acceleration in age-related changes in gene expression and early death. | Rogina et al. 1997 |
| InR | Insulin/IGF-I-like receptor | First step in insulin-like signaling pathway; mutations increase life expectancy. | Tatar et al. 2001 |
| chico | Insulin receptor substrate | Second step in insulin-like signaling pathway; mutations increase life expectancy. | Clancy et al. 2001 |
| Indy | Dicarboxylic acid transport protein | Partial loss of function increases life expectancy; caloric restriction mimetic? | Rogina et al. 2000 |
| hsp70 | Heat shock protein | Catalyzes renaturation of proteins denatured by heat; overexpression increases life expectancy. | Tatar et al. 2001 |
| sod1 | Cu/Zn-superoxide dismutase | Overexpression increases life expectancy; overexpression need occur only in motor neurons; total ablation reduces life expectancy. | Phillips et al. 1989<br>Parkes et al. 1998<br>Sun and Tower 1999 |
| mth | Transmembrane protein | Partial loss of function increases life expectancy and resistance to stress. | Lin et al. 1998<br>Jonassen et al. 2001 |
| Pcmt | Protein carboxyl methyltransferase | Overexpression increases life expectancy at high temperature. | Chavous et al. 2001 |

# Longevity Genes Identified in Mice

**Table 3**

| GENE | BIOCHEMICAL FUNCTION | COMMENTS | REFERENCES |
|---|---|---|---|
| Pit1$^{dw}$ | Transcription factor required for pituitary development | Mice are deficient in GH, prolactin, and TSH; are infertile and cold intolerant, and grow slowly; mutations increase life expectancy and delay immune function and collagen aging. | Bartke et al. 2001a, b<br>Flurkey et al. 2001 |
| Prop1$^{df}$ | Required for pituitary development | Mutations increase life expectancy; phenotype same as pit1$^{dw}$ mice. | Bartke et al. 2001a, b |
| Ghr | Growth hormone receptor | Loss of function increases life expectancy; high circulating growth hormone levels. | Coschigano et al. 2000 |
| Ghrhr$^{lit}$ | Growth hormone releasing hormone receptor | Have low circulating growth hormone levels and increased life expectancy. | Flurkey et al. 2001 |
| Klotho | Membrane protein with β-glucosidase activity? | Mutations lead to arteriosclerosis, osteoporosis, and reduced life expectancy; mutants appear to be more insulin sensitive. | Kuro-o et al. 1997 |
| p66$^{shc}$ | Not known | Mutations enhance resistance to apoptosis and increase life expectancy; phenotype not yet well characterized. | Migliaccio et al. 1999 |
| Sod2 | Mn-superoxide dismutase | Mutant mice die very young with evidence of extreme oxidative stress. | van Remmen et al. 2001 |
| MsrA | Methionine sulfoxide reductase | Converts methionine sulfoxide to methionine on the surface of proteins. | Moskovitz et al. 2001 |
| Upa | Urokinase-type plasminogen activator | Overexpression in the hypothalamus decreases appetite; caloric restriction mimetic? | Miskin and Masos 1997 |
| p53 | Tumor suppressor protein | Partial deletion of gene leads to premature development of age-related pathology and reduced life expectancy; the +/m mouse is very tumor resistant. | Tyner et al. 2002 |

# Genes Associated With Avoiding Late-Life Disease in Humans

**Table 4**

| GENE | BIOCHEMICAL FUNCTION | COMMENTS | REFERENCES |
|---|---|---|---|
| APOE | Lipoprotein metabolism | E2 variant is frequent in centenarians while E4 variant as a risk factor for Alzheimer's disease is rare in centenarians. | Schachter et al. 1994 |
| ACE | Angiotensin-converting enzyme | Plays a role in regulating blood pressure. | Schachter et al. 1994 |
| PAI1 | Plasminogen activator inhibitor 1 | Plays a role in blood clotting, thus affecting risk of stroke and heart attack. | Mannucci et al. 1997 |
| HLA-DR | Histocompatability locus antigen | DR variant is frequent in centenarians; resists infection and inflammation? | Ivanova et al. 1998 |
| WRN | Possesses both DNA helicase and exonuclease activity | Gene responsible for Werner's Syndrome; mutation leads to a variety of aging-related pathologies, e.g., cataracts, cancer, osteoporosis, slow wound healing, etc. | Yu et al. 1996<br>Huang et al. 1998<br>Martin and Oshima 2000 |
| B3AR | B-3 adrenergic receptor | Allelic form present affects time of onset of Type 2 diabetes. | Walston et al. 1995 |
| MTHFR | 5-, 10-methylenetetra-hydrofolate reductase | Deficiency leads to increased levels of homocysteine and DNA hypomethylation; increases risk of cardiovascular disease and cancer. | Heijmans et al. 2000 |
| KLOTHO | Membrane protein with β-glucosidase activity? | Homozygous variant form is underrepresented in elderly individuals. | Arking et al. 2002 |

