GENETICS OF AGING IN THE FRUIT FLY, DROSOPHILA MELANOGASTER

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Key Words life span, oxidative stress, insulin/IGF-like signaling, caloric restriction, gene expression

Abstract Research into the mechanisms underlying the process of aging is emerging as an exciting area of biomedical research. Observations challenging the fundamental assumptions of aging have begun to rejuvenate the field, opening up aging research to fresh ideas and approaches. Genetic approaches, which have been successfully used to understand other complex biological phenomena, are beginning to reveal important patterns and conservations between the processes of aging in a variety of species including yeast, nematodes, flies, and mice. A combination of candidate and random gene alteration approaches, particularly in the fruitfly model system, Drosophila melanogaster, should prove to be especially valuable for elucidating the primary physiological systems involved in aging and life span determination.

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If we kept throughout life the same resistance to stress, injury and disease which we had at the age of ten, about one-half of us here today might expect to survive in 700 years’ time (10–15% live past 2,000 years). The reason that we cannot is that in man, and in many, but probably not all, other animals, the power of self-adjustment and self-maintenance declines with the passage of time, and the probability of disease and death increases. The increase in man becomes eventually so steep that while exceptional individuals may outlast a century, there is an effective limit, depending upon our present age, upon the number of years for which any of us can reasonably expect to go on living. The uniformity of this process is one of the earliest unpleasant discoveries which every individual has to make, and although, we have many psychological expedients to blunt its impact, the fact of this effective fixity of life-span, and of the decline in activity and health which often determine it, is always in the background of the human mind (14).

WHY ARE SCIENTISTS INTERESTED IN AGING?

Understanding the process of aging is the next great frontier of biomedical science. This is not only because of the possible benefits for humankind but also for the challenges of understanding this pervasive complex biological phenomenon. Over the past 30 years we have learned a great deal about some of the long-standing mysteries of life such as development and behavior. Through genetic and molecular approaches, many of the essential features of how a single fertilized egg develops into a mature individual are already or will soon be understood. The beginnings of our understanding of how the brain functions and the elements of behavior have also yielded to combined molecular, genetic, anatomical, and physiological approaches. Yet the mechanisms underlying how we age, a topic of personal and scientific interest for more than two millennia, remain largely a mystery. Such elemental questions, as to whether aging is a process or only a byproduct or epiphenomenon of life, are not definitively known. Do the characteristic stereotypic changes that are seen with age in most organisms represent some underlying program or the final common outcome or canalization of accumulated random stochastic events? There is no reason to suspect that the process of aging will represent fundamentally different laws of nature. Aging should follow the normal chemical and physical laws that all life and other complex biological phenomena obey, and thus should yield its mysteries to a combined genetic and molecular approach.
WHAT IS AGING?

Much of the confusion about aging is wrapped up in attempts to define it. Descriptions of aging phenotypes often seem less like a process than a list or accumulation of often unrelated changes over time. Most definitions of aging emphasize a steady progressive, irreversible decline in organismal performance. Even our choice of words to describe aging implies deterioration and decline, as Sir Peter Medawar noted:

“It is a curious thing that there is no word in the English language that stands for the mere increase of years; that is, for ageing silenced of its overtones of increasing deterioration and decay. At present we are obliged to say that Dorian Gray did not exactly ‘age’, though to admit that he certainly grew older” (57).

Attempts to provide neutral language, such as age-related changes, have not caught on (17). The point is not simply semantic; changes with age are not all deteriorative, and to assume that they are may be limiting and misguiding research directions and ideas (17).

MEASURING AGING

Given the difficulty in defining the process of aging, the next-best approach to provide information on aging might be to examine how it is measured. Often how a phenotype is measured helps to better define it. Here too there are problems. There are no direct methods of measuring aging. Most often aging is measured in an indirect demographic manner. Rather than measuring how individuals change with age, demographically based approaches measure a single outcome, usually the age at death of individuals in a cohort. This information provides no direct information on what leads to death, and thus no information is available on the dynamic nature of the aging process. Nonetheless, the examination of age of death of individuals does identify some important aspects of the aging process, such as the stochastic nature of life span and the relationship of mortality to age. Although these measures may be several steps removed from describing the process of aging, they are the most commonly used methods available. Much of what we know about the aging process is thus based upon measurements of age at death or survivorship curves, where comparisons are made between the mean, median, and maximum life spans of different populations (species, strains, etc.) under different conditions (environmental and genetic alterations) (30).

