The 2002 Kleemeier Award from the Gerontological Society of America was awarded to Thomas E. Johnson, PhD, of the University of Colorado at Boulder. Dr. Johnson was the pioneer who first applied genetic analyses to the study of the aging processes in *Caenorhabditis elegans* and who introduced the nematode as an aging model. Longer life span was chosen as a surrogate marker for slowed aging. Here Dr. Johnson describes his role(s) in the isolation of *age-1*, the first longevity mutant, which can more than double the life span and which slows the rate of aging more than twofold. He also reviews research suggesting conservation of function and applicability to intervention by pharmacological targeting of the Age-1 pathway. Current work by biotechnology companies targets this and other basic discoveries in an attempt to postpone human aging.

**Finding the Right Approach**

I think perhaps the key issue for me in initiating this research was deciding what species to work on. Now I had the misfortune of having an unsuccessful experience during my first postdoc and deciding to switch to another organism for a new postdoc, which at that time was fairly unprecedented. So I went to Bill Wood, an eminent molecular geneticist then at Cal Tech, asking him if I could join his lab as a new postdoctoral fellow. One of the reasons that I did that was due to an influential discussion with Don Murphy, a new Program Director at the recently formed National Institute on Aging. (This was 1976, the Institute on Aging was formed in 1974.) Don Murphy had the insight to develop a program for the NIA, emphasizing *C. elegans* work. There were only about 35 “*C. elegans* people” in the world at that time; I was actually at a meeting with all of them present at Wood’s Hole in 1977. All of the Nobel Prize winners—Sydney Brenner, John Sulston, and Bob Horvitz—were there; they all gave talks. It was very insightful of Don
Murphy to recognize that *C. elegans* was a great model system for aging research. I’ll tell you why in a little bit. By the way, those of us working on *C. elegans* don’t ever really call it *C. elegans*, we call it “the worm”; so that’s probably the way I’ll refer to it. Here’s a picture of a nice, svelte, young hermaphrodite *C. elegans*; these are hermaphrodites, not true females. I set about trying to study the process that turns one of these hermaphrodites—young, beautiful—into an old animal, and you can see that this happens in only 21 days (Figure 1). This is quite fortunate because I didn’t want to spend 2 or 3 years, which is the time it takes for mice to die, doing one experiment, or even worse 70 or 80 years, which is the time it takes one of us to die. So a key part of the choice of this model system was the short life of *C. elegans*. Whether or not this species shared a lot of mechanisms of aging with humans was much less important and is something about which I wish to be very cautious; we’ll discuss that in a bit. But there really are a lot of reasons for working on *C. elegans*. I won’t go into too much of this [for a review of this, see (1)]. In addition to its short life span of a couple of weeks, one of the real advantages is that we can freeze these animals to keep them viable forever. Really, cryogenic preservation works, at least in one species. I can thaw a tube of worms that I froze 20 years ago and a few of them are still viable and will crawl out and live for a few days, long enough to reproduce and maintain the strain. So this is effective immortality, but not one that most of us would want. Another reason for working on the worm is that, about 5 years ago, the complete genome sequence was determined; at the time, the worm was the only metazoan for which the genome sequence was available. The sequence has increased the genetic tractability of the worm 10-fold, maybe 100-fold. There is just an incredibly facile molecular genetics; I won’t get into this in detail. But there’s one more big reason for working in this species: it’s a self-fertilizing hermaphrodite, meaning that there is no inbreeding depression and we can easily find and maintain mutants, but that’s for the specialists and we’ll leave that alone for now, even though this has been a sizable part of my research.

**Genetic Approaches to Aging**

I was trained as a geneticist and I took advantage of the fact that there was great genetics in the nematode, even when I started in 1977. So I started using a genetic approach to study aging and initiated an analysis of the genetics of aging but really I was focusing on the genetics of life extension. Genetics is a terribly powerful approach. As we all know, genes are the blueprints, the instructions for the cell, to create every aspect, every protein found in that cell. And, the phenotype that we were interested in here was longevity. So we set off on a project in which we were going to start developing genetics of longevity. Genes only explain about 20% of overall life span in humans; this is from studies on identical twins. There is a similar fraction of the overall variation in length of life in a population of nematodes, only about 30% or 40% that is due to genetic variation (2). Nevertheless, that’s controllable and it’s very replicable, so genes give a very good handle. Another reason that we use genetics is that there are at least 20,000 interventions. In other words, we can delete any gene in the genome of the animal one at a time and see what effect that deletion has on life span. So genetics is an exquisitely sensitive way to dissect an organism’s biology. By the 70s, all of those handles either were in place or it was clear that they were going to be in place for use in *C. elegans*. So, we set off to find mutants and genetic variants for life span.