# Cross-Species Comparisons of Processes and Genes That Influence Longevity and/or Aging

**Table 5**

| | Insulin-Signaling Pathway | Stress Resistance | Metabolic Rate |
|---|---|---|---|
| Nematode | daf-2<br>age-1 (daf-23) | old-1<br>old-2<br>ctl-1<br>mev-1 | clk-1<br>eat-1<br>eat-2<br>sir-2 |
| Fruit fly | InR<br>chico | sod1<br>mth<br>hsp70<br>Pcmt | Indy |
| Mouse | $Pit1^{dw}$<br>$Prop1^{df}$<br>$Ghrhr^{lit}$<br>Ghr<br>Klotho? | Sod2<br>$p66^{shc}$<br>MsrA<br>p53? | Upa<br>(caloric restriction?) |
| Primates (including humans) | PROP1*<br>GHR* | ? | Caloric restriction<br>in primates?≠ |

*** Human PROP1 and GHRmutations have been identified,but evidence that they increase life expectancy is fragmentary.**
**≠ Preliminary results suggest that caloric restriction may extend life expectancy in Rhesus monkeys (Wanagat et al. 1999). Although some mutants have pleiotrophic effects and could be placed in two or more columns,they are listed in the column most relevant to the primary defect.**

# Lessons From Invertebrate Model Systems

**NEMATODES (Caenorhabditis elegans)**

age-1 was the first single gene mutation shown to extend life expectancy in any organism (Klass and Hirsh 1976; Friedman and Johnson 1988). This was followed by the demonstration by Kenyon et al. (1993) and Larsen et al. (1995) that Caenorhabditis elegans daf-2 and daf-23 mutants are also long-lived. Subsequently, age-1 and daf-23 were shown to be the same genetic locus based on their failure to complement (Malone et al. 1996; Morris et al. 1996).These results were quickly followed by the identification of other C. elegans mutations that either increase life expectancy or modify the extension of life expectancy by other mutations (see Table 1).

The next major breakthrough occurred with the cloning and sequencing of these genes and the identification of the proteins coded by them. As seen in Table 1, daf-2 codes for a protein with homology to the gene family that includes the insulin receptor in mammals (Kimura et al. 1997), daf-16 codes for a protein with homology to a known transcription factor (Ogg et al. 1997; Lin et al. 1997), and age-1 (daf-23) codes for a protein with homology to the enzyme phosphatidyl-inositol 3-kinase (Morris et al. 1996). This protein is involved in transducing the signal from the insulin-like receptor to downstream effector molecules called Akt kinases. Phosphorylation of the DAF-16 gene product by Akt kinase activity inactivates this transcription factor by preventing its nuclear translocation. A few genes that are regulated by the DAF-16 gene product have been described (Yu and Larsen 2001; Murakami and Johnson 2001).

How daf-2 and age-1 (daf-23) genes in C. elegans globally influence aging is not known. Even though the natural ligand for the insulin-like receptor in C. elegans has yet to be identified, the above results indicate that this insulin-like signaling pathway can influence longevity regulation. Of particular interest is the observation that normal daf-2 expression need be restored only in neurons to specify wild-type life expectancy (Wolkow et al. 2000).

The old-1 and old-2 genes code for putative receptors with tyrosine kinase activity. Over-expression of either gene confers increased resistance to heat and UV-irradiation (Murakami and Johnson 1998; Rikke et al. 2000). old-1 and old-2 are about 90 percent identical, and these mutations extend life expectancy by 65 percent and 20 per-cent, respectively (Murakami and Johnson 1998). In C. elegans, increased life expectancy and resistance to environmental stress appear to be closely associated, but how old-1 and old-2 mutants increase stress resistance is not known. In contrast, the ctl-1 gene codes for cytosolic catalase activity, and the life expectancy of ctl-1 mutants is decreased by about 25 percent, presumably because of increased oxidative stress (Taub et al. 1999). The ctl-1 mutation also prevents the increased life expectancy characteristic of daf-2, age-1, and clk-1 mutants.The age-1 mutation increases the levels of both catalase and superoxide dismutase activity in C. elegans (Larsen 1993), but it is not clear whether this increased resistance to oxidative stress is required for the increased life expectancy of the age-1 mutant. Furthermore, overproduction of superoxide

anion by mitochondria due to mutations in the mev-1 gene shortens life expectancy (Senoo-Matsuda et al. 2001); mev-1 codes for a component of the mitochondrial complex II.

Finally, slowing down mitochondrial metabolism also appears to extend life expectancy in C. elegans. Clk-1 mutants are slow growing but long-lived when grown on E. coli (Lakowski and Hekimi 1996). The clk-1 mutant fails to grow when coenzyme Q is removed from the diet altogether because it is unable to synthesize coenzyme Q9 (Jonassen et al. 2001). Overexpression of the clk-1 gene shortens life span (Felkai et al. 1999). In contrast, the life expectancy of wild-type C. elegans is extended about 60 percent by growth on a diet lacking coenzyme Q (Larsen and Clarke 2002).There are also many C. elegans eat mutants with defects in feeding. Most of these eat mutants are long-lived, presumably because they mimic caloric restriction; the strongest effect was seen with eat-2, which lives about 50 percent longer than wild type (Lakowski and Hekimi 1998). The eat-2 mutation further extends the life expectancy of daf-2 mutants but not clk-1 mutants.

