The Aging Enigma

Scientists probe the genetic basis of longevity.

Is aging necessary? Are the wrinkles and gray hair, weakening muscles, neurodegeneration, reduced cardiovascular function, and increased risk of cancer that afflict organisms toward the end of their lives inevitable? Or are these age-related changes part of a genetic program that can be altered?

Molecular biologists experimenting with organisms such as yeast, roundworms, fruit flies, and mice have found that they can dramatically extend life span by tweaking single genes. The altered organisms don't just live longer, they age more slowly, in many cases retaining youthful characteristics even after normal individuals have died. More remarkable, the genetic manipulations that cause these changes seem to work through a common pathway across all species. This suggests that if there is a program that controls aging, it must be ancient indeed: in evolutionary terms, yeast and mammals diverged about a billion years ago.

Separately, geneticists studying long-lived people appear to be narrowing in on a gene common to centenarians that promotes longevity. Given these advances, the possibility that the human life span could be extended seems tantalizingly close. But some scientists caution that for all the genetic similarities between model organisms and humans, the differences may be greater than we imagine. Researchers still don't know what causes aging in any animal. Evolutionary biologists, who theorize about why some organisms naturally live longer than others, ask if there is any reason to believe that maximum human life span, already at the upper end of longevity among mammals, could be increased at all—even as researchers on aging, spurred by new experimental breakthroughs, increasingly ask, Why not?

The Elegans Solution

The experimental evidence that suggests aging is under genetic control, rather than a consequence of normal wear and tear, *is* compelling. So much so that when Cynthia Kenyon, a professor at the University of California, San Francisco, gave a lecture at the Radcliffe Institute last year describing her research on roundworms, she began her slide presentation by projecting an image of *C. elegans* on the screen and asking provocatively, "Could this little animal eventually lead us to the fountain of youth?"

During development, roundworms exposed to environmental stress "stop the clock" by becoming dauers, the term for a spore-like state akin to hibernation. They can remain in this suspended condition for long periods, until their surroundings again become hospitable to growth and they can become normal adults. Dauers don't eat or reproduce but, Kenyon discovered, they are extremely long-lived. When she announced this finding in 1993, says Harvard Medical School (HMS) professor of genetics Gary Ruvkun, it seemed at first a restatement of the obvious. But Kenyon's larger point, he now says, was not just that dauers are long-lived, but that perhaps animals, as part of normal physiology, can regulate their own life span.

That meant that genes controlled longevity. Sure enough, in 1996 Kenyon demonstrated that roundworms missing one copy of a gene called *daf*-2 during development will enter the dauer state regardless of environmental conditions. But what would happen if she knocked the gene out of adult roundworms that had already passed the developmental stage during which they might have become dauers? Would they revert to the dauer state? They did not—but they did live about 50 percent longer than normal, and suffered none of the physiological tradeoffs seen in dauers, such as infertility and cessation of eating. What this meant, she realized, was that the program that controls longevity can be uncoupled from other physiological processes.

Ruvkun, meanwhile, published a paper in 1996 showing that *daf*-2 and a gene called *age*-1, discovered years earlier in a long-lived roundworm mutant, were part of the same molecular pathway. "They code for proteins that send signals down the same transmission line," Ruvkun says. "That said that even though we had just dipped our toe in the water about aging—we had just started to study it in any systematic way—there were not going to be a million genes in the same pathway that regulate life span, there were going to be a few, and that it was a solvable problem."

But no one knew the genes' precise role. A year later, Ruvkun showed that *daf*-2 encodes an insulin receptor. (The hormone insulin is best known for its role in maintaining stable blood-glucose levels.) This was a galvanizing moment, because in an instant it linked aging in roundworms to the only known protocol that will extend life span in any organism: caloric restriction.

"I was shocked by all of this," admits Ruvkun, who says he had previously been "completely dismissive of aging researchers in general because I didn't think they were going about it systematically." But the data on the effects of caloric restriction are well-established, and suddenly the field became much more credible.