**Focus on Life Extension**

The next thing in terms of finding the right approach was not just using “the worm”, but deciding what measurement of aging I would use. To do this I had to have a way of knowing how fast the worm was aging, and measuring the “biologic age” or rate of aging of an organism was not (and still is not) easy. However, we know that death is the final...
observable outcome of aging, and most biologists agree that aging can be defined as increased risk of death with chronological age. So I decided to focus on life span and mortality rate.

The final (and perhaps most important) thing that led to success was my decision to focus on life extension. Many different variants and many different mutations can lead to shortening of life span; this is clear in humans and lots of other animals. In contrast, life extension is more likely to be real in the sense that we detect a true anti-aging function or have identified a true aging gene. In the first paper that I published on aging in 1982 with Bill Wood (2), we said that “genetic variants that lived longer than parental strains seemed more likely than shorter-lived variants to be altered in primary, rate-limiting processes that determine life span”.

This has become the paradigm now that has driven and is driving all of us doing aging research or genetics of aging research to look for longevity mutants—that is, mutants that have longer than normal life spans. We were actually amazingly successful in identifying longevity variants. Within a year or two we demonstrated that, in contrast to what the literature had suggested at that time, we could detect genetic effects on longevity and thus on aging. [Actually half a dozen papers were published in the mid 1970s saying aging is different; there are no genes that control aging, e.g. (3,4)]. One very well known scientist said, “Aging may be ineluctable” (3). (You can go home and look up that word in your dictionary.) But, it turns out that it was actually very easy to intervene and to find longevous strains and mutants. So within a year of initiating this project, we had made estimates that 20%–50% of the individual variation in life span is genetic (2). We developed recombinant inbred (RI) strains, which carried different combinations of the two parents’ genes and we obtained RI strains with mean life spans of up to 31 days, an increase of 70% over the mean parental life span of 18 days. This 70% increase of life span turned out to be observed in a number of different single-gene studies as well (5,6).

**Mutants that Extend Longevity and Slow Aging**

The next landmark was moving from the polygenic approach that I started to the single-gene approach. [It’s proven remarkably difficult to clone and identify the causal alteration in the polygenic system I started with; nevertheless, hundreds of us are still using this approach and trying to understand the basis of quantitative variation in a variety of traits, including aging (7–10)]. But single genes are really the way to go, and there is a long history of mutations in single genes being used to study various aspects of biology. This had not previously been applied to aging, so a few of us decided to start looking for critical genes by identifying long-lived mutants. But there was a huge theoretical problem with looking for single genes that increase longevity. Evolutionary biologists had stated that evolutionary theory precluded the existence of a single gene with a large effect on aging. The theory says that such genes can’t possibly exist because aging is caused by the combined effect of hundreds, if not thousands, of different genes. So, I wasn’t very enthusiastic about doing this and about the probability of success. Fortunately a friend of mine, Michael Klass, ignored these evolutionary-biological warnings and started to search for such mutants. He was very successful!

Mike Klass was a student of Joan Smith-Sonneborn. He came from the right background and had an understanding of aging. Mike and I were actually postdocs together in different labs across the hall from each other at the University of Colorado at Boulder. In 1983, he published a paper in Mechanisms of Ageing and Development that showed that he could identify longevity genes. Now, Michael had submitted that paper to *Nature* (and I had helped him rewrite that paper several times). *Nature* wouldn’t even consider reviewing it; they were so convinced that there must be some flaw in the research that it wasn’t even sent out for review. Not only that, but Michael was not tenured as a result of, primarily I think, his working in the aging field. He left to work at Abbott Labs, leaving aging research never to publish again in the field. So, things have really changed in the 20 years since this paper was published. Certainly this would not happen today; there are a few labs on the coasts that can call up *Science* or *Nature* and tell them about their results, and these journals would review the papers immediately. Aging is very hot science. When Michael left academia, he sent me his strains to work on. At that time I still thought Mike had been wrong. I thought that his mutant strains were probably polygenic; i.e., they had mutations in several genes. I started working with his mutant strains to convince myself that there really were several mutations and not just a single mutated gene. I had an undergraduate student, David Friedman, who worked on this with me for 5 years, and he finally convinced me that there was just a single mutant. We showed that when you crossed a long-lived “mutant” and a strain with normal life span that the strains you got out were either completely long-lived or completely short-lived. (This “all or nothing” conservation of the phenotype rather than getting intermediates was an observation that Mendel used as well to show that there was a single gene for a phenotype.) We concluded that there was only one mutation in the long-lived strains and we called that mutation *age-1*, the first aging mutant (11). The third paper that I published on *age-1* did make the big time, I guess, in the sense that it was published in *Science* in 1990 (12). Here we showed that a single mutation in *age-1* could result in a dramatic extension of life expectancy (almost 60%) (Figure 2) and a maximum life-span increase of about 95%, and (most importantly) that the rate of increase in mortality (which some have called the rate of aging) was decreased more than twofold.

