Genes, Aging, and the Future of Longevity

by Colin Rundel

We present another undergraduate paper that caught our attention from among the many excellent papers to come out of this year's Core 1 Science Writing course.

The Sphinx's riddle, "What is it that has four feet in the morning, but two at midday, and three, when the evening comes, and has only one voice; when it has most feet, it is the weakest?," to which Oedipus answered, "Man," illustrates the natural progression of life from the development of childhood, to the strength of adulthood, to the final decline of old age. This progression is not at all unique to humans. All life undergoes some form of aging, slowly deteriorating over time until finally succumbing to the inevitability of death. The common signs that most people recognize as aging, such as the appearance of a wrinkle or a slight creakiness when getting out of bed, are larger manifestations of the aging process as it occurs in every cell of the body. In the scientific world the aging process is defined as the general deterioration over time of the cells, tissues, and organs of an organism, ultimately reducing normal function and increasing the probability of death. Aging is a complex process that involves a multitude of both environmental and genetic factors that result in the determination of an organism's life span. But why does aging happen? All life is the product of evolution and, as such, all natural processes in one way or another have developed as a result of natural selection. This implies that, as deleterious as it might seem, aging serves some beneficial function.

Aging, Evolutionary Biology, and You

While the arguments about the evolutionary sources of aging are far ranging and highly speculative, there are two predominant theories. The first suggests that aging evolved as a process of planned obsolescence. Much like a car or a flashlight, organisms are thought to have been designed to wear out over time and eventually need replacing. While such an argument seems counterintuitive at first, it does have some grounding in evolutionary biology. Within a population, it is important that there be at least a small amount of turnover, with older members of the group dying and being replaced by newborns. While this borders on a group selectionist argument, it still has important implications. Firstly, it is important that new individuals are born into the population so that natural selection has something on which to operate. Without the mixing of genetic material that occurs with sexual reproduction, the gene pool stagnates and the population's ability to adapt to new conditions is diminished. Secondly, turnover allows the population to maintain a more stable growth rate and a more evenly distributed demographic. For example, a population in which there is no loss of individuals due to old age increases the stress on the population, as the ever-increasing population must waste limited resources on post-reproductive individuals who are not contributing in any significant way to their offspring's reproductive success. This implies that aging plays a role in creating turnover within a population in order to promote genetic diversity and limit the rate of population growth.

A second line of reasoning for the evolutionary basis of aging is that the forces of natural selection are weaker for organisms that have reached a post-reproductive stage of life. The simple idea behind this is that natural selection is mostly focused on producing an organism that can successfully produce as many offspring as possible, thereby spreading its genes as widely as possible. So once an organism has passed the reproductive age, any mutations that negatively affect survival are not subjected to the same rigorous selective pressures as early-acting deleterious mutations. Therefore, over the billions of years of the evolution of life there has been a tendency for the accumulation of late-acting mutations that negatively affect survival. Given enough of these mutations, an organism will exhibit rapid deterioration after the reproductive stage of life; that is, it will age. While these two theories are presented separately, they are not

The woodcut *Oedypus Sphynge*, above, is from the 1687 edition of *Atalanta Fugiens*, a book of alchemical emblems by Michael Maier first published in Frankfurt in 1617. Each emblem is accompanied by a piece of music, making this book one of the first attempts at a multimedia production. By permission of the Huntington Library, San Marino, California.

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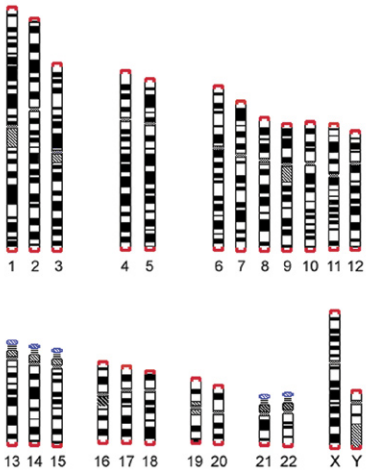
mutually exclusive and have probably both played a role in the evolution of the aging process.