By Jonathan Shaw

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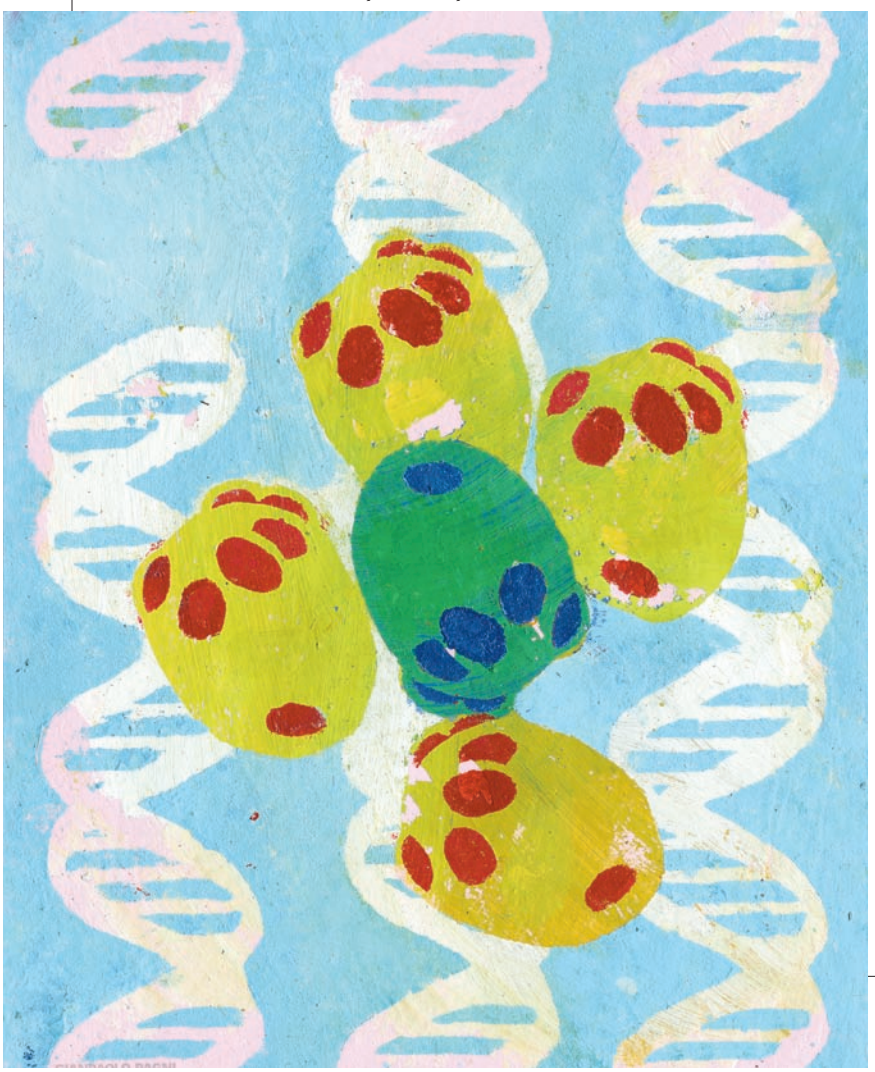
Caloric restriction (CR) was discovered 70 years ago when Clive McKay, a professor at Cornell, underfed rats in a lab. When given 40 percent fewer calories, the rats lived 30 percent longer—and they were healthier. While normal animals became scruffy and lost their hair as they aged, the food-restricted rats retained beautiful coats and didn't get common ailments such as cancer, heart disease, or diabetes. In fact, all aspects of aging in the rats were slowed down—even cataracts and gray hair.

Caloric restriction has since been found to slow aging in every organism on which it has been tested, from yeast cells to dogs. Several continuing studies using nonhuman primates, our closest relatives, have shown that the regimen protects against disease and probably slows aging as well. Not surprisingly, animals on food-restricted diets have lower levels of circulating blood glucose, insulin, and triglycerides. But they are generally infertile, and near-starvation is not a regimen that any organism would follow by choice.

Death of a Cell

While Ruvkun and Kenyon were publishing their work on roundworms, David Sinclair, then a graduate student in the laboratory of MIT professor Leonard Guarente, was pursuing parallel genetic experiments on aging in baker's yeast. When he started in 1995, he says, "It was considered preposterous by most that you could even *study* aging at the genetic level and find single genes that could extend life span." His contention that yeast cells might lead to insights into human aging was considered even more unlikely. But 10 years later, now an HMS associate professor of pathology, Sinclair has found the same genes he identified in yeast "playing important roles in biology and possibly health and aging in ourselves."



Nobody knows what causes aging in any animal, though there are many theories. The most familiar of these posits that life span is tied to metabolic *rate.* Ordinary metabolism generates free radicals—reactive oxygen species (ROS)—that can damage DNA and proteins. Animals that live fast, so the theory goes, will die young, because high metabolism produces free radicals at a high rate. According to this model, which is known as the metabolic rate/oxidative stress theory, long-lived animals should have high concentrations of antioxidant enzymes in their tissues and low concentrations of free radicals. This has not been found, however. And there are other anomalies that the theory cannot explain, such as why antioxidant supplements, which should increase life span by reducing ROS concentrations, do not work, and why mice live three years, while bats, with a similar metabolic rate, live 10 times longer. A recent, competing theory has been proposed by Lloyd Demetrius, an associate of the department of population genetics in Harvard's Museum of Comparative Zoology. Demetrius's hypothesis, which has been favorably reviewed by S. Jay Olshansky of the University of Illinois at Chicago and other leading theoreticians of senescence, argues that metabolic *stability* is a better predictor of longevity than metabolic rate. The metabolic stability hypothesis proposes that an organism's ability to *maintain stable levels* of free radicals is more important than *how fast* it produces them (see page 91). Accordingly, pharmacological agents that simply act to reduce ROS concentrations may even be harmful, because they could perturb the delicate balance necessary for normal cell function.

Theories are one thing, but Sinclair and Guarente decided to tackle experimentally the question of what causes aging by starting with a simple yeast, a single-celled fungus whose life span is defined by the number of times it can divide. They discovered that a reorganization of DNA over the course of the cell's lifetime is linked to its death. A yeast cell divides 20 times on average—40 times at most. But when the cell's DNA is stabilized (prevented from rearranging), both the average and maximum life span increase. One of the proteins that stabilizes the chromosomes of a yeast cell, encoded by a gene of the same name, is called sir2.