Let’s look in more detail at what this single experiment tells us. The fact that these mutants in *age-1* live longer than the wild type means that the normal *age-1* gene confers a short life span. This is what, I think, made the observation so difficult for people to accept and why journals like *Nature*, at that time, assumed that it couldn’t possibly be true. Our intuition tells us that mutations negatively affect an individual. They couldn’t possibly prolong life, which is perceived to be a great benefit to the organism and to the species. We now know that this life prolongation effect that is associated with *age-1* is a detriment to the species and that individuals carrying this *age-1* mutation are not as evolutionarily fit as the wild-type, short-lived individual (13). So, it’s consistent with evolutionary theory, but at that time it...
seemed very controversial. One of the reviewers actually contacted me personally and replicated some of this research before the paper was published. This was 8 years after Michael Klass’ work, and it was still pretty controversial. We’ll come back to these aspects when we start talking about drugs and interventions into the aging process through pharmacological approaches.

**DOES age-1 AFFECT AGING?**

So, I set off to try to demonstrate that this gene actually affected the aging process. Anyone who has ever been in a discussion with more than one biogerontologist about what aging is knows that no two agree on the definition of aging. Indeed, at least one eminent biologist seems to so define aging that it becomes a law of the physical universe, impossible to modulate without changing the Laws of Physics (14). It was not easy to come up with some ways to demonstrate that these genes actually affected fundamental aging processes. I settled on one aspect of aging: mortality rates and the increased risk of death as organisms age. We showed that aging decreased the mortality of *C. elegans* (Figure 3). This is really what evolutionary biologists and demographers define as aging, an increase with chronological age in the probability of death. In the human population, by the time an individual is 85 years old their annual mortality is in excess of 10%. And it’s not just overall probability of death, it’s probability of death from any one of many, many different causes. All of these, or many of them at least, go up exponentially. On a semi-log plot, this is a straight line, meaning an exponential rate of increase. As Finch and colleagues (15) showed about 10 years ago, every 8 years our risk of death from aging or from many other diseases doubles. Well, the same thing is true in nematodes (Figure 3). Now we don’t know what kills nematodes, and a number of us are still trying to understand really what nematode pathology is all about. But if you compare the rate of increase in mortality as a function of age in the normal, wild-type nematodes and in the age-1 mutants you can see that there’s about a 2.7-fold change in the slope of this line (12). The slope of this line has been referred to by some as the rate of aging. But in any case, the rate of increase of age-specific mortality was altered by the age-1 mutation. Now let’s look in larger populations (this is work that I’ve done in collaboration with Jim Vaupel). In the previous slide we looked at 200 nematodes in each population, while in this particular slide (Figure 4), we’re looking at 1.2 million nematodes and we’re looking at age-1, wild-type, and a couple of other longevity mutants. But let’s just look at
age-1 and wild type here. Now these are no longer plotted on an exponential scale, but in an arithmetic scale to emphasize the huge difference in mortality between an age-1 population and a wild-type population. So that, for instance, at the time the wild-type population expires, the age-1 mutant has an aging rate one tenth the rate of the normal. The wild type has a force of mortality of about 80%; age-1 has a force of mortality of about 4%. So! Huge differences!