In summary, regulation of life expectancy of C. elegans can be influenced by at least three important pathways or processes: insulin-like signaling, stress resistance, and caloric intake/metabolic rate. These processes overlap to some extent, and all have putative mammalian parallels (Table 5).

### FRUIT FLIES (Drosophila melanogaster)

The discovery that nematode mutants with an attenuated insulin-like signaling pathway have increased life expectancy spurred attempts to determine whether the same would be true in fruit flies. Support for this point of view has been obtained (Tatar et al. 2001), but it is not clear why some effects are much greater in females than in males (Clancy et al. 2001).

Many approaches have been tried to demonstrate that Cu/Zn-superoxide dismutase (SOD1) plays a role in aging. Early experiments showed that mutants lacking SOD1 live only 20 percent as long as normal (Phillips et al. 1989). A later report concluded that overexpression of SOD1 increased life expectancy if accompanied by over-expression of catalase (Orr and Sohal 1994), but concerns about the life expectancy of the control strain compromise this interpretation. Parkes et al. (1998) found that selective overexpression of the human SOD1 gene alone in motor neurons increases life expectancy, and Sun and Tower (1999) reported that overexpression of the fruit fly sod1 gene in all cells is positively correlated with life expectancy. Although the molecular basis for the extended life expectancy of the flies selected by Rose (1984) is unknown, these long-lived flies are resistant to oxidative stress in the form of superoxide anion (Harshman and Haberer 2000). In view of these diverse results, the conclusion that sod1 is a longevity gene remains equivocal.

Another possible example is the gene that codes for the repair enzyme protein carboxyl methyl-transferase (PCMT). PCMT converts isoaspartyl residues back to aspartyl residues, and isoaspartyl residues accumulate in proteins with age if PCMT is absent in mice. When this enzyme is overexpressed in fruit flies at a high temperature (29°C), mean life expectancy is increased by about 35 percent (Chavous et al. 2001).This enzyme presumably helps to maintain appropriate protein structure and function during aging in a variety of organisms, and its activity may be rate limiting in fruit flies stressed by growth at 29°C.

Research on fruit flies has identified several new longevity genes.These include (a) the methuselah (mth) gene that codes for a putative transmembrane signal transduction protein whose specific role in vivo is unknown, although these mutants have increased resistance to stress (Lin et al. 1998),

and (b) a gene coding for a dicarboxylic acid transport protein that is conserved in mammals and C. elegans (Rogina et al. 2000). This transport protein may be required for uptake of citric acid cycle intermediates such as succinate, citrate, and a-ketoglutarate by the mitochondria. In the mutant called Indy (I'm not dead yet), partial decreases in this transporter protein significantly increase life expectancy without a significant change in either reproduction or physical activity. This mutation is particularly interesting because of its possible relevance to the mechanistic basis of caloric restriction. Rogina et al. (2000) suggest that "these mutations may create a metabolic state that mimics caloric restriction, which has been shown to extend life-span." Another way to extend life expectancy is to overexpress the hsp70 gene, which codes for a heat-shock protein (Tatar et al. 1997). Such proteins are assumed to increase survival by preventing denaturation of proteins in response to stress.

The mth and Indy mutations are both partial loss-of-function mutations, suggesting that normal levels of these proteins are not optimal for life expectancy. These genetic interventions clearly are not examples of supplementation of proteins or functions lost during aging, and knowledge of the physiological functions they alter might lead to the development of nongenetic interventions that could be useful in people.

Rogina et al. (1997) have also described a short-lived fruit fly mutant called drop-dead that appears to age prematurely and rapidly, as judged by patterns of age-related changes in gene expression. The life expectancy of the mutant is only about 20 percent of normal, though the reason for this accelerated aging is not known. Helfand et al. (1995) have identified a number of genes having temporal expression patterns in antenna that scale with life span, but most of these have not been further characterized, and it is not yet clear whether the temporal pattern of gene expression is a biomarker of aging in Drosophila.

In summary, regulation of life expectancy in fruit flies may be influenced by attenuation of the insulin-like signaling pathway and/or metabolic rate and is associated with stress resistance, all of which mirror the findings with nematodes (Tables 2 and 5).

### YEAST (Saccharomyces cerevisiae)

Although yeast was not discussed at the workshop, it is briefly included here because of a unique life-extending process uncovered by Leonard Guarente's laboratory (Tissenbaum and Guarente 2001). In yeast, the SIR2 gene codes for a histone deacetylase activity that silences gene expression, and overexpression of this gene extends life span of not only yeast but also C. elegans. The histone deacetylase activity requires NAD+ as a cofactor, establishing a possible link between increased life expectancy and nutrient availability. Mutations in the Akt protein kinase gene SCH9, which operates in the insulin signaling pathway, also extend life span and increase stress resistance in yeast (Fabrizio et al. 2001).