Age at death of individuals in a population or survivorship provides a specific measurable phenotype for studying elements of the process. It is assumed that alterations (genetic or environmental) that shorten or lengthen life span may be doing so as a result of an effect on the process of aging itself. Hence, screens dependent upon life span as the output are potential enrichment schemes for identifying
age-related mechanisms. Interventions, genetic or environmental, that alter life span may not truly provide direct information on the process of aging itself. The distinction between life-altering interventions and rates of aging can be illustrated by looking at how survivorship and mortality curves are used to examine the demographic rate of aging. The slope of the mortality curve defines the demographic rate of aging. Alterations that change the slope of the mortality curve are thought to reflect changes in the rate of aging, at least the demographic rate of aging. In cold-blooded animals, such as the fruitfly *Drosophila melanogaster*, changes in ambient temperature dramatically alter the rate of physiological changes that take place with age (fertility, physical activity—negative geotaxis, etc.) (59) and change the slope of the mortality curve (30). Other interventions, such as alterations in reproduction in *Drosophila*, for example, which can result in twofold changes in life span, do not appear to affect the slope of the mortality curve, but instead alter only the $y$-intercept, a measure of the initiation of mortality (30). In cases where the $y$-intercept changes but the slope does not, it is thought that the intervention has altered the baseline mortality, but once mortality begins to increase, it does so at the same rate as the unaltered condition. Hence some interventions that alter life span may do so by changing the demographic rate of aging, whereas others do not alter the demographic rate of aging but instead affect the timing of its onset—these interventions are thought not to alter the “normal” course of the aging process. Despite these important caveats, life span analysis is presently the most commonly employed and best assay for measuring age-related phenotypes. It is our hope that the development of age-related physiological and molecular changes will ultimately supplant life span as the primary assay in aging research (30, 31).

**MYTHS OF AGING**

Several assumptions about the aging process have taken on near-axiomatic status, but upon closer inspection may not be quite so clear-cut. Some of these assumptions have had a profound influence on directing aging research for years, providing either guidance on where and how to examine aging or setting up barriers or obstacles to potentially important investigations into the aging process (27). The following are examples of four such assumptions.

It is a commonly held notion that an inevitable consequence of life is a progressive functional decline with age. In recent years, the demonstration of a number of species, from several different phyla, that do not appear to decline in function with age casts some doubt upon the universality of functional decline or senescence and thus its inevitability. As is noted by Finch, organisms such as fish, turtles, tortoises, lobsters, clams, and plants do not appear to decline with age and have negligible senescence (17, 18, 27). The demonstration of such organisms suggests that the functional decline so commonly seen with age is not a biological necessity, and these exceptions may help us to understand how aging does occur.

A second assumption about aging refers to the character of adult life. As stated by Sir Peter Medawar:
“It has several times been pointed out that the changes which an animal may undergo after it has ceased to reproduce are never directly relevant, and are in most cases quite irrelevant, to the course of its evolution. A genetic catastrophe that befell a mouse on the day it weaned its last litter would from the evolutionary point of view be null and void” (56).

Thus, it is often stated that after the peak of reproduction, the force of selection declines toward zero, and the remaining portion of an organism’s life is thought to follow a course of random passive decline. However, gene-expression studies in both mammals and fruitflies, rather than showing a random passive decline, document dynamic, well-regulated change in the signature pattern of many different genes throughout adult life (26–29, 31, 72). This suggests that, regardless of the force driving the process of aging, many of the organism’s responses are not random or passive, but dynamic and well-regulated (27, 31).

The third assumption, based on principles of engineering and evolutionary theory, is that aging is associated with a global loss of homeostasis with subsequent age-dependent decline of all organ systems. The notion is that all things fall apart and do so at nearly the same time. Measuring transcriptional activity with age, a sensitive physiological measure, using $\text{lacZ}$-tagged genes or microarrays has, however, failed to show an age-dependent increase in global dysregulation in $Drosophila$ (72, 76). In addition, the observation that overexpression of human SOD1 in only motor neurons of $Drosophila$ causes a 40% increase in life span directly casts doubt on the idea that all organ systems fail at or near the same time (62).