**MOLECULAR ANALYSIS OF age-1**

I think the next landmark was the cloning of the first gerontogene. In genetics, the absolutely essential thing to do after finding a mutant is to clone it. That means to identify the gene and purify huge amounts of the particular gene that was mutated so that we can sequence it to understand what that gene is and the function that gene plays in the overall biology of the species. So, I actually began the cloning process using genetic mapping in 1986 and proceeded extremely slowly because we were doing all of our mapping based on the only phenotype we had, longevity. So, this moved very, very slowly even with an animal that had a life span of 1 month or 2.5 months, depending on whether you were looking at the normal or mutant version. The real breakthrough came when two things happened. First, Cynthia Kenyon discovered that a much better studied mutant, daf-2, which I’ll tell you a little bit about, also is an Age mutant; it has extended life span. She published that research in *Nature* in 1993 (6). Her paper was immediately accepted and has been cited much more than the age-1 paper; it got people much more interested in the process now that we knew there were other genes and that age-1 was not a complete oddity. About 3 years later, Jim Thomas, another nematode geneticist at the University of Washington in the Genetics Department, and Gary Ruvkun at Mass General showed that the mutant that I’d been working on for years, age-1, encoded a phosphatidyl-inositol 3 kinase (17,18). So, this resulted in my being completely scooped in identifying the gene that I’d now spent 11 years working on. Gary has been responsible, actually, for cloning almost every single longevity gene that’s been cloned, and Cynthia has gone on to address some very intriguing questions about the “genetic” program and to popularize these studies, to bring the work into a biotechnology company called Elixir Pharmaceuticals, and to keep the nematode aging story in public view. About 6 weeks ago, she claimed a new record for life extension, more than six-fold life extension, published in a Brevia in *Science* (19). However, she didn’t set the record; about 3 months earlier, a publication (20,21) in another journal, *Experimental Gerontology*, by Jacques Vanfleteren and colleagues in Belgium, showed more than a sevenfold increase in longevity. Let’s talk a little bit more now about the function of these gerontogenes (that is, a gene that specifies aging; I use a working definition: “a gene is a gerontogene if modifying its action can result in life extension). Here we have a cartoon that we made in which each of these boxes is meant to illustrate the protein that is synthesized by one of the genes. The DAF-2 protein (encoded by the daf-2 gene) is homologous to an insulin receptor (22). The word “homologous” in genetics has a formal meaning: “derived from the same ancestral gene.” In practice, we think of “homologues” as having similar, even identical, molecular functions; this is a powerful result, one of the most important inferences in genetics. Once you show homology, you get a big clue to the function of the newly identified gene. The DAF-2 protein is encoded by *age-1* and a PI3K that complexes with this “insulin/IGF-1 receptor” or another substrate of that receptor, carrying the signal from the external environment to the interior of the cell through several additional proteins. A gene encoding each of these proteins has been identified in
the nematode (23). The final target of this “signal transduction pathway” is the transcription factor called DAF-16, where the protein just above it is a kinase whose role is to add a phosphate to the protein. Thus, when the insulin receptor is full, DAF-16 gets phosphorylated and stays out there in the cytoplasm (Figure 5A); it doesn’t go into the nucleus (24). However, in the absence of insulin, it carries no phosphates and goes into the nucleus, where it catalyzes transcription of many genes. This is the way around the evolutionary argument that one would have to intervene in hundreds of different genes simultaneously because that’s exactly what the regulatory genes in this pathway do. By impinging upon the DAF-16 transcription factor and regulating its location as being extranuclear or intranuclear, these genes can modulate (both up and down) as many as 700 different genes that are targets of that transcription factor (25,26). When you mutate any of these “upstream” genes, you block the flow of cellular information which phosphorylates DAF-16, so the non-phosphorylated DAF-16 moves to the nucleus. This nuclear localization is in response to the absence of this insulin signaling. The absence of that signaling is a result of hard-times conditions in the wild, when the nematode is starved there is no insulin present; that triggers nuclear localization. In the Age mutants, this same nuclear localization of DAF-16, due to the lack of some component of the signaling process, causes increased longevity (24).

**HUMAN HOMOLOG OF age-1**

So, do those of us in this room have this gene? Does it function in the same way? In other words, is the function of this gene evolutionarily conserved? Here is where there is much disagreement among those of us in the field.

The evolutionary literature in aging (e.g., 27) suggests that aging in each species should be unique; aging should be something that happens differently among different species. There is no question that this age-1 pathway has been evolutionarily conserved between nematodes and humans. Indeed, for these genes that I’ve just been talking about, we know that there are homologs in humans of every one of these genes and I’ve referred to them; for instance, the homolog of daf-2 is a gene encoding an insulin or an IGF-1.
receptor. However, the question is whether the human pathway functions in the same way as it does in worms.

Nematodes and humans, believe it or not, are very distant cousins. About 600 million years ago, they shared a common ancestor, and that common ancestor had ancestral versions of these genes. For example, we’ve studied the sequence of the three human proteins that are similar to DAF-16; they’re actually called FOXO 1, 3, and 4. The nematode daf-16 transcription factor has very similar amino acids throughout, and in some regions more than 50% of the amino acids are perfectly conserved. The only way that this can happen is for both nematodes and humans to have inherited this gene from their distant ancestor. This gene must have been so important in promoting survival of that distant ancestor, and of all of its offspring, that the gene could not be lost in evolution because losing it was a lethal event. That gene duplicated itself twice in humans. (Those of us in nematode biology propose humorously that humans are really just a tetraploid worm, because there are a number of nematode genes that are found in three or four copies in humans; this is one example.) So there are three copies in humans of this transcription factor.