# Lessons From a Mammalian Model System

Because of their more complex anatomy and our better knowledge of their physiology and metabolism, mice provide a very useful model system for a bridge between invertebrates and humans. At least four distinct genes (Pit1$^{dw}$, Prop1$^{df}$, Ghr, Ghrhr$^{lit}$) have been identified in which loss-of-function mutations lead to dwarfism and increased mean and maximum longevity (Table 3). These all affect either the production of growth hormone or the ability to respond to it, and the first three have been shown to reduce the levels of circulating IGF-I, insulin, and body temperature (Bartke et al. 2001a). All of these dwarf mice live longer than normal, in agreement with results found in nematodes and fruit flies. Of considerable interest is the observation that caloric restriction further increases the life expectancy of Prop1$^{df}$ dwarf mice (Bartke et al. 2001c), suggesting that the mechanism by which these two pathways influence the rate of aging may be at least partially distinct.

Many genetically altered mice have been generated to determine whether enzymes involved in either preventing or repairing damage to cellular components (such as proteins, DNA, and membranes) play critical roles in aging. Foremost among these are DNA repair enzymes and antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, and methionine sulfoxide reductase. However, some genetic interventions that extend life expectancy in invertebrates, such as SOD1 overexpression in fruit flies, apparently do not have the same effect in mice (Huang et al. 2000). In contrast, mice totally deficient in the mitochondrial superoxide dismutase 2 activity are extremely vulnerable to oxidative stress because mitochondria are the primary site of generation of reactive oxygen species in vivo (Li et al. 1995). Such mice die within 30 days after birth, depending on their genetic background, but it is unlikely that studying such mice will provide much information about normal aging because of their extreme oxidative stress and very early death. A more benign, but still prematurely fatal, intervention is to ablate methionine sulfoxide reductase activity; the life expectancy of these mice is about 40 percent less than normal (Moskovitz et al. 2001), suggesting that deficiency of this enzyme compromises protein structure and function and thereby affects the rate of aging. The p66$^{shc}$ mutation occurs in a gene that codes for a signal transduction protein that regulates apoptosis in response to oxidative stress, and in so doing extends life expectancy (Migliaccio et al. 1999). An interesting short-lived mouse model was recently reported by Tyner et al. (2002). This heterozygous mouse carries one good allele of the p53 gene and one truncated, but still active, allele of p53. These mice are very cancer resistant but also develop age-related pathologies (e.g., osteoporosis, loss of subcutaneous fat, reduced rate of wound healing, muscle atrophy) and die sooner than usual. Another interesting but very short-lived mutant is the klotho mouse that exhibits a similar syndrome resembling human aging (e.g., osteoporosis, atherosclerosis, skin atrophy) and dies by 10 weeks of age (Kuro-o et al. 1997).The klotho gene is homologous to a gene for a putative membrane protein with β-glucosidase activity. Humans

with polymorphisms in this gene are known, and homozygous individuals with two variant alleles of this gene are underrepresented in the elderly population (Arking et al. 2002).

A genetic intervention that appears to mimic caloric restriction is overexpression of the gene coding for the urokinase type of plasminogen activator in the hypothalamus. This apparently down-regulates appetite, leading to lower food intake and smaller body size (Miskin and Masos 1997).

In summary, putative parallels have been observed among nematodes, fruit flies, and mice, assuming that the growth hormone-related mutations act by reducing insulin-like signaling in mice (Table 5). At least some of nematode, fruit fly, and mouse mutations may mimic changes induced in mice by caloric restriction.

# Transition From Animal Models to Humans

**MAJOR QUESTIONS ARISING FROM THESE STUDIES WITH ANIMAL MODELS:**

- Can these results be extrapolated to humans?
- If so, will this information lead to the development of safe and effective nongenetic interventions to delay some of the adverse aspects of aging in humans, or at least provide clues about modulating mechanisms of aging in humans?
- What other genes can be mutated or overexpressed to produce delayed aging, and does natural variation at these loci influence the rate of aging and risk of late-life diseases in mice?
- Most important, do any of the loci where null mutations greatly extend longevity in model systems provide leads to the genetic differences responsible for the much greater aging rate differences among species?