The fourth assumption concerns one of the central elements of aging research—Gompertz’s law of mortality. In 1825, Gompertz, a British actuary, showed that in populations of humans the rate of mortality increases exponentially (21). As one gets older, the chances of dying in the next time period continuously increase. The observation of an exponential increase in mortality was shown in a variety of different species and has also provided the data for a related assumption, species-specific life spans. The idea of a species-specific life span states that each species has its own fixed or limited life span. For example, flies live for months, mice for two to three years, and humans for decades. However, in the 1990s, largely through work using $Drosophila$ and related insect species (medflies, Ceratitis capitata), it was demonstrated that if a large enough population is examined, rather than showing a constantly increasing rate of mortality, many different species, including humans, show a late-life mortality plateau (5, 15, 97, 98). In addition, when a large enough population is examined, the species-specific life span hypothesis also breaks down. The average life span of medflies was thought to be approximately 30 to 40 days, but when the cohort size was in the millions, it was noticed that there were a number of medflies that were older than 160 days (5).

Although there is yet no ready explanation for the late-life mortality plateau, (density effects and genetic heterogeneity have been ruled out), its existence strongly challenges the idea that there is a fixed biological limit to life span for each species (10).
The fruit fly *Drosophila melanogaster* is one of the most common genetic model organisms used for examining complex biological phenomena. The benefits of using *Drosophila* for studying aging in particular include (a) its relatively short life span (3 months), (b) ease of maintenance, (c) environmental and genetic manipulations that alter life span, (d) already available information on aging, (e) availability of stocks containing altered genes, (f) powerful molecular genetic techniques, (g) sequence of the full *Drosophila* genome, and (h) proven success in dissecting apart complex biological phenomena such as development (31). Additional aspects of the biology of the fly that are of particular advantage for aging work include its life history, which is divided up into distinct morphological stages, so that the periods of growth and development can be readily distinguished from the sexually mature “aging” adult phase. In many organisms, visually distinguishing mature aging adults from immature or juvenile stages is not simple. In *Drosophila*, growth and development are restricted to the embryo, worm-like larval stages, and the pupae. Within 24 h of emergence from the pupal case, the adult fly is sexually mature and hence considered a mature “aging” adult. A second feature of note is that the adult fly consists almost entirely of postmitotic fully differentiated cells. Except for cells in the gonads and a few cells in the gut, no other cells are thought to continue to divide (4, 33). Thus aging in the fly may be reduced to the aging of a set of postmitotic cells that are present from the time of adult emergence until death (2). In other organisms, such as humans, many organ systems such as the gastrointestinal, dermatological, and hematopoetic systems are constituted by nearly continuous cell replacement. Other organs, including the brain and heart, rarely have the addition of new neurons or cardiac cells. Aging may mean different things in each of these two different situations. If aging research is primarily involved in trying to understand the changes that take place in cells and organs over time, then organisms such as *Drosophila*, which are almost entirely postmitotic, are excellent model systems for such studies.

**DROSOPHILA’S PREVIOUS CONTRIBUTIONS TO AGING RESEARCH**

*Drosophila* and related insects have been used as a model organism for aging research since at least 1916. Loeb & Northrop used *Drosophila* to show that life span obeyed the normal laws of chemistry and physics, similar to other biological processes (49, 50). By changing the ambient temperature at which these poikilothermic flies were cultured, they showed an inverse relationship between ambient temperature and life span. In the 1920s, Pearl and colleagues used *Drosophila* to show that life span is an inherited trait (67–69). Experiments using *Drosophila* also figured prominently in Pearl’s “rate-of-living” hypothesis, a precursor to the
modern-day oxidative stress hypothesis (1, 66, 84, 88). In the 1950s, J. Maynard Smith and colleagues showed the importance of reproduction in determining life span and highlighted *Drosophila* as a model system for studying fitness trade-offs and life span (82, 83). In the 1980s, laboratory selection experiments showed the plasticity of life span in *Drosophila* (52–54, 78–80). By selecting for late-life reproduction, they developed long-lived lines that lived twice as long as the original outbred stock from which they were derived. Over the past 80 to 90 years, research utilizing *Drosophila* has been essential for recognizing the heritable and plastic nature of life span and instrumental in the development of many of the more important hypotheses of the aging process, including the rate-of-living and oxidative stress hypotheses.