**Drugs for Life Extension**

Finally, let’s talk about whether or not we can perform this kind of dramatic life extension in humans. First, let’s be clear; we would not alter the human genes directly. Not only does that seem ethically untenable and foolish, it is technically impossible. However, we could potentially mimic the effects of mutations with drugs that would inhibit the function of the protein by complexing with it, just like a mutation inhibits the function of the protein by changing amino acid sequence. Does this pathway have efficacy as a drug target? Now we know that the insulin-signaling pathway in humans is an exquisitely good target for drugs. There are all sorts of drug interventions into this pathway. As a matter of fact, the main thing you have to worry about is cross-reactions and side effects of drug intervention. So there’s no question that we could intervene pharmaceutically in this pathway, if there is good reason to. Now I’ve constructed a slide showing companies currently working in the area (Table 1). I’ve taken the liberty of listing GenoPlex first. GenoPlex was my company, and it went defunct about 4 years ago, so don’t tell the CEO that I’m showing you our business plan, but this was something we floated to the investment community, starting in 1995. Unfortunately, other than from our local investors, we never got a bite. We tried for about 3 years to convince the investment and pharma companies about the value of this investment. We talked to 17 of the top 20 pharma companies around the world. It was great for my frequent flyer miles, but it was devastating in terms of the amount of dollars we got; we got no investors. So we closed the doors of GenoPlex in about 1999. One of the strategies that we had was to intervene using the gerontogene pathways that I just showed you, as an interventional strategy. You now know we had lots of reasons for doing this; we knew that defects in a lot of these proteins play a role in diabetes, cancer, and many other age-related diseases.

Even more importantly, and this is what I’ve been very interested in in my academic research, we knew that these gerontogenes increased the ability of the nematode and animal or mouse models to withstand environmental stress. George Martin, Steve Austad, and myself have a review that we wrote in 1996 in *Nature Genetics* (28), in which we proposed that one of the conserved functions of these pathways is to increase or modulate the ability of the individual organism to resist environmental stressors and endogenous stressors. That’s since been proven to be emphatically true, and there are probably 20 papers in the last year validating that hypothesis. We suggested that these nematode genes are master regulators of multiple stress responses. So human gerontogenes may well be master regulators; indeed, this has proven to be true as well. Stress proteins are widely becoming recognized as good pharmacological targets.

Here’s a partial list of companies that are currently involved or have been involved in the last 5 years that I know of. I found many of these in a *New York Times* article (September 21, 2003), and many of these are companies that I and several others of you in this room have been involved with from time to time over the years (29). All of these are (or were) valid anti-aging companies that try to make drugs to intervene in one, or usually many, aspects of aging. Now I’m not convinced that any of these companies actually have the key, but the combined force of these companies and other ones that we don’t even know about, I think, is going to prove successful, eventually. If proven successful soon enough, I expect my kids to actually start taking these drugs. I’m not sure that I will, well I’m sure I will, but I’m not sure it will be soon enough to really slow down my aging rapidly enough. I would love to be playing tennis 100 years from now, but I don’t think I’m going to make it; hopefully, my kids will.

With that, I will leave you. We’ll continue discussions of whether or not these anti-aging interventions might be real
and, more importantly, what will society when they do show themselves to be real. Finally, I want to thank the people who actually do the work; I just go give talks.

ACKNOWLEDGMENTS

My work has been supported over the years by grants from the National Science Foundation, the National Institutes of Health (RO1 AG01234, RO1 AG05720, RO1 AG08332, PO1 AG08761, RO1 AG10248, RO1 AG12423, RO1 AG12021, KO1 AG09409, and KO2 AA00195), The American Federation for Aging Research, and by gifts from the Glenn Foundation for Medical Research, the Ellison Medical Foundation, and the Jared Polis Foundation, as well as by personal contributions from myself and friends. I want to also acknowledge the support of a few dozen hard-working postdocs, graduate students, and technicians who have been in my lab over the years and who actually did most of the work I have talked about above, especially Pat Tedesco, who has kept the lab running in good times and bad. David Friedman who believed early on, and Gordon Lithgow and Simon Melov who have developed their research careers around the concepts we talked about a decade and a half ago.

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Received October 27, 2004
Accepted December 7, 2004
Decision Editor: James R. Smith, PhD