Aging in a Nutshell

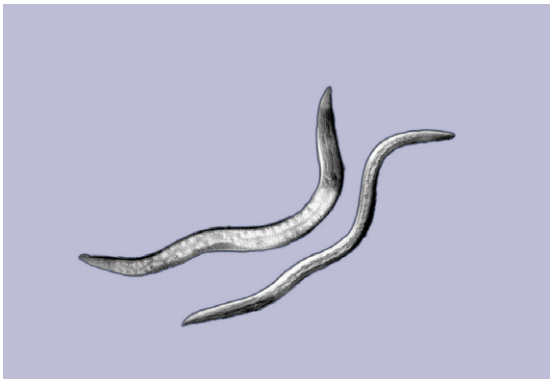
Speculation about the evolutionary reasoning behind aging does little to address the proximate causes of aging. Aging is an amazingly complex process in which a multitude of different factors act over time to slowly reduce viability. All over the world, laboratories of renowned scientists are studying the processes and diseases associated with aging, and still no clear picture has emerged of any single "aging pathway." Much noise has been made in recent years by the popular press about the role of telomeres in aging. Each time a cell divides it is necessary that it make copies of its chromosomes, but every time this duplication occurs, a small portion of DNA at the end of the chromosome is not copied, due to the nature of the replication mechanism. To combat this, organisms have evolved telomeres, stretches of junk DNA at the tips of their chromosomes, portions of which can be lost without any ill effects. The problem is that the telomere is only so long, and after a certain number of cellular divisions the chromosome will have shortened to the point where pieces of critical genes will be lost. At this point most cells will simply commit suicide or be destroyed by the immune system. This fits nicely with one of the theories of aging, since the older we become the greater the number of divisions our cells have undergone. Therefore, as we reach old age, more and more of our cells die because of telomere shortening. Yet this programmed cell obsolescence is not enough to explain the majority of degeneration associated with aging. And the argument implies that as the shortening occurs, it should remove pieces of the same genes on the end of each chromosome in everyone. Such a process cannot possibly explain the seemingly random way in which aging affects each individual in a unique way.

So what might be a more fundamental and inclusive theory of aging? The surprising answer seems to lie in free radicals, small reactive molecules that surround us every day of our lives. They are in our food, they are in the air we breathe, and even our bodies produce large quantities of them. This theory was originally proposed by Denham Harman in the 1950s as the "free-radical theory of aging" and has subsequently gathered support from numerous sources throughout the scientific community. The basic principle of this view of aging is that throughout our lives, our cells are bombarded by various free radicals, which bind to and damage a whole host of critical cellular factors like DNA and proteins. Over time this damage accumulates and reduces the functioning of the cell, which will subsequently reduce the organism's ability to function as more and more cells are damaged. Therefore, what we recognize as the aging process is the gradual accumulation of this cellular damage.

Although generated in a variety of ways, the majority of free radicals have an unexpected source—the cells' mitochondria, which release reactive species of oxygen. So the production of these radicals occurs as an accidental by-product of normal respiration. During the final stages of respiration the chemical energy derived from the breakdown of sugars is used to synthesize ATP, the basic unit of energy used in the cell. However, during this process electrons can be inappropriately transferred to molecular oxygen, thereby generating superoxide, a highly reactive species of oxygen. Whether this occurs due to an inherent flaw, or as part of some greater evolutionary design is unclear, yet it seems to fit within the context of



Map of the 23 human chromosomes, with the telomere caps shown in red or blue. Courtesy of the Human Telomere Mapping and Sequencing Project.



The well-fed roundworm on the left will age faster than the skinny dauer larva on the right.

the evolutionary necessity of aging. The implication is that through the process of living we are slowly poisoning ourselves with each breath we take. While this paints a somewhat bleak picture of existence, it at least identifies who the enemy is and offers some hope that in the future something could be done to postpone the inevitable without reducing the quality of life.

The connection between aging and oxidative stress due to superoxide agrees nicely with previous connections drawn between aging and metabolism. It has long been known that life expectancy and metabolic rate are inversely related, as animals with high metabolic rates tend to have very short life spans, whereas larger animals with lower metabolic rates can expect to live far longer. For example, the average mouse kept in captivity can expect to live about two years, while a captive elephant in a zoo has an expected life span of upward of 70 years. Regardless of the medical attention administered, or how carefully the mouse is treated, at present there is no medical treatment available that can extend its life span much beyond those two years. In light of the free-radical theory of aging this makes sense. The mouse simply has a far higher metabolic rate than the elephant and therefore the rate of superoxide production is increased. The net effect of this is that the mouse's tissues will accumulate damage at a far greater rate than those of the elephant and as such will display a more rapid onset of aging. Yet, like most models, there are also certain marked exceptions to the rule that complicate the explanation. In particular, some birds and bats exhibit life spans that far exceed what would be expected for their metabolic rates. This would imply that the basic tenets of the free-radical theory are correct, but that the effects of the stress are modulated by certain genes that can increase the life spans of some organisms. Once again, evolution has taken a very heavy hand in determining how and when the aging process occurs.