**MANIPULATIONS THAT EXTEND LIFE SPAN IN DROSOPHILA: NONGENETIC**

A number of interventions are known to affect life span in *Drosophila*. These include (a) mild stressors, (b) ambient temperature, (c) physical activity, (d) reproductive status, and (e) dietary status (30, 31). Different mild nonlethal stressors, such as periods of heat shock (37°C for less than 60 min), cold stress, or low levels of radiation, can significantly extend life span in *Drosophila*. The mechanisms by which these manipulations alter life span are not known. Mild stressors may induce protective systems that result in short-term beneficial effects and life-span extension. Periodic heat shocks increase the expression and efficiency of a number of different protective systems. Experiments investigating the molecular mechanisms underlying how this increases life span initially suggested that *hsp70* might be involved in the positive effect on life span. A brief heat shock of transgenic animals with multiple additional copies of the *hsp70* gene showed a transient decrease in the slope of their mortality curve (92); however, subsequent studies have failed to show a positive affect on life span or physiological markers such as motility (58). In some cases, the positive life-span effects of stressors may be through their effect on other physiological systems. For example, the life-span extension seen with nonlethal irradiation in females is probably due to the effect irradiation has on reproduction, causing a temporary sterility. In support of this conclusion is the lack of life-span extension after irradiation of genetically sterile females (43, 44).

Decreases in ambient temperature to 18°C can more than double life span and change the rate of age-related physiological markers such as reproduction and motility (31, 59). The mechanisms by which ambient temperature alters life span are probably through a direct effect on metabolic rates. Interestingly, in *Drosophila*, more severe reductions in ambient temperature to 11°C can induce a state of reproductive diapause in young flies (94). At temperatures of 11°C, females arrest their egg development at the previtellogenic stage, and both males and females can remain in this state for at least 9 to 11 weeks, after which, upon rewarming, they become reproductively active and live a completely normal life span (94).
features of this diapause state show a similarity to the dauer stage of the nematode *Caenorhabditis elegans* (94). Changes in physical activity, known to affect life span, may also do so through an effect on metabolic rates. A forced reduction in physical activity in houseflies, by housing single flies in small containers that did not permit them to fly, increased life span (85). Neurological mutants, in K⁺ channels (*Hyperkinetic*, *Shaker*), increase physical activity and shorten life span (96).

Reproductive status has a major effect on life span, particularly in females. Virgin females live up to twice as long as mated cohorts (82, 83). The mechanism behind this extended life span is probably a combination of the reduction in energy costs from lower or delayed egg production, as well as the costs of mating itself. The cost of mating includes several factors in addition to wear and tear (7, 8). For example, the seminal fluid transferred by males to females during mating has a direct negative effect on female life span (8). Substances in the seminal fluid, likely intended to improve sperm utilization and decrease remating frequency, have negative repercussions on female life span. Male virgins also live longer than females, although the extension, depending on the strain, is usually significantly less than in females (63).

Caloric or dietary restriction extends life span in a variety of organisms, including mammals, flies, and nematodes (19, 41, 51). In *Drosophila*, dilution of their diet of yeast or other components of their food leads to extension of life span, particularly for females (9, 12, 22, 55). However, despite dilution in the calories or quality of the food, the flies still have continuous access since they are living in or on their food, and could therefore eat more to make up for the dilution. Although this issue awaits resolution, these manipulations of the food cause at least two important physiological responses in female flies: life-span extension and reduction in egg laying (55). Regardless of whether this is the same as caloric restriction in mammals, flies clearly respond to a reduction in yeast or other food components by adjusting two important life-history parameters. Despite over 60 years of studies on caloric restriction in mammals, we are not much closer to understanding the molecular or physiological mechanisms by which life-span extension occurs. Demonstration of the life-extending effects of dietary dilution or restriction in flies will allow us to use the powerful molecular genetic system of *Drosophila* to dissect out the relationship between food intake, utilization, and life span.