One example of the difficulty encountered in comparing the effect of natural human mutations with targeted mouse mutations is provided by the growth hormone receptor gene (GHR). Humans lacking growth hormone receptor function are known, and these Laron syndrome patients are characterized by short stature, facial dysmorphism, obesity, low serum glucose and IGF-I, and delayed puberty (Zhou et al. 1997). More than 220 cases have been reported so far, but these represent at least 25 different mutations (partial gene deletions and nonsense and missense point mutations), so there are also various phenotypes (Kopchick and Laron 1999). Furthermore, longevity data on these human cases appear to be scarce. The oldest patient with Laron syndrome in the Israeli cohort is only 70 years old. However, fragmentary information for the "Little People" of Krk with mutations in the PROP1 gene suggest that these individuals may be long-lived (Krzisnik et al. 1999; Bartke et al. 2001a); at least one patient lived for 91 years. Thus, while either complete growth hormone deficiency or the inability to respond to growth hormone might not be the optimal strategy for manipulating life expectancy and aging in humans, it is at least possible that moderate depression of circulating growth hormone and IGF-I levels at early ages might lead to lower risk of age-related diseases at older ages. Such a preventive strategy would most likely involve direct manipulation of the IGF-I signaling pathway by nongenetic interventions. However, although decreased insulin-like signaling appears to increase life expectancy in invertebrates, similar mutations in humans might cause insulin resistance, which would be life shortening. It is not clear how to resolve this interspecies dichotomy.

Although the exact number of human genes remains in dispute, it is known that there are substantially more than exist in the invertebrate species discussed in Lessons From Invertebrate Model Systems (p. 10). Because humans have much longer life expectancy and a much more complex physiology, and because invertebrate species are not known to die from well-characterized pathology and disease, the spectrum of identifiable human longevity genes may differ substantially from those shown in

Tables 1 and 2. It is also likely that the genetics of longevity in humans is much more complex than in invertebrate species. Furthermore, age-related pathology clearly differs in mice and humans, so even murine and human longevity genes may have limited overlap. Seemingly, this might discourage one from engaging in a search for longevity genes in humans, but several recent discoveries, coupled with advances in genetics and genomics technology and progress made on the Human Genome Project, could greatly accelerate the search for human longevity genes.The following section describes current efforts to identify human longevity genes. It is already clear that many genes associated with avoiding late-life disease will be discovered (Table 4), and these will provide valuable information for reducing age-related disease and pathology.

# Identifying Human Longevity Genes

### WHO SHOULD BE SELECTED AS SUBJECTS FOR THE DISCOVERY OF HUMAN LONGEVITY GENES?

Some success has been achieved in identifying genes that increase the risk of early-life pathology or that affect what kind of old person one becomes. One approach to detect such gene variants is to determine their frequency at older ages in cross-sectional studies (Schachter et al. 1994); the "oldest old" or centenarians may be the best subjects for such longevity research. Although 100 years of age has no special gerontological significance, many studies have arbitrarily used this milestone in their design. These studies have produced the following results:

- Several studies have shown a very strong relative risk (fourfold to seventeenfold) among siblings of centenarians for becoming a centenarian (Kerber et al. 2001; Gudmundsson et al. 2000; Perls et al. 1998). Such relative risks, especially those greater than eight, are consistent with an important genetic component to exceptional longevity (Rybicki and Elston 2000).
- The proportion of people who reach age 100 is only about 1 per 5,000–10,000, and thus the trait is less common than many other complex syndromes with genetic underpinnings, such as type 2 diabetes.
- Centenarians usually maintain good health to very old ages. Most centenarians report minimal or no medical expenses through seven or more decades of life, even though centenarians often disclose unhealthy lifestyle practices (e.g., obesity and cigarette smoking) and usually indicate no overt effort to achieve longevity, suggesting a genetic rather than an environmental disposition to exceptional longevity.
- Centenarians typically have shorter periods of morbidity than people who die at younger ages (Hitt et al. 1999).

### WHAT RECRUITMENT STRATEGIES SHOULD WE USE?

Although centenarians appear to be enriched with longevity genes, investigators face significant challenges in recruiting large numbers of such individuals for genetic studies. Traditionally, gene localization efforts have utilized families in which several members share the exceptional longevity trait. However, recruitment from founder populations that are more genetically homogeneous offers the advantage that fewer genes may be responsible for longevity and thus they may be easier to identify. A second challenge is that although age of death is an obvious endpoint for study, unequivocal biomarkers (or intermediate traits) relevant to aging and longevity in humans have not been identified (International Longevity Center 2001).

### EFFECTIVE ASCERTAINMENT STRATEGIES FOR LONGEVITY GENE LOCALIZATION EFFORTS

- Recruitment of centenarians and their long-lived (affected) siblings for affected sib-pair linkage analysis.

- Recruitment of long-lived multigenerational multiplex families for linkage analysis (both parametric and model-free).
- Recruitment of large numbers of unrelated long-lived individuals and ethnically matched "controls" for association analyses.
- Recruitment of the offspring and spouses of long-lived probands in order to identify and measure biomarkers of the longevity phenotype for quantitative trait linkage analysis and also for candidate gene studies. These individuals may also be useful for longitudinal follow-up.