Sinclair and Guarente found that if they introduced one extra copy of the Sir2 gene into a yeast cell, generating about twice as much sir2 protein and stabilizing the DNA, the yeast lived about 30 percent longer. (In yeast, genes are uppercased and proteins are lowercased. In roundworms, this convention is reversed.) This suggested, like the work of Kenyon and Ruvkun in roundworms, that a small set of genes could control life span.

Sir2 is believed to be the founding member, in evolutionary terms, of a family of genes known as sirtuins that are present in "all complex life forms on the planet," says Sinclair. "We think that they evolved about a billion years ago to protect organisms during adversity, when the environment became harsh." Work done at Harvard has shown that the Sir2 gene is activated when yeast cells are stressed. "This mild stress could be too much heat or too much salt or not enough calories," Sinclair says. "In any of

these conditions, the Sir2 gene will act to stabilize the chromosome and make the cells live longer." (More recently, Sinclair's research group has identified another gene that controls Sir2, a "master regulator" called PNC1. Stress turns on the PNC1 gene, the activity of which turns on Sir2.)

Having described the genetics of this longevity pathway in yeast, Sinclair began to wonder how he could "artificially turn on that defense pathway, that survival-longevity pathway, without having to stress the cell." Might there be a drug that would turn on these genes? "We screened through Harvard's library of molecules at the ICCB [Institute of Chemical and Cell Biology]," he says, "and found a set of plant molecules that binds to the sir2 protein, tricking the cells into thinking that they are under mild stress. You get the benefits, without actually having to *be* stressed" (see "Messages from the Plant World?" page 50).

"We have fed these molecules to yeast and they live longer," Sinclair reports, speaking of these sirtuin activating compounds, or STACs. He is convinced the molecules are acting through Sir2, because when that gene is deleted, the effect vanishes. "When you feed the molecules to much more complicated organisms, like roundworms and flies," he adds, "*they* also live longer." Flies, for example, live 40 percent longer, but as with yeast, when the Sir2 gene is deleted, "the flies don't respond to our STACs anymore." Sinclair has linked all his genetic and small-molecule work to caloric restriction using the same technique of gene deletion. Yeast and flies which aren't getting enough food don't live longer if the Sir2 is missing.

The link to caloric restriction, already proven to increase longevity in many species, leads Sinclair to believe that his STACs may also be universally efficacious, even in humans, because they trigger natural defense mechanisms against environmental insults. "What we have really discovered here is that the body has its own innate defense system. It could be a new era of medicine," he says, in which harnessing these defenses "could be combined with traditional medicine."

If sirtuins are part of the same insulin-signaling pathway identified by Kenyon and Ruvkun, as the connection to CR suggests, what exactly is the relationship? Sinclair believes the sirtuins are controlled by insulin and a closely related hormone called IGF-1 (insulin-like growth factor-1), a link to the work of Kenyon and Ruvkun; a paper he published in *Science* last year showed that SIRT1 (the mammalian equivalent of Sir2 in yeast) rises when levels of these hormones fall, as they would in a calorie-restricted organism. But when sirtuins are triggered by STACs they don't cause infertility, as occurs with caloric restriction. "We thought they might cause infertility," Sinclair says, "because if we were really mimicking the pathway high up, we would have had all the effects." In the chain of responses to CR, "it looks as if we have come in at the right level with sirtuin [i.e. far enough down] so that we can get all the benefits without the tradeoffs." His worms and flies not only lived longer, they ate as much as they wanted and had no decline in reproductive capacity. "If anything," he says, "these flies were laying more eggs than usual."

Of Mice and Men

Will the interventions that work in laboratory organisms really work in higher organisms, even humans? Extending the life span of a fly or a worm or a yeast cell is exciting, but extending life span in a mammal without the use of CR would be even more so. "There is a lot of real progress," notes Iaccoca professor of medicine C. Ronald Kahn, president and director of the Joslin Diabetes Center, "and a lot of papers on *C. elegans* and *Drosophila* [fruit flies], but you are not seeing a lot of experiments on mammals, because the experiments are so much harder. What we really need to do is make the jump to higher organisms to see if the same mechanisms and pathways, or different mechanisms and pathways, affect longevity." Fruit flies and roundworms, for example, have just one type of receptor for both insulin and growth hormones. But as animals became more complex, these two pathways diverged: mammals have separate insulin and insulin-like growth-factor receptors, both descended from this common ancestral form. Although these receptors in mammals are structurally and functionally very similar, one is part of a system that regulates metabolism, while the other primarily mediates growth. In mammals, changes in either pathway can lead to long-lived mutants. Nevertheless, "There aren't too many of us looking at aging even in animals as sophisticated as mice," says Kahn, "because every experiment takes three or four years."

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Kahn himself is at the forefront of such research. Scientists at Joslin are very interested in insulin signaling for metabolic reasons and because of the connections to diabetes, which frequently leads to early cardiovascular disease, other complications, and early death. About five years ago he started breeding mice in which he had genetically knocked out insulin signaling from one tissue at a time: muscle, in the MIRKO (muscle insulin receptor knockout) mouse; fat in the FIRKO mouse; liver in the LIRKO mouse; and brain (neural tissue), in the NIRKO mouse.