**IS AGING GENETICALLY DETERMINED, AND WHAT DOES THAT MEAN ANYWAY?**

It is generally thought that genetics plays a major role in the process of aging and the determination of longevity (17). The fact that aging is hereditary, however, does not necessarily mean that a fixed genetic pathway determines aging. While it is possible that genetic elements could provide instructive information and drive the process of aging directly, as it does in development, there are other possibilities by
which genetic elements may influence aging and life span. Genetic elements could simply limit the extent to which an organism can respond to stresses or damage. If such were the case, genetic elements would not be controlling the process, but instead would be defining the possible responses, thereby determining the pace of aging, yet would not be responsible for directing the process itself. For example, if aging is due to the accumulation of damage from some toxic agents, simply by setting the genetic endowment for determining the amount of protective antitoxic responses of the organism, genetic elements could play a major role in determining life span without actually being responsible for setting the course of the process.

GENETIC APPROACHES TO UNDERSTANDING AGING

Whether it is ultimately determined that genetic elements participate by an active (instructive) or passive (endowment) mode, the use of genetic approaches remains an invaluable method for identifying the physiological systems responsible for the aging process. Genetic approaches, altering single genes and examining the resultant phenotype, are particularly powerful methods for determining causal relationships and dissecting complex biological phenomena such as aging. Genetic approaches can thus be used to confirm already available hypotheses using candidate gene approaches, or be used to explore for new ones, using random single-gene alteration approaches. An additional advantage of genetic approaches is that they require little to no knowledge of the system being studied—a particularly valuable asset in studying aging.

Concerns with Genetic Studies on Aging in Flies

A number of considerations must be kept in mind when using genetic approaches to study aging, particularly in flies. First is the question of what is the phenotype being examined; what is to be measured? As noted in an earlier section, measuring the actual process of aging is not simple, and is rarely done. What is usually done is to measure one aspect of the aging process, life span. Determination of life span to assess the value of specific genetic alterations has important caveats. Genetic interventions that shorten life span may be doing so through pathological reasons, unrelated to the aging process itself, and hence be of little value for studying aging. Therefore, the preferred approach has been to look for genetic interventions that extend life span. However, life-span extension alone is not sufficient to be certain that an intervention is altering the process of aging in a meaningful way. As noted in the section on nongenetic interventions, there are a number of ways in which life span can be extended that may or may not tell us much about the process of aging. For example, if a genetic intervention reduced the metabolic rate of the animal or left the animal so debilitated that it could barely move, the fly might be long-lived, but is this really extending life span? The trade-offs on quality of life such as movement and reproduction would clearly be unacceptable. It has been proposed
that the relevant aspects of life span can be quantified as the product of metabolic rate \times life span or metabolic potential \((86, 87)\). Under this definition, interventions that increase the metabolic potential of the animal could be identified, as opposed to those that may be slowing down normal processes, giving the impression of longer life at the expense of activity or other important life span elements such as reproduction. Reduction in female reproduction, which is known to increase life span, is another common means of extending female life span. Therefore, measurements of metabolic rate, physical activity, and reproduction are critical to assess the value of any life-extending intervention.

Genetic approaches usually require a uniform genetic background, raising the theoretical concern of inbreeding and the accumulation of deleterious mutations. A potential consequence of the creation and maintenance of genetic homogeneity in inbred stocks is that a particular fly stock may accumulate deleterious mutations, giving the stock an artificially shorter life span. If this were to occur, then a genetic intervention that improved or rescued one or more of these deleterious mutations could extend life span back toward “normal,” but might be misinterpreted as a genetic alteration that extends life span and defines a pathway important in “normal” life-span extension. There are a number of ways of evaluating this possibility, such as testing the genetic intervention in other genetic backgrounds, including other inbred stocks separated for decades, as well as in outbred stocks. This approach has been successfully used by a number of investigators reporting single gene alterations that extend life span, including \textit{Indy}, \textit{InR}, \textit{chico}, and \textit{rpd3} \((13, 74, 75, 93)\).

Logistical considerations also influence the design and success of genetic studies of life span in the fly. The stochastic nature of life span necessitates that the assessment of life span is a population-based assay, precluding studies on single individuals. The number of individuals needed for each assay raises additional difficulties when trying to use traditional genetic approaches of gene exploration, random single gene alterations. In order to adequately assess life span, between 200–300 male and an approximately equal number of female flies need to be studied for each life span. Thus, not only must the genetic study be an F2 screen, but large numbers of flies must also be grown up in order to obtain a big enough cohort for life span studies. An additional element is that life span at \(25^\circ C\) for most wild-type stocks (Canton-S, Oregon-R) is from 3 to 4 months. We estimate that under normal culturing conditions \((25^\circ C, \text{etc.})\), a medium-sized PI-based laboratory would only be able to screen on the order of a few hundred lines a year. Most genetic screens seek to test thousands of different random gene alterations, hence the likely reason why there have been few screens for life-span extension in the fly. Using environmental methods to accelerate the assay, such as growing the animals at higher temperatures, \(29^\circ C\), introduces other problems \((30)\). The uses of a sensitized genetic background \((73)\), surrogate markers for aging such as stress resistance \((48)\), or the development of biomarkers of aging have been suggested \((31)\).