Genetics of Aging: The Beginning

It is evident that within different organisms the levels of free radicals produced must be regulated in some manner. Therefore, if there are factors that directly affect the amount of reactive oxygen species present in the body, they should have an indirect effect on life span. The implication is that there are specific genetic factors within an organism that have been programmed by evolution to set a specific life expectancy, one that is most beneficial for reproductive success. Experimental confirmation of this has come out of Michael Rose's laboratory at UC Irvine, where researchers were able to selectively breed fruit flies (*Drosophila melanogaster*) for extended life span. Just like eye color and height, aging must therefore be at least partially under the control of certain genes, which can be selected for, just like an animal breeder can select for increased size or a gentler disposition. This opens up a whole new world of possibilities for studying the aging process. Through genetic dissection, it becomes possible to identify genes that affect an individual's life span and to fit them into an overall picture of the aging process.

Suddenly, the study of aging is switched from a top-down view of the effects of aging to a bottom-up approach, where causes can be determined. Now individual genes can be identified as contributors to the aging process and their products characterized, opening the door for the creation of drugs that can slow aging. However, there is one problem with Rose's work. By selectively breeding his flies, Rose has significantly altered the genetic diversity of the new long-lived population. It is therefore impossible to differentiate between the genes that extend life span and the genes that were selected for, due to the population of flies he bred for the experiment. This then leads us to the current state of genetic aging research, the analysis of single-gene mutations that have a significant impact on an organism's life span. The benefits of such an approach should be immediately apparent, as the alteration of the properties of a single gene should give a definitive answer as to its effects on aging.

Enter the Worm

Single-gene aging research is still a relatively new field and, at the present time, only a handful of genes that affect aging have been identified in a variety of model organisms. It was not until 1988 that the first aging gene was identified, *age-1.* This is a gene in the nematode (roundworm) *Caenorhabditis elegans* or *C. elegans*, one of the model organisms commonly used in genetic research. The effect of reducing the level of *age-1* expression in a nematode was a 110 percent extension of maximum life span at 25°C, while also reducing reproductive output.

This discovery was followed by the identification of a variety of other nematode genes that made a direct connection between stress response, mating, and aging. One of the surprising results to come out of this work was the discovery of the connection between aging and a molecular pathway known as the insulin signaling pathway. Everyone has heard of insulin, the protein that regulates the level of blood sugar and plays a central role in diabetes. But how does it connect to aging? The basic role of insulin within a cell is to measure the amount of sugar that is present in the blood at any given time, and then to instruct the body how to respond appropriately. This has one useful feature that has been latched onto by aging. Since this molecule is constantly measuring the amount of sugar present, it could also be used to measure the amount of food being consumed by the organism at any given time. So it is believed that a pathway has evolved that is capable of reporting on the level of food availability in the environment. In combination with other signals, it allows the organism to monitor biochemically if it is undergoing food stress.

Transgenic fruit flies bred by Colin Rundel to investigate the genetics of aging.

This connects to aging and reproduction in a somewhat subtle way. By constantly monitoring whether food supplies are plentiful, an organism can decide if it is a good time to mate or not. The general strategy is that during times when food is plentiful an organism should make the energetic investment in reproduction and produce as many offspring as possible, but when times are not so good, it should batten down, save most of its energy, and hold out until a better opportunity presents itself. This type of strategy is beautifully demonstrated by *C. elegans* in its ability to form dauer larvae. For those not familiar with the development process of *C. elegans*, the worm goes through several larval stages, much like a butterfly (without the cocoon). During one of these early stages there is the potential for the worm to adopt a dauer form, which is an alternative type of larva whose formation occurs under stressful conditions, most notably when food is scarce. These dauers have several amazing properties, the first of which is that, as soon as food is available, the worm is able to continue development and become a fully functional adult. What is more interesting to the study of aging and free-radical stress is that during the dauer period, the worm exhibits a dramatically slower aging process and increased resistance to stress. It is because of this connection to dauer formation that several of the most notable aging genes, *daf-2* and *daf-16* for example, were found in worms. As an interesting side note, it has also been shown that the genes linked to dauer formation can be altered in such a way that they no longer cause dauer formation but still impart the benefits of stress resistance and longevity.

What Good is a Fly?