"What struck us about the FIRKO mouse," Kahn says, "is that it remains lean as it ages, protected against obesity even on a high-fat or high-calorie diet." This provided an opportunity to dissociate the two things that happen in caloric restriction. For example, in CR, leanness is associated with decreased food intake. "But in the FIRKO mouse we had an animal that ate as much as a normal mouse and yet remained lean. In fact, it ate even more than normal relative to its body weight." He could then ask the question, would being lean by itself promote longevity in a mouse that was eating normally? "Sure enough," says Kahn, "the animals lived longer, by 18 to 20 percent." The reason for their longevity might be related to the leanness, but could also be related to the disruption of insulin-signaling, Kahn allows, even though, in the FIRKO mouse, insulin signaling has been disrupted in only *one* tissue of the body. To Kahn, this suggests that in mammals, the links between insulin signaling, caloric restriction, and obesity could be centered on fat tissue.

Kahn's lab has decided to ask several other questions "to try to get to the bottom of this," he say. First, what is it about fat that makes a difference? "We have separated appetite from fat mass," he notes, "but why does having more or less fat mass make you live more or less long?" One possibility is that fat either makes or accumulates something that is toxic. Fat is known to make hor-

mones called adipokines. If you are lean, and the balance of adipokines in your body is changed, might this act on other tissues to promote longevity? Alternatively, could the fat be a source of molecules involved in oxidative stress, such as free radicals? Or does leannesss protect against free radical damage? "And what about genes involved in longevity like the sirtuins?" asks Kahn. "Are they up, down, or unchanged in the FIRKO mouse?"

The latter question, at least, has been answered, because Kahn has found no change in the level of sirtuin proteins in the fat of FIRKO mice, though he notes that the protein's activity could have changed. He and his colleagues have, however, observed other cellular changes. "In these fat cells that lack insulin receptors, there are changes in some of the pathways [that result in] oxidative stress factors." (As noted above, oxidative stress is often cited as a possible cause of aging—cells burn oxygen to make energy, but in the process produce toxic free radicals.) "The reason we find this particularly interesting," explains Kahn, "is because of the links to diabetes." Earlier studies at Joslin have shown that there is decreased expression of a number of genes involved in mitochondrial oxidation (mitochondria are the energy-producing structures within a cell) in the muscle of patients with type 2 diabetes. "And a group at Yale led by [Professor Gerald] Shulman has shown that in human muscle, there is a decrease in oxidative metabolism with age as well." Within mitochondria, the ratios and levels of certain cellular metabolites such as NAD/NADH change, and thereby regulate both sirtuin proteins and the generation of free radicals. Given these emerging connections among diabetes, oxidative metabolism, and aging in muscle, and now perhaps in fat, Kahn wonders if there is a common oxidative pathway that becomes less effective with age. If so, that might explain why animals that are protected remain active over a longer period as well.

The Metabolic Conundrum

The FIRKO mouse eats a lot yet remains skinny, suggesting it has a high metabolism. How is it burning all the extra calories? "We haven't figured this out yet," admits Kahn. "The obvious answer would be that they are more active." But they aren't: "If you put them in a cage that has light beams that measure how much they move around, FIRKO mice are not more active than normal mice." Even their internal body temperatures are the same. "Obviously, they must be burning off the energy in some way," continues Kahn, "because if you take in the calories, you either have to store them, burn them, or excrete them. They are not excreting them, so we believe they are being burned up in excess energy utilization by some mechanism that does not involve being more active."

One hypothesis is that the FIRKO mice are metabolically inefficient. Kakn has observed that normal mice, like humans, vary in how much weight they gain for a given amount of food that they eat. "Some mice will literally gain 30 percent more weight on the same amount of calories than another mouse," he says. "Others are just like friends who say that they can eat anything and never gain weight" (though he notes that quantifying and correcting for varying activity levels can be difficult).

There is some evidence, albeit controversial, that suggests that calorie-restricted animals exhibit an altered metabolism. In this state, says David Sinclair, they are slightly less efficient at converting food into energy, but produce fewer free radicals and so experience less oxidative

Messages from the Plant World?

The idea that mild stress might lead to health benefits is not new. In fact, the concept has a name: *hormesis.* Plants given low doses of an herbicide, for example, can actually become stronger and grow better. Harvard Medical School associate professor David Sinclair's discovery that a family of plant molecules will increase longevity in yeast, roundworms, and flies made him wonder, "Why do molecules from plants extend life span? Are they just being nice to us, or is there some explanation?"

"When you look at these molecules, without exception they are produced by plants when they are stressed or starving," he says. "We think that the plants make these molecules to turn on their own protective sirtuin genes in order to defend themselves." Sinclair suspects that other organisms have evolved to pick up on stress signals from the plant world, using them as a chemical cue for the state of the environment. "The idea is that when our food supply is stressed out," he explains, "we turn on our own defenses against a loss of food or other potentially adverse condition.

"Pretend there is a yeast on a grape," he continues. "How would a yeast know if the water table of that grape vine is drying up? It wouldn't, unless it could pick up on the plant chemicals that the grape is producing in response." If the yeast does sense the grape's chemical messages, then it might, "two weeks in advance, start hunkering down and building its own resources to get ready for the adversity that is about to come."

Maybe that is why we love organically grown fruits, Sinclair speculates. Because they haven't been chemically protected from insects and disease, "They're stressed out, they are full of these compounds. We know they are good for our health, just based on thousands of years of evidence, but no one really knows why."