## WHAT GENETIC APPROACHES CAN BE USED TO IDENTIFY HUMAN LONGEVITY GENES?

The two main approaches that are utilized to identify genes for diseases and other traits, including longevity, are the candidate-gene and the genome-wide approaches. The candidate-gene approach requires knowledge of the underlying biology of the age-related trait and, based upon this understanding, identification for study of those specifically involved in the underlying biological processes. The candidate gene is examined for sequence variants (mutations and single nucleotide polymorphisms). If a gene variant is found, one may then determine whether the gene variant is shared more often in pairs of long-lived siblings or tracks with longevity in families (linkage analysis). Alternatively, one can ask whether the identified gene variant is more common in long-lived individuals than in controls (association analysis). Once an association or linkage of the gene variant with longevity is demonstrated, studies to investigate how the variant alters function, and how the altered function leads to exceptional longevity, are performed. Candidate genes might include those whose products are involved with lipoprotein metabolism, oxidative stress, DNA repair, cell death, insulin resistance, and metabolism. However, given our very limited understanding of the aging process, our choices of which candidate genes to study are no more than educated guesses.

The candidate-gene approach can also be applied to the mitochondrial genome as well as the nuclear genome. For example, Tanaka et al. (2000) have identified a longevity-associated polymorphism in the gene coding for NADH dehydrogenase, a component of the mitochondrial complex I. The mutant form of this gene suppresses the accumulation of mitochondrial DNA mutations and is enriched in centenarians, possibly because it reduces oxidative stress-induced mutagenesis. This is likely to increase resistance to adult-onset diseases caused in part by age-related mitochondrial dysfunction.

With the progress of the Human Genome Project, it is now possible to perform a search throughout the entire genome (nuclear and mitochondrial) to find regions of chromosomes that are shared more often in long-lived family members than would be expected by chance. The elegance of this approach is that it makes no a priori assumptions about biological processes or candidate genes. Once a chromosomal region is localized, the genes in that region can be examined for mutations. Once a gene is identified, linkage analysis and/or association analyses can be performed; and if the gene variant associates or is linked to longevity, then functional studies, as outlined above, can be performed.

## CURRENT EFFORTS AND SUCCESSES

Some progress has already been made in recruiting and studying long-lived individuals and their siblings for genetic analyses. For example, the New England Centenarian Study has recruited 250 long-lived sibling pairs from throughout the United States. As mentioned above, there

are advantages to performing studies in more genetically homogeneous populations. To this end, researchers are now collecting and studying long-lived individuals and their family members from the Ashkenazi, Amish (Mitchell et al. 2001), and Sardinian populations. Also, a genome-wide scan for linkage to human exceptional longevity was performed among 308 long-lived individuals comprising 137 families recruited for the New England study. One region of the genome that the sibling pairs shared more than would be expected by chance was on chromosome 4 (Puca et al. 2001).The next step in this research is to define which of the many genes in this region is linked to exceptional longevity.

Through use of high-throughput genomics technology, large numbers of gene variants throughout the human genome have been investigated for over- or underrepresentation in unrelated individuals of varying ages. Several gene variants have been identified with this approach, and the relevance of gene variants to age-related disease and the aging process is now under investigation. Centenarians may represent an "ideal genome" in terms of lacking genetic variants that predispose them to age-related diseases at younger ages. As such, the frequencies of informative single nucleotide polymorphisms could be compared between centenarians and individuals with specific age-related diseases, thus aiding in the discovery of disease-disposing genes.

Some progress has been made in defining age-sensitive traits associated with successful aging. While most biological parameters in centenarians at the end of life are in the normal range, it does not necessarily mean that this was the case when they were 30 years younger. Indeed, whereas high-density lipoprotein (HDL) cholesterol levels in Ashkenazi centenarians are normal, HDL cholesterol levels in offspring of centenarians (some of whom also are enriched for longevity genes) are much higher than an age-matched cohort. The pattern of inheritance of high HDL cholesterol appears to be autosomal dominant. It is likely that their centenarian parents also had high HDL cholesterol levels earlier in adult life and that, although HDL cholesterol levels decreased into the normal range over time, this "biomarker" either directly protected the individual from killing diseases (e.g., cardiovascular disease) or was a marker for other biological traits that enhanced longevity (Barzilai et al. 2001).Candidate-gene approaches in which genes that are involved in the regulation of HDL cholesterol levels might unveil gene variants that promote longevity.

## FUTURE DIRECTIONS AND RECOMMENDATIONS FOR HUMAN GENETIC RESEARCH

There is convincing evidence that human longevity has a genetic component (Finch and Tanzi 1997; Ljungquist et al. 1998). Recent progress in identifying longevity genes in animal models, the success of the Human Genome Project, and the development of improved genetic and genomics technology should make it possible to identify longevity genes in humans also. These factors justify moving human longevity research forward in parallel with research in animal model systems. Success of the effort will depend on:

- Recruitment and characterization of large numbers of long-lived individuals and their family members.
- Use of genome-wide and candidate-gene approaches.The former requires access to high-throughput genomics technology, while the latter requires increased knowledge of biological processes of aging and disease.
- Defining human longevity biomarkers (probably through the study of offspring of long-lived individuals).
- Investigation of how genes interact with environmental factors such as physical activity, diet, and other exposures.

