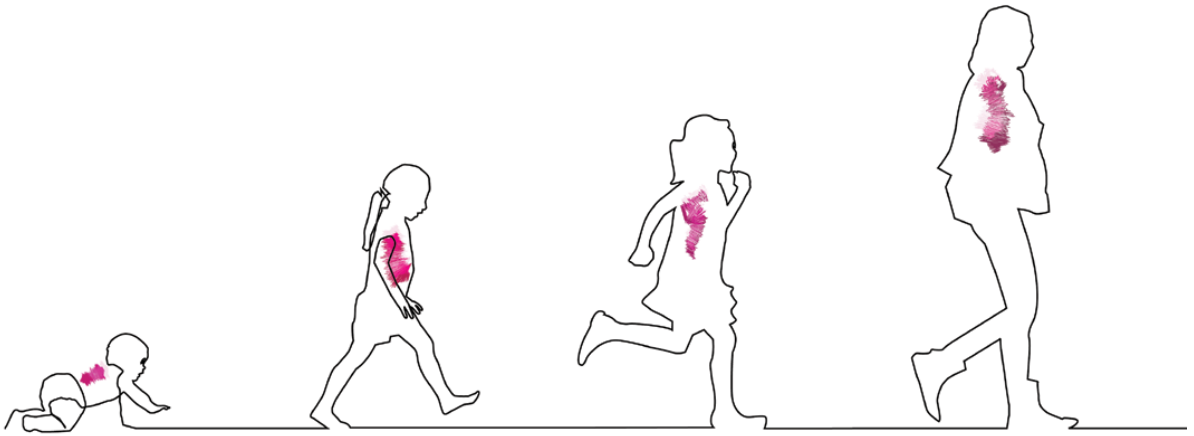
Aging from the inside

by Jessica Stoller-Conrad

Researchers at Caltech peer beneath our wrinkles and gray hair to add to our understanding of aging.



Aging. It's about more than just wrinkles, stiff knees, and hair loss; it's about a process that begins at birth and starts from the inside, affecting how we function at the level of our DNA, our cells, and our tissues. Despite claims made about creams and potions, it's unlikely that science will ever provide us with unending youth. Yet, thanks to improvements in medicine and our understanding of health, humans around the globe are living longer today than ever before. In fact, according to the World Health Organization, in 2011 the average global life expectancy was 70 years, an increase of six years since 1990.

For researchers who study aging, increasing the human life span isn't the only priority. In their studies of how aging affects organisms ranging from yeast to mice to human beings, researchers at Caltech are working to understand the basic, fundamental changes that happen *under* the surface as we age. "Researchers on aging don't really think they're going to find the 'fountain of youth,'" says biochemist Judith Campbell. "Instead, they're looking at ways to try to improve your health span—or, the number of healthy years in the life span."

CLIPPING AND REPLENISHING

Campbell has spent the past three decades at Caltech studying DNA replication—the process necessary to make sure that new cells in your body carry all the same genetic information that the old cells carried. During an embryo's development, cells divide to create organs and tissues; in adults, cell division replaces old, worn-out cells with new ones. Every time one of your cells divides, the double-stranded DNA that makes up that cell's 26 chromosomes unzips into two single strands, each of which then serves as a one-sided template, attracting the molecules needed to build the second, complementary strand.

However, the machinery involved in DNA replication always leaves a little piece of DNA at the end of each strand uncopied—meaning that every time a cell divides, the chromosomes carrying the cell's genetic material get a little bit shorter. To guard against clipping off genes at the end of the chromosome during cell division, the chromosome has protective end caps made up of special DNA sequences called telomeres; during every round of cell division, the telomeres are clipped shorter. After a cell ages—and has divided many times—the telomeres become so short that more divisions and replication would threaten to cut into important gene sequences. At that point, the cell stays alive but ceases to divide, reaching a stage called replicative senescence—a marker of aging in cells.

As it turns out, it was a gene that interacts with these telomeres that led Campbell and her colleagues into aging research. "We were driven to look at aging because the protein produced by one of the genes we were working with at the time, called Dna2, was actually massively concentrated at the telomeres," Campbell says.

Dna2 is a DNA binding protein, Campbell says, but at first she and her team didn't know why it is so strongly attracted to telomeres—or what it does once it reaches them. So they decided to find out, using a tiny, single-celled organism as their guide.

Campbell and her colleagues first encountered Dna2 while working with *Saccharomyces cerevisiae*, or brewer's yeast—the organism best known for turning water and grain into beer. But in addition to its role in crafting fine brews, *Saccharomyces* has also provided an important model for the study of aging, Campbell says.

"In the early 1960s the people studying yeast noticed that even this single-celled fungus showed signs of aging," she says. "Every time that a dividing yeast cell, called the mother cell, grows and divides, it gets older. Then, after about 23 divisions, it goes into replicative senescence."

At least, that's what happens in "normal" yeast cells. But in the 1990s, researchers in Campbell's laboratory noticed that in mutant yeast cells—cells in which the Dna2 gene was absent—the telomeres were shorter than those in a Dna2-laden yeast cell that had been through the same number of divisions. And the mutant cells also entered into replicative senescence after fewer divisions: their aging process seemed to have accelerated.