If Sinclair's theory—which he calls *xenohormesis*—is correct, it would explain why so many of our medicines come from the plant world, and why they seem to be "almost miraculous." Aspirin, for example, cures headache, dulls pain, and even prevents heart attacks. "All the molecules in the aspirin family come from plants," notes Sinclair. "Could it be that we have evolved to pick up on those?"

To test the hypothesis, one of his graduate students, Natalie Arkus, is working with professor of genetics Frederick Ausubel to compare the longevity of aphids feeding on stressed and unstressed groups of the mustard plant *Arabadopsis thaliana.* Under the mild stress of excessive light, the plants produce sirtuin-activating compounds (STACs) that turn the leaves purple; the leaves of unstressed plants remain green. "We predict that aphids feeding on the stressed plants will live longer," says Sinclair. If he's right, it would not only place humans and other species squarely in a broader ecological context, it would mean that the place to look for new medicines is in stressed plants, rather than in well-cared-for specimens.


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damage. Like a car with pollution controls, the mitochondria of a calorie-restricted animal, and perhaps of the FIRKO mouse, may produce less energy but burn fuel more cleanly.

Is accumulation of free-radical damage, then, the key regulator of life span? Studies in roundworms suggest that the ability to resist free-radical damage is just *one* of many effects that arise from genetic alterations to the insulin-signaling pathway. Says Ruvkun, "In my view, the reason that *daf*-2 is so potent is that it triggers everything that would make an animal live longer, not just part of it." Kenyon describes the cascade of changes that she sees taking place in her research as a "life-span regulatory module." While some genes downstream from *daf*-2 encode antioxidant proteins thought to protect the body against damage from free radicals, she reports that others "code for protein 'chaperones,' which help proteins fold [folding is critical to function] and take them to the garbage can when they are damaged." Some genes encode antimicrobial agents that kill bacteria and fungi, while a set of metabolic genes, when turned down, also promotes longevity.

These experimental observations can be united within a single idea: that increased ability to withstand environmental insults overall increases longevity. Combined with the observation that FIRKO mice, with their apparently *higher* metabolism, nevertheless live longer, it suggests that a reduced rate of metabolism, and hence a lower rate of free radical production, may be less important than other factors contributing to longevity—and it lends support to a prediction of Lloyd Demetrius's longevity theory that metabolic stability is more important than metabolic rate in determining life span. The metabolic stability idea, as Demetrius has argued, may provide a unified perspective for understanding why organisms with different life spans differ in their ability to withstand both internal and external stresses.

In fact, aging research has shown that long-lived animals are more resistant to pathogens and other environmental stresses. In rat studies, and even in research with monkeys, at the point when control animals are suffering from diseases of old age like cancer and heart disease, most of the food-restricted animals remain "totally normal, fit, and healthy. That is important," emphasizes Sinclair, "because we are not adding years onto an unhealthy state, we're adding *healthy* years."

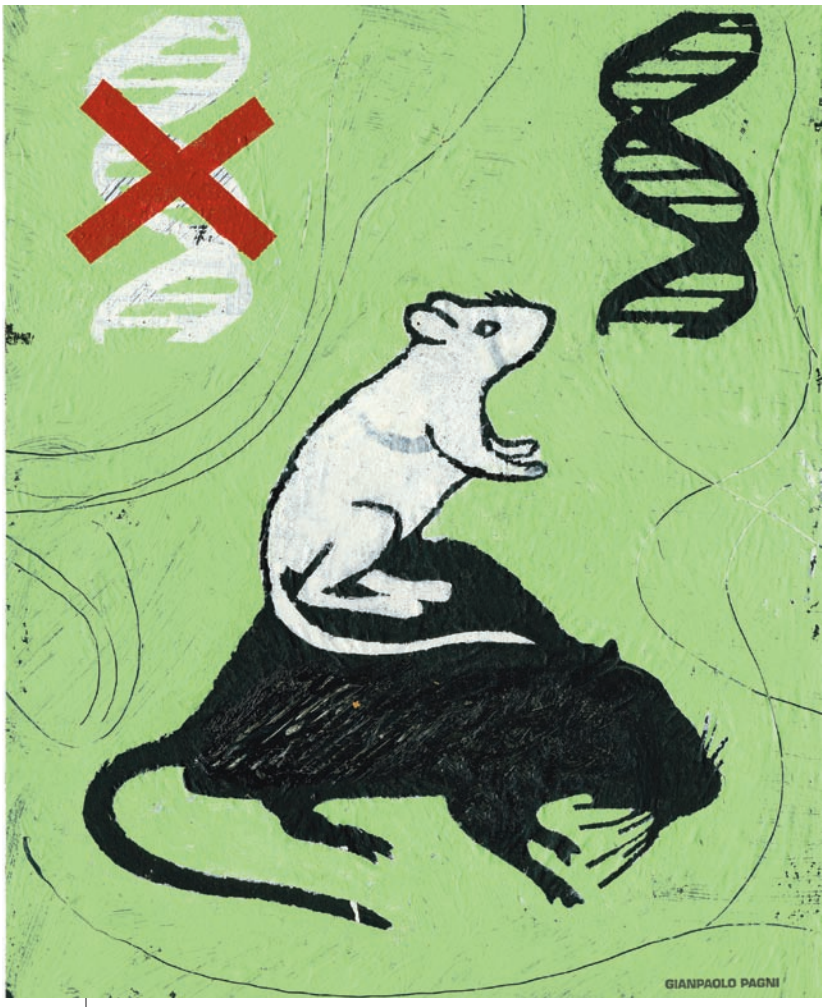
The correlation between disease-resistance and longevity has led him to test the efficacy of sirtuins against various diseases of old age. He has created a series of transgenic mice that overexpress each of the seven mammalian sirtuin genes (yeast has only five). He can turn the genes on and off in any tissue, such as the brain or the cardiovascular system. What does he expect to find?