# Research Agenda

The previous sections have documented the enormous advances made in the past 10 years to identify genes and processes that influence longevity in both animal model systems and humans. Research on invertebrates has been particularly successful because of their short life spans and well-developed genetic systems. The good news is that apparent parallels have already been demonstrated between invertebrates and mammalian systems, including, to a very limited extent, humans. *However, it is important to note that the goal of these studies is not to find ways of altering human genes but to learn enough about aging to extend the years of active healthy life without genetic alterations.* The ultimate payoff for genetic research is to learn enough about aging to identify promising avenues for nongenetic interventions to delay, prevent, or even reverse the adverse age-related changes leading to disease and disability. With this in mind, the workshop participants identified the following avenues for future research, in addition to those listed in the previous section:

- Caloric Restriction (CR)
  The molecular mechanisms by which caloric restriction increases healthy life expectancy must be understood. Although it would be useful to know whether CR increases the life expectancy of humans and other primates, how CR delays age-related disease in rodents is also an important intermediate goal. Similar arguments can be made for understanding the roles of the insulin-like signaling pathways and stress resistance in regulating life expectancy.
- Identify Biomarkers of Aging
  This need was discussed in a Canyon Ranch report (International Longevity Center 2001). Success would provide an important tool for validating promising interventions as well as characterizing longevity genes.
- Comparative Biology of Aging
  The promise of this approach is consistent with the results summarized in Table 5. Increased emphasis should be given to understanding how some species either escape aging or appear to be longer-lived than expected based on known physiological parameters. For example, it would be useful to know how most bird species deal with high circulating glucose levels, body temperature, and metabolic rate, all of which are thought to be risk factors for aging.
- Use of Mammalian Models
  Invertebrate model systems such as nematodes and fruit flies have been extremely useful for identifying genes that influence aging and longevity, but it is important to increase the emphasis on mammals, including nonhuman primates, in future research. Possibilities include the lemur and, because of available colonies in the United States, baboons and rhesus monkeys.
- Single Nucleotide Polymorphisms
  The elucidation of the sequence of the human genome, and subsequent resequencing of selected genes in a population of individuals, indicates that there is great genetic diversity among individuals. It is a high priority to continue the

search for alleles in candidate genes that significantly influence the rate of aging and life expectancy and the risk for age-related disease.

- Environmental Factors
  Genetic factors have been estimated to account for at most 30 percent of human risk factors in aging (Ljungquist et al. 1998).The remaining risk is likely due to environmental and behavioral factors, so it is important to identify these and how they interact with the known genetic factors. An example is the role of exercise in aging.
- Drug Discovery
  The translational value of understanding the genetic basis of aging and longevity is to increase the potential for rational drug discovery related to reducing the incidence and/or delaying the onset of age-related disease. Thus, emphasis should be placed on translational research leading to discovering useful drug interventions to improve the health of older people and to prevent disability.

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# Glossary

**ALLELE** – one of the variant forms of a gene at a particular locus on a chromosome. Different forms of a gene (one from each parent) produce variation in inherited characteristics.

**ALZHEIMER'S DISEASE** – an aging-dependent disease characterized by loss of memory. Risk factors include both genetic and environmental factors. Age of onset varies from the late forties for patients with early-onset genetic risk factors to 65 and older for most other patients.

**ANTIOXIDANT** – a compound and/or enzyme that neutralizes reactive oxygen species, thereby reducing oxidative stress.

**APOPTOSIS** – programmed cell death, the body's normal method of disposing of damaged, unwanted, or unneeded cells. In general, apoptosis is the result of oxidative stress and free radical damage.

**BIOMARKER (OF AGING)** – an age-related change that reflects the physiological age of an individual in contrast to the chronological age.

**CALORIC RESTRICTION** – a diet strategy to limit caloric intake while supplying all essential dietary ingredients. This extends life expectancy and delays the onset of age-related disease in rodents.

**CATALASE** – an antioxidant enzyme that converts hydrogen peroxide to water and oxygen, which are less damaging.

**CENTENARIAN** – a person who has lived for at least 100 years.

**CHROMOSOME** – a threadlike package of genes and other DNA in the nucleus of a cell. Humans have 23 pairs of chromosomes; each parent contributes one chromosome to each pair.

**DEACETYLASE** – an enzyme that removes acetyl groups from molecules.

**daf** – a class of genes in nematodes in which mutations cause developmental defects.

**DELETION MUTATION** – mutation in which a portion of a gene has been removed or lost.

**DNA** – deoxyribonucleic acid, the double-helix chain that makes up chromosomes and carries the genes and other genetic instructions for making living organisms.

**FOUNDER POPULATION** – a population in which certain mutations or alleles occur frequently because of a shared single ancestor or a small number of ancestors.

**GENOME** – all the DNA contained in an organism or a cell, including the DNA in chromosomes within the nucleus and the DNA in mitochondria.