Finally, when performing genetic studies the genetic background of the experimental lines and controls must be carefully managed. Genetic background is
profoundly important in determining life span in flies. Many of the new molecular
genetic manipulations available in the fly that make it such a powerful molecular
genetic model system introduce changes in the genetic background in ways that
are difficult to control for, so it is essential to be particularly careful and fastid-
ious about matching controls to each experimental condition (35). One potential
method for eliminating the problem of genetic background is to use one of the sev-
eral methods of inducible or conditional drivers such as doxycycline or RU-486,
where the difference between the experimental and control conditions will be the
presence or absence of the inducing agent, but the genetic backgrounds will be the
same (61, 77, 89, 90). Examination of, and controlling for, any effects that these
inducing agents may have on life span directly will of course be needed.

MANIPULATIONS THAT EXTEND LIFE SPAN IN
DROSOPHILA: GENETIC

The recent explosion in the use of single gene approaches to understand aging
may be traced to the 1993 report showing that mutations in a single gene, *daf-2*,
in *C. elegans* could double the adult life span of the nematode (37). Previously, it
was thought that it would be difficult to identify a single gene that would have a
significant affect on life-span extension (65). A number of reports using candidate
and random gene-alteration approaches have subsequently been used to identify
genes and physiological systems important in aging in the fly. The candidate gene
approach is most often used to test already available theories on aging. These
include the oxidative stress hypothesis, role of insulin/IGF-like signaling, role
of protein repair mechanisms, role of chaperones and heat-shock proteins, and
contribution of chromatin structure.

Antioxidants and Life Span

The oxidative stress hypothesis is presently the most prominent theory in the field
of aging research (20, 86, 88). This hypothesis states that the imbalance between the
formation and detoxification of reactive oxygen species generated during normal
cellular metabolism results in the accumulation of oxidative damage to a variety
of macromolecules, including DNA, proteins, and lipids. The accumulation of
damage to macromolecules is thought to be the driving force behind the process
of aging. The ability to slow the accumulation of oxidative damage should delay
or slow the rate of aging and extend life span. A decrease in the accumulation of
oxidative damage could be imagined to occur through either a decrease in reactive
oxygen species production, an increase in detoxification of reactive oxygen
species, or the repair and/or removal of oxidatively damaged macromolecules. *Drosophila*
has been at the forefront of studies testing the oxidative stress hypoth-
esis. The primary approach to date has been to decrease or increase antioxidant
enzymes important in detoxifying reactive oxygen species before they can cause
damage. Decreases in some of these enzymes, such as catalase and superoxide dismutase (SOD), shorten life span, suggesting the importance of detoxification of reactive oxygen species and life span (39, 60, 70, 71). However, in all cases the mutation was also present during development, and hence the shortened life span of the adult flies could, in part, be due to an accumulation of damage accumulated during development, and not due to the effect of oxidative damage during adult life. Therefore, more attention has been placed on increasing antioxidant enzymes in the anticipation of showing an increase in adult life span. The results on these studies have so far been mixed. Either a combined increase in SOD and catalase or SOD activity alone has been reported to show modest increases in life span (reviewed in 31). One notable finding is that overexpression of human SOD in the motorneurons of the fly resulted in a 40–50% increase in life span (62). Importantly, some of these studies have used techniques to increase SOD or catalase activity only during adult life in order to eliminate the effects of overexpression during development and to more precisely examine their effect on aging per se. At first glance, the relatively modest effects on life span when increasing antioxidant enzymes could provide ammunition for opponents of the oxidative stress hypothesis. However, since the relevant outcome, decrease in oxidative damage, is rarely measured, it may be that these particular transgenic animals either do not increase the antioxidants to levels high enough to have an effect on the relevant oxidatively damaged macromolecules, or the temporal or tissue distribution of antioxidants is critical to life-span extension.