Sinclair believes that SIRT1 will slow the progression of cancer and prevent Alzheimer's disease, as well as other neurodegenerative disorders. "When you culture cells in a dish and subject them to the toxicities of Huntington's and Alzheimer's, and you turn on SIRT1, those cells survive much better," he reports. "It looks like SIRT1 is a pro-survival protein and it looks like the brain is a very good place to start [testing its efficacy against disease.]"

Sinclair has begun feeding mice resveratrol, the best-known of his sirtuin-activating compounds derived from plants, and reports that it suppresses the growth of implanted cancer tumors. He is also feeding it to healthy mice to see whether it increases their longevity. The molecule "seems to be a very potent cancer-preventive agent," he reports, and is currently in clinical trials for colon cancer on the one hand, and, because of its antiviral properties, for oral herpes on the other. "It should also have benefits for diabetes," he says, and it has been shown to be effective in animals "against heart disease, stroke, and high cholesterol. It looks like it is going to become a super-aspirin in the future.

"Where I hope this type of research leads," he adds, "is to new medicines that people can take safely throughout their lives to *prevent* diseases, not just treat them. But it is very hard to do a preventive trial through the FDA," he notes, so such a drug is more likely to come to market as a treatment for a specific disease. "My prediction is that within five years we will see the first of these drugs used to treat severe disease, perhaps neurodegenerative disorders or problems with the optic nerve. But once they are on the market, I could imagine them being widely used against other disorders and maybe, eventually, it will be proved safe enough that people can use it on a daily basis for prevention as well."

Ruvkun, however, urges caution. The discovery of resveratrol, like the discovery of *daf*-2, was a kind of "gold strike," he says; whether it will work in mammals, either to fight disease or promote longevity, is still unknown. Demetrius concurs, noting the differences between mice and humans both in the types of cancer they develop and in the ability to resist it as they age. Mice tend to develop the sarcomas and lymphomas that, in humans, are characteristic of children (epithelial cancers predominate in older people). Furthermore, cancer incidence in mice increases exponentially with age, while in humans, such an increase doesn't begin until age 40. Beyond age 80, incidence of cancer in humans levels off with increasing age. Ruvkun says he's "not impressed by all the biotechnology companies that have assembled around [the sirtuins]. We don't know enough about [human] aging to make drugs around it." Sinclair himself acknowledges that the fortuitous discovery of STACs has allowed researchers to tweak regulators of aging without understanding the underlying causes.



Humans at One Hundred

Genetic studies of human centenarians may be the best way to understand longevity in man. HMS professor of pediatrics Louis Kunkel, who heads the genomics program at Children's Hospital, entered the field of aging research almost by accident in 1997, when he met Thomas Perls, director of the New England Centenarian Project. Perls, then at Harvard but now based at Boston University Medical Center, realized during the process of gathering information about centenarians' lifestyles and family history that a tendency toward longevity clusters in families. He asked Kunkel to try to identify genes that extend life span.

In many centenarians' families, longevity appears to be a dominant trait, says Kunkel, as multiple individuals live past 100. He is currently trying to map the gene common to a European family in which the parents lived into their mid 90s and all the children also fit the criteria: five are still in their 80s, but the others are 95 or older, even into their hundreds.

One in 10,000 people alive today will have longevity genes, says Kunkel. But they are not as rare as those numbers suggest, because the population a century ago was much smaller than today's. "What you really have to do is compare the numbers to the population totals when they were born; then it comes out to about 1 in 100 to 200." "A person born today," he says, "could have a 1 in 100 chance of having such genes. They would also have good gene variants at all the loci that would otherwise predispose you to premature death."

"We all have the same genes," says Kunkel. "We vary from each other based on our SNPs" (pronounced "snips"), or single nucleotide polymorphisms. The differences among us encoded in SNPs are statistically tiny. The average gene contains 50,000 base pairs (two nucleotides joined by hydrogen bonds across two complementary strands of DNA or RNA), and may contain as many as 100 SNPs. The vast majority of these SNPs have no impact on longevity. But a few of them might increase the likelihood of high cholesterol, cardiovascular disease, or Alzheimer's. Negative mutations can accumulate in the course of evolution, as long as they don't affect fertility or life span during an organism's reproductive years. "To have reached 100, centenarians have escaped most of those problems by definition," he says. In addition to being free of the negative genetic variations common in other human beings, Kunkel believes centenarians also have "some positive mutations that increase the possibility of longer life span."