**GENE** – functional and physical unit of heredity passed from parent to offspring. Humans have two copies of each gene—one inherited from the mother and one from the father. Genes are located at specific places on each of an individual's 46 chromosomes (23 pairs). Genes are pieces of DNA, and most genes contain the information for making a specific protein.

**GENETIC LOCUS** – the place on a chromosome where a specific gene is located.

**GENOTYPE** – an individual's genetic characteristics as opposed to outward physical characteristics (see phenotype).

**GLUTATHIONE** – a natural antioxidant made in cells that helps prevent oxidative damage.

**GLUTATHIONE PEROXIDASE** – an antioxidant enzyme that uses glutathione to prevent or reverse oxidative damage.

**GROWTH HORMONE** – a hormone produced in the pituitary that is essential for normal growth; circulating levels decrease with age, and growth hormone replacement has been promoted as a possible "anti-aging" intervention.

**GROWTH HORMONE RELEASING HORMONE** – a hormone that directs the pituitary gland to produce growth hormone.

**HETEROZYGOUS** – having two different alleles of a gene, one inherited from each parent.

**HOMOZYGOUS** – having two identical alleles of a gene, one inherited from each parent.

**HORMONE** – a substance produced in one tissue but usually acting on another.

**HUTCHINSON-GILFORD SYNDROME** – a human syndrome characterized by abnormal development and premature vascular disease. Children usually die of heart attacks before reaching the age of 20.

**INSULIN-LIKE GROWTH FACTOR I (IGF-I)** – a factor that resembles insulin and stimulates cell growth.

**INSULIN-LIKE SIGNALING PATHWAY** – the series of biochemical events that occur inside the cell when insulin binds to its receptor on the cell surface.

**KINASE** – an enzyme that catalyzes addition of a phosphate group to another molecule, often another protein.

**KNOCK-OUT (MOUSE)** – disruption or deletion of a specific genetic locus from the mouse genome. Genes can also be "knocked out" in nematodes and fruit flies.

**LIFE EXPECTANCY** – the average length of life of a population of individuals.

**LIFE SPAN** – the maximum life span defines the age of death of the longest-lived member of a population.

**LIGAND** – a molecule that binds to a receptor and initiates a specific signaling pathway inside the cell. Hormones are examples of ligands.

**METHIONINE** – a sulfur containing amino acid vulnerable to oxidation, resulting in the production of methionine sulfoxide.

**METHIONINE SULFOXIDE REDUCTASE** – an antioxidant enzyme that converts methionine sulfoxide back to methionine, including on the surface of proteins.

**MISSENSE MUTATION** – the mutation results in a change in the structure of the protein coded by the gene.

**MITOCHONDRIA** – the energy-producing power plants of cells. Cellular degeneration may be caused by mitochondrial dysfunction due to oxidative stress.

**MUTATION** – a permanent structural alteration in DNA; an individual carrying a mutation is referred to as a mutant.

**NEMATODE** – a microscopic worm, usually soil dwelling, that has been developed for biomedical research because of its well-characterized developmental program; it is a useful model system for studying aging because of its short life expectancy.

**NONSENSE MUTATION** – the mutation results in a protein shorter than the normal protein because of premature termination during synthesis of the protein.

**OSTEOPOROSIS** – an age-related pathology characterized by decreasing bone density leading to susceptibility to bone fracture.

**OXIDATIVE STRESS** – the process whereby cellular macromolecules are damaged by reactive oxygen species, produced mainly in the mitochondria, leading to dysfunction.

**PHENOTYPE** – the observable traits or characteristics of an organism; the phenotype is determined by the combination of the genotype and environmental factors to which the individual is exposed.

**PITUITARY** – a gland in the brain that produces several hormones, including growth hormone.

**SARCOPENIA** – an age-related pathology characterized by loss of muscle mass and strength.

**SIGNAL TRANSDUCTION** – the general term for a biochemical pathway that transmits the response to a biological stimulus.

**SINGLE NUCLEOTIDE POLYMORPHISMS** – single base changes in genetic sequences that usually have only subtle, if any, effects on the function of the protein coded by the gene. Every gene contains such polymorphisms, which can be used as genetic markers.

**SUPEROXIDE DISMUTASE (SOD)** – an antioxidant enzyme that converts the superoxide anion to hydrogen peroxide.

**SYNDROME** – the group of symptoms that occur together to characterize a particular abnormal condition.

**TRANSGENIC (MOUSE)** – an experimentally produced mouse, in which DNA has been artificially introduced and incorporated into the mouse's germ line, usually by injecting the foreign DNA into the nucleus of a fertilized embryo. Transgenic nematodes and fruit flies can also be produced.

**TRANSCRIPTION FACTOR** – a protein that helps specify which genes will be expressed at any given time or in response to a biological stimulus.

**WERNER'S SYNDROME** – a human syndrome characterized by premature age-related pathologies, usually starting in one's twenties.

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# Notes

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