**Insulin/IGF-Like Signaling and Life Span**

The findings in *C. elegans* that mutations in *daf-2* and other *daf*-related genes extend life span demonstrated the importance of the insulin/IGF-like signaling pathway for determination of life span. In *Drosophila*, specific mutations in the insulin receptor gene (*InR*) and the insulin receptor substrate (*chico*) extend life span of females (13, 93). Both male and female mutants are dwarf, and the long-lived females are sterile or subfertile. Life-span extension in mice has also been shown when IGF-1 is reduced ubiquitously or selectively in adipose tissue (3, 32). The positive effects of manipulations of the insulin/IGF-like signaling pathway in nematodes, flies, and mice show the conservation of this longevity-determination pathway across distant species, and highlight the power of model systems for exploring and understanding aging and life span.

**Protective/Repair Systems and Life Span**

The combination of observations of age-related accumulation of damage to a variety of macromolecules (including those associated with oxidative damage) and the life-extending effects of a variety of nonlethal stressors has suggested the possibility that the state of protective or repair pathways might affect aging and longevity. Will an enhancement of protective or repair pathways prolong life span? Increased expression of two different protein repair systems, protein carboxy
methyltransferase (PCMT), which is involved in repairing damaged isoaspartyl residues in proteins formed during aging, and methionine sulfoxide reductase, which is involved in repairing oxidized methionines in proteins, have both been reported to extend life span when overexpressed in transgenic flies (11, 81). The positive effects of mild heat shock on life span led to the evaluation of specific chaperone and heat shock proteins on life span. The use of transgenic animals to test the life span-extending capabilities of hsp70 have shown limited success, although chaperones and heat shock-related proteins play a role in protecting Drosophila from the induction of neurodegenerative diseases (reviewed in 6, 25, 31).

Chromatin Structure, Silencing, and Life Span

In Drosophila and other species, there are a variety of different genes whose proteins are important in the maintenance and integrity of chromatin structure and gene expression. A set of diverse proteins, including histone deacetylases, are important in controlling the packing of DNA, and hence a gene’s accessibility to gene expression machinery. Some of the histone deacetylases, including rpd3 and Sir2, appear to be involved in life-span determination of yeast, C. elegans, and Drosophila. In yeast, reductions in rpd3 or increases in Sir2 extend life span (23, 38). Increasing Sir2 also extends life span in C. elegans (95), whereas a decrease in rpd3 increases life span in Drosophila (74). In addition, drugs such as phenylbutyrate (PBA) that inhibit histone deacetylases also appear to increase life span in Drosophila (36).

Random Single Gene Approaches

An even more useful genetic approach for understanding the aging process may be the unbiased random gene alteration screen. This approach can confirm previously recognized aging-related systems, but more important, can identify new physiological systems involved in aging. Drosophila is particularly well suited to this approach because of the highly developed molecular genetic tools available, short life span, and well-defined, age-related physiological changes (fertility, motility, behavior, etc.). Although small and often viewed simply as an innocuous pest, Drosophila is not a primitive organism. These animals are highly complex, with nervous systems possessing millions of neurons as opposed to several hundred in the nematode, and engage in highly sophisticated behaviors such as flight, courtship, learning, and memory, all of which can be assessed in an age-dependent manner.

In the random single-gene alteration approach, one gene at a time is altered, either directly (mutagenesis) or by ectopic expression, and its effect on the phenotype of choice is then measured. This method has proven successful in helping to unravel other complex biological processes such as development. In the case of aging, the standard phenotype has been life span, particularly life-span extension, but this approach can be used to investigate any age-related phenotype, such as late-life preservation of motility. One benefit of the single-gene alteration approach, as
opposed to the quantitative trait locus (QTL) or the candidate gene approach, is that the method of altering the gene may create a new protein product or distribution of protein expression that would not have been suspected and engineered in a candidate gene, or may never be seen in nature, and thus not be accessible using selection or QTL approaches.