Last year, Kunkel and his colleagues thought they had found one of these positive mutations in many of the New England centenarians they were studying. "We mapped a gene to an interval [of DNA] on chromosome four," he explains. The variant they found involved changes in a lipid-packaging protein called microsomal transport protein (MTP) that the pharmaceutical industry had already targeted for study because of its role in cardiovascular disease. "It was a great candidate," says Kunkel.

But when Kunkel tested French, and another group tested German, populations, both found the variant occurring at the same frequency in the controls as in the centenarians. This raised the possibility that the gene was not the one they were looking for. The problem might have been caused by American genetic heterogeneity: a possible Anglo-Irish bias in the New England control group that skewed the results, something Kunkel is now testing. ("If you think about it," Kunkel reflects, "it is very difficult to match a U.S. centenarian population with an ethnically matched American population of controls.")

But Kunkel still believes there is a mutation on chromosome four common to centenarians. In the previous study, the interval he focused on contained more than 50 genes (spaced over 10,000,000 base pairs). Now, with improved genetic techniques and twice as many centenarian subjects, he should be able to narrow the interval in which to search for the variant. Kunkel's experience with the centenarians "shows you the difficulty of doing the genetics of complex traits."

If Kunkel does find a SNP that promotes longevity, what could be done with the knowledge? "It might be possible to target the pathway in which the gene product [i.e., a protein] acts," he says, "but how could you clinically trial that drug? You would have to test it over the life span of a human. No pharmaceutical company is going to want to do a 30- or 40-year trial on some drug. You'd have to have a specific disease target." That is not the only difficulty. When pharmaceutical companies tried earlier to target MTP, they found that "if you hit it with a hammer, the result could be highly toxic." Kunkel's genetic studies revealed that subtle variations, perhaps beyond the reach of a drug, cause changes in the way the gene works.

Centenarians are a diverse group, so identifying shared traits that may play a role in their longevity has not been easy. So far, sirtuins have not been found to play a role in the New England group. But Kunkel notes that "genes work differently in different populations," depending on environmental influences. A gene that leads to high blood-lipid levels in primitive, physically active, food-limited populations might promote longevity in that context, but cause heart disease and lead to early death in a

sedentary modern European. Nevertheless, the clustering of genetic variations among centenarians suggests to Kunkel that there may be one or two genes common among long-lived individuals that have a much stronger influence than others.

The Power of Positive Thinking

How might such a gene work? Most researchers on this subject agree that insulin signaling is the most potent longevity pathway discovered so far, but they disagree over what sort of genes might control it in humans. Asked to speculate, their pet theories tend to reflect the workings of their own favorite model organism. Sinclair's findings from yeast naturally lead him to favor the sirtuins as key regulators of life span, while Kahn, with his FIRKO mouse, suspects fat plays a critical role. Ruvkun, whose roundworms have insulin receptors only in their nervous systems (none in the fat), thinks that the brain is the key to aging. As Sinclair puts it, "All these groups have been describing the same elephant, but from different ends."

How might these diverse approaches coalesce in our understanding of human aging? Ruvkun offers two different possibilities, both purely speculative and not necessarily consistent with each other. For starters, he suspects that aging is controlled by a kind of clock in our brains. In worm genetics, he notes, life span is essentially regulated by a hibernation cycle. "What is the closest thing to hibernation that humans do?" he asks. "Sleep."

"When animals enter hibernation, they are responding to their environment and essentially shutting themselves down," Ruvkun explains. "We do it every night when we go to sleep, and that is regulated by your nervous system, not your kneecaps. If I were to guess why some people live to 100, I would guess that they do something very different when they are sleeping—whether their body temperature goes down, or how they burn fat, changes. So the idea that there is a central clock regulating the rate of aging strikes me as very reasonable."

What sort of a gene then, would link hormonal signals regulating sleep and insulin to longevity? "One of the observations that Tom Perls has made of centenarians is that they are optimistic," says Ruvkun. "They don't have any one body type, but they are all kind of positive people. You can say, 'Of course! They have been healthy their whole life.' On the other hand, maybe [that quality] is pointing to a hormonal sense of well-being, and the hormonal state that is consistent with living a long time is a hormonal state of happiness."

Ruvkun guesses that whatever Kunkel may find will be something high up in the longevity pathway, not something that would affect one little thing. "It might be a peptide hormone like insulin that triggers high-level responses," he says, "or the sorts of things that signal satiety. There is nothing that makes you happier than a good big meal with some wine. What if there is variation in that, so that some people feel well-fed without necessarily having eaten much? If you hallucinate a full belly, you're a happy person and you'll be thinner."

Psychosocial factors like attending church or owning a pet have been linked to longer life, so some longevity pathways may indeed be under social control, activated through hormones. This is an area of continuing research, one that Sinclair has pursued by creating mice with additional SIRT1 in the brain. "Will that make the whole body healthier?" asks Sinclair. "If so, it could be that the brain is secreting hormones [that cause this]." Says Ruvkun, "Happiness is quantifiable. Not yet—but we will be able to measure it some day with a blood test, and say, 'Hmmm, you have some problems here—we've got some drugs that will make you happier.' Which is, of course, what Prozac does, but it is not very sophisticated."

Ruvkun thinks that the really interesting question, in trying to understand longevity, is not why our bodies (soma) die, but why our germline (the genetic legacy we pass from generation to generation through our children) is immortal. "The germline is a living system," Ruvkun points out. "Yours is an extension of your parents' and it goes back in an unbroken line to the very first animals." So why does the soma destroy itself? Some evolutionary biologists have argued that we die because the soma hasn't been selected to maintain itself beyond reproductive age.