It is now time to return to the organism that is at the center of so many other genetic stories, the fruit fly, *Drosophila melanogaster*. Research into aging in flies got off to a far slower start than one would expect. The first single-gene mutant that extended life span in the fly was not identified until 10 years after the discovery of *age-1*. This mutant came to be known, appropriately, as *methuselah (mth)*, and while the extension in life span was less than that seen in *age-1*, only a 35 percent increase, it was still significant because the mutation exists in an organism another step closer to humans. There has been only one problem with *mth*: it shows no significant similarities to any other known gene. After four years, its cellular function has yet to be characterized. It is known that the gene negatively influences longevity and stress resistance but is critical for the survival of the fly.

In the last few years, the rate of discovery of new aging genes has sped up considerably, but the number is still hovering around a half-dozen or so published mutants. While this is certainly progress, the numbers available are simply too small to construct any type of detailed biochemical pathway. One interesting finding, however, has been the identification of a gene involved in insulin signaling that also affects aging, implying that the system originally found in worms is conserved, and that related genes may prove to extend life span in higher organisms.

Aging and Mammals

So, you are probably asking yourself, what is the point of all this? While to a geneticist it is wonderful that the life of a worm or a fly can be extended, I am certainly not either of those, so how is this going to affect *my* life? The truth of the matter is

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that at the moment it is not going to in any significant manner. Progress is being made on a variety of fronts, but for now the field is too new, and there is much more that remains to be discovered about the genetics and biochemistry of the aging process. Two recent discoveries do show a great deal of promise for the future of a direct application of aging research to the human population. The first of these is the identification of the first known single-gene aging mutant in a mammal, $p66^{shc}$, found in mice in 1999, only six years after the journal *Nature* published an April Fools' Day article on just such a long-lived mouse. The second encouraging finding is the recognition of a link between a group of French centenarians and a region on the fourth human chromosome, implying that there may be a gene in that area responsible for longevity.

While there is little hope of any kind of genetic treatment in the near future, there may be some opportunities for a more pharmaceutical approach. Before discussing the chemical compounds that seem to affect aging, it is important to mention one surefire way to extend your life span without having to do anything at all to your genes, or having to take any drugs. The process is known as caloric restriction, and all that is involved is limiting the amount of caloric intake to approximately 60 percent of the normal amount. The amazing part of this process is that it seems to exist universally in all organisms, from mice to worms, and everything in between. The conclusion that can be drawn from this takes us back to what we first saw with the worms: our bodies can pick up on when food is in short supply and respond by increasing all of our molecular defenses to better survive until a period of greater abundance.

For those individuals who do not find this prospect appealing, there appear to be other options, but it will be a long time before they become available as antiaging therapies. For example, a drug known as PBA (4-phenylbutyrate) has been shown to extend the life span of *Drosophila* flies when mixed into their food. It is thought to function by inhibiting enzymes responsible for winding up DNA and packing it together into chromosomes. This alters gene-expression levels because regions of the DNA that would typically have been inaccessible become available to express their genes. How this causes an increase in life span is very unclear. However, the drug seems to achieve a goal similar to that of a protein known as sir2, which has also been shown to increase life span in worms and yeast. But we are once again confounded by our lack of understanding of how the aging process works, and any possible use of PBA is thus limited. It is hard to appreciate how complex biological systems are, and even the slightest alteration can cause far-reaching effects that are amazingly difficult to predict.

Where This Leaves Us

At the present time, doing research into aging is like trying to do a jigsaw puzzle with half the pieces and the box missing. You do not know what it is supposed to look like or where everything is supposed to go, but connections are being made and new pieces are being found all the time. Aging is a complex and intricate process that is entangled in a multiplicity of other systems in unexpected ways. The genetic approach to aging holds great promise, but it needs to shift from the question of what will extend life to the more important question of how life is extended. Society also has to investigate how slowing the aging process may influence our demographic, economic, and environmental conditions before any therapy is made available for human use. With an ever-expanding world population bringing us ever closer to the earth's carrying capacity, extending life span may have disastrous consequences for humanity and the planet as a whole. □

Colin Rundel is a senior majoring in biology. He has spent the last two years attempting, with some success, to extend the lifespan of fruit flies in the laboratory of Seymour Benzer, Boswell Professor of Neuroscience, Emeritus. His science writing mentor was Paul Sternberg, professor of biology and Howard Hughes Medical Institute Investigator. Colin is planning to go to graduate school and continue "forcing helpless insects to do his bidding."