To date, two different life-extending mutations have been identified using screens that made use of random genetic alterations, the *methuselah (mth)* and *I'm not dead yet (Indy)* mutants. Partial loss-of-function mutations in either *mth* or *Indy* increase life span of both male and female flies, without a loss of fertility (48, 75). The *mth* gene, mutations of which increase mean life span by 35%, codes for a G-coupled transmembrane, receptor-like molecule that is a member of the secretin subfamily (99). In addition to long life, the *mth* mutant animals are resistant to heat stress, starvation, dessication, and paraquat (oxygen radical generator) (48). The ligand for MTH protein is not yet known, nor is the mechanism by which decreases in MTH lead to life-span extension or stress resistance. However, the association of life-span extension with stress resistance supports the hypothesis that stress resistance is related to longevity (34).

The *Indy* gene is a dicarboxylate transporter, found in the plasma membrane of organs important in intermediary metabolism of the fly, including the midgut, fat body, and oenocytes (40). Partial decreases in INDY protein in these tissues are associated with a near-doubling in average life span of both males and females (75). In addition to the preservation of normal or supranormal fertility in *Indy* long-lived females, there are no measurable decreases in resting metabolic rates or important physiological measures such as flight performance and 24-h activity levels (55; J. Hendricks, personal communication). However, under conditions of poor food quality, the *Indy* long-lived females lay substantially fewer eggs than normal-lived control females under similar low-quality food conditions, perhaps helping to explain why *Indy* has not arisen and taken over in the wild (55). It has been suggested that life span-extending interventions will always have unacceptable trade-offs in metabolic rate, growth, physical activity, or early-life fecundity. It is therefore of great interest that under normal laboratory conditions, when conditions are constant and food is abundant, the *Indy* mutation dramatically extends life span without any observable trade-off. The *Indy* mutation has been postulated to reduce the uptake, utilization, or storage of important nutrients, somehow altering metabolism in a way that increases life span.

**EVOLUTIONARY CONSERVATION OF LIFE SPAN-EXTENDING SYSTEMS**

Even at this early period in the molecular genetics of aging in *Drosophila*, important principles are emerging (19, 31, 64). At least three different physiological systems involved in determining life span appear to be conserved across distant species. The insulin/IGF-like signaling pathway in nematodes, flies, and mammals
(3, 13, 24, 32, 91, 93) and the histone deacetylases rpd3/Sir2 pathway important in yeast, nematodes, and flies both appear to be involved in longevity determination (23, 38, 74, 95). A third intervention, which may be the point of convergence for each of these systems, is caloric restriction. Caloric restriction, the only known means of extending life span in mammals, also extends life span in yeast, nematodes, and flies (9, 12, 19, 22, 41, 42, 45–47, 51, 55). Of the five single gene alterations that significantly increase life span in the fly (mth, Indy, InR, chico, and rpd3), at least three (Indy, chico, rpd3), and possibly four (InR), have been linked to caloric restriction or postulated to involve metabolic changes similar to caloric restriction.

CONCLUSIONS

In an era when we can sequence the genome of any organism, understand the detailed processes associated with normal and abnormal cell physiology and development, and have great insight into complex biological issues such as the workings of the brain including learning, memory, and behavior, it is ironic that our understanding of the aging process is so far behind. New revelations challenging some of the fundamental assumptions of aging have begun to rejuvenate the field, opening aging research to fresh ideas and approaches. Genetic approaches, which have served so well to elucidate the mysteries of development, and have provided insight into other complex biological phenomena, are beginning to reveal important patterns and conservations between the processes of aging in a variety of distant species, including yeast, nematodes, flies, and mice. These genetically based studies have already provided important clues about the aging process and are good reason for excitement and optimism. A combination of candidate and random gene alteration approaches should be successful in elucidating the primary physiological systems involved in life span determination and aging.

“It is one of the most remarkable things that in all of the biological sciences there is no clue as to the necessity of death. If you say we want to make perpetual motion, we have discovered enough laws as we studied physics to see that it is either absolutely impossible or else the laws are wrong. But there is nothing in biology yet found that indicates the inevitability of death. This suggests to me that it is not at all inevitable, and that it is only a matter of time before the biologists discover what it is that is causing us the trouble and that that terrible universal disease or temporariness of the human’s body will be cured” (16).

ACKNOWLEDGMENTS

Supported by NIH grants AG-14532 to S.L.H., ES-11463 and AG-20816 to B. R., Donaghue Investigator Award from The Patrick and Catherine Weldon Donaghue Medical Foundation (S.L.H.), and an Ellison Medical Foundation Senior
Investigator Award (S.L.H.). We apologize to those colleagues whose work has not been referenced owing to space limitations.

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