But as the converging research of people like Sinclair, Kenyon, Kahn, and Ruvkun suggests, certain elements controlling longevity have been conserved from the simplest organisms all the way up into mammals. In other words, "our common ancestor had a life span," says Ruvkun, "and we inherited it. Aging is an active mechanism that has been under selection because it works well. Most of those animals that were immortal are no longer with us, because that doesn't work as well, presumably because it doesn't allow diversification and adaptation."

How Long a Life Span?

If aging is actively under selection, what are the implications for human life span? Has evolution set limits on the longevity of each species—and if so, how much longer might a human be able to live?

The fact that some people are now following a near-starvation regimen, in the hope that it will extend their life spans, derives from the belief that what works in animals like mice and rats will also work in humans. But this is a controversial proposition. Evolutionary biologist Lloyd Demetrius believes that life-span potential is related to an organism's ability to maintain stable levels of *(please turn to page 91)*

Wine: White or Red?

David Sinclair, associate professor of pathology at Harvard Medical School, is often asked about sources for resveratrol. "The molecule you can buy at a store, or on the Web, is a plant extract of 50 percent unknown composition," he says. In fact, when his laboratory discovered resveratrol's role in yeast longevity, they tested the various products containing the molecule that were available on the market at the time, and were surprised to find that none had any resveratrol in them. "The molecule is very sensitive to light and air," Sinclair explains, "and probably its shelf life is so short that by the time you buy it, it is gone."

The highest levels of resveratrol available to consumers occur in red wines. The molecule, which is concentrated in the skins of grapes, is highly insoluble. But red wine is made from grapes processsed with their skins, and alcohol helps extract it. And because the wine is stored in dark, light-proof bottles, corked to keep oxygen out, the resveratrol is preserved. Sinclair doesn't admit to taking resveratrol himself, since it hasn't been tested in humans. "But," he says, "I've switched from drinking white wine to red."

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critical cellular metabolites, not to its metabolic *rate*. The traditional theory that longevity and rate of aging are determined by metabolic rate and the rate of production of free radicals has had broad appeal as an explanation for why some animals live longer than others. But numerous exceptions to this rule (including the FIRKO mouse) have undermined the idea over time.

Demetrius's metabolic-stability hypothesis argues instead that longevity is determined by the stability of free-radical levels. He points out that an increase in ROS can damage DNA and lipids, thus accelerating aging, while also noting that some level of ROS is necessary for cell-to-cell signaling. This suggests that the capacity of cells to maintain ROS within an optimal range may be a better way of thinking about the links between oxidative stress and aging. Recent work by HMS research fellow Javier Apfeld has shown that metabolic stability in roundworms declines with age.

> "PROGRESS AGAINST AGE-RELATED DISEASE COULD ADD FIVE TO 10 YEARS ON AVERAGE TO HUMAN LIFE SPAN."

Demetrius's hypothesis (see "A New Theory on Longevity," November-December 2004, page 17) links evolutionary history to longevity, arguing that organisms that mature late sexually, have fewer offspring, and spread their reproductive activity over a longer period will also be long-lived, because the metabolic stability of their cells and cellular networks have evolved to accommodate this life history. And because such animals already enjoy high levels of metabolic stability, interventions like CR (and, presumably, related genetic manipulations)—which he believes work by increasing the stability of cellular networks—will not benefit them as much as it will benefit species characterized by early sexual maturity, a narrow reproductive span, and large litter size: traits that reflect a survival strategy of the sort that one finds in mice, which evolved to cope with feast-or-famine circumstances. "Darwinian fitness in a mouse is characterized by flexibility," he explains, "the ability of a population to respond to unpredictable resource conditions," whereas "Darwinian fitness in humans derives from being robust. The stability of cellular networks has evolved in concert with population stability," he says. And, in fact, human cells have been shown to be more resistant to stress than the cells of mice. His theory also explains why, in humans and other long-lived species, the rate of death ceases to increase exponentially after a certain age, which is not the case in mice. (Human mortality decelerates after about age 85.)

If Demetrius is right, then interventions that increase longevity will have large effects on the mean and maximum life span of mice. In rhesus monkeys, which share many genes with humans, he expects that results of a continuing caloric-restriction experiment will show a 15 percent increase in mean life span and have no effect on the maximum. In humans, he predicts the effect will be much less, adding perhaps 5 percent to average life span, and none to the maximum.

David Sinclair, however, does not rule out changes to the human maximum, although he believes that "We are not going to see any super-long-lived people in our lifetimes." Progress against age-related disease could add five to 10 years on average to human life span. "Who wouldn't be happy," he asks, "with an extra five years?"

Among humans, the longest-lived person ever documented was a Frenchwoman named Jeanne Calment, who lived to be 122. The maximum possible human life span may have hovered around this age for a very long time. Moses was said to be 120 when he died (ignore the fantastic life spans mentioned in the Old Testament, which range as high as 969 years). "There is a Jewish toast—'May you live to be 120'—but presumably not longer," Kahn says, grinning. "No one should live longer than Moses!"

Jonathan Shaw '89 is managing editor of this magazine.