These findings suggested that, in normal yeast cells at least, Dna2 might have a role in maintaining the length of telomeres. An already-known enzyme called telomerase can slow a cell's aging process by adding a short sequence of DNA to the end of a shortened telomere; this partial restoration of telomere length is especially important in frequently dividing cells, like immune cells and skin cells in humans. Campbell and her colleagues hypoth-

esized that Dna2 might be interacting with telomerase.

Further work has backed up this hypothesis, showing that the telomerase in yeast cells lacking the Dna2 protein was ineffective, unable to add length to the telomeres. In other words, they found that the Dna2 protein was necessary in order for telomerase to function properly.

And that's not only the case in yeast. "We also work on mouse and human cells, and so more recently we've been showing that, in mice, Dna2 does the same things," Campbell says. "I can't tell you if the mice that have a reduced amount of Dna2 age more rapidly, but I can tell you that their telomeres get short."

Dna2 has other tasks in the cell as well; Campbell and her colleagues have recently shown that, in addition to lengthening telomeres, Dna2 plays a role in repairing damaged DNA in yeast, mouse, and human cells.

So, if Dna2 and telomerase work together to replace clipped telomeres, and Dna2 can repair DNA damage, are these two proteins the key to increasing an organism's life span?

They likely have an important role in longevity, says Campbell. But, as she points out, a longer life isn't necessarily a healthier one. Cells that continuously divide, multiply, and never die are dangerous—these are the characteristic traits of cancer. "One of the most important diseases of aging is cancer," she says. "Cancer cells need telomerase, and all tumor cells reactivate telomerase—otherwise, they would only go through a few divisions and you wouldn't get a tumor."

A healthy cell has to strike a delicate balance between clipping its telomeres through cell division and replenishing its telomeres via telomerase and Dna2—in other words, each cell needs to find its balance between aging and cancer. Although Campbell's work began investigating the aging side of this balance in yeast, her lab's focus has now shifted to the role of Dna2 in telomeres—and cancer—in mice.

"I could say that we focus much more now on cancer than on aging, but it's very difficult to dissect those two processes because we're working on machinery that functions in both kinds of biology," Campbell says.

FUSION AND FISSION

While telomeres shrinking on chromosomes are associated with aging, they are not the only age-related DNA changes your cells undergo. Outside a cell's nucleus are free-floating mitochondria—organelles best known as cell "power plants," converting nutrients from the food we eat into usable energy. These organelles contain their own genetic material, called mitochondrial DNA, or mtDNA. And according to biologist David Chan, whose lab at Caltech focuses on the role of mitochondria in health and disease, that genetical material is also involved with aging and, in particular, diseases of aging.

"Mitochondria have their own genome," Chan says. "That genome is very small; it only has 37 genes and only 13 of those are protein-encoding. But all of those 13 proteins are essential to the cells' generation of energy."

For that reason, Chan says, it's crucial that the genes for these 13 important proteins remain relatively free of mutations—acquiring just enough changes to allow evolution to occur but not so many that the cell can no longer function. Indeed, if such negative mutations accumulate, they can have drastic effects on the cell, with those effects being most devastating in muscle cells, in which large numbers of working mitochondria are needed to produce the energy needed for physical activities like walking and running.

"In a young person, all of the muscle cell fibers have varying degrees of respiratory activity from their mitochondria. But then, in an older individual, some muscle fibers start losing their mitochondrial function—and aging people tend to have an increased number of these defective fibers," Chan says. "If you look at the mtDNA in these nonfunctional fibers, there is invariably a mitochondrial genome that has deletions in the DNA sequence—the accumulation of mtDNA mutations increases with age."

These mutations arise as a result of the inevitable interactions between the hundreds or even thousands of mitochondria that are needed to power the routine activities in just one cell, Chan says. "You can have two mitochondria fusing together, becoming one mitochondrion," he says. "And you can also have the opposite process, in which a mitochondrion divides, which is called fission. They constantly come together and separate, come together and separate, as a way to exchange contents."

This give and take between fusion and fission—one aspect of the field called mitochondrial dynamics—can be beneficial for the organelle. "Say there is a mitochondrion with a mutation that prevents it from making an essential protein. After it fuses with a mitochondrion that *can* produce the protein, you get a mixing of gene products—the proteins coded by those genes," Chan says. By conjoining, the mitochondria create a single mitochondrion that has all of the essential proteins—which can then be combined into protein complexes necessary for respiration, he adds.

Chan's laboratory investigated how fusion can benefit mitochondria by studying two genes that control mitochondrial fusion in mice and humans—Mitofusin 1 (Mfn1) and Mitofusin 2 (Mfn2). In a study published in the journal *Cell* in 2010, Chan and his colleagues showed that the muscle cells of mice with mutations in both Mfn1 and Mfn2 were much smaller than those of normal mice of the same age. Although the animals had the same number of muscle cells, or fibers, the muscles were smaller overall, due to the smaller muscle cells. Furthermore, as the mutant mice grew older, they also experienced an increase in mtDNA mutations compared to normal mice of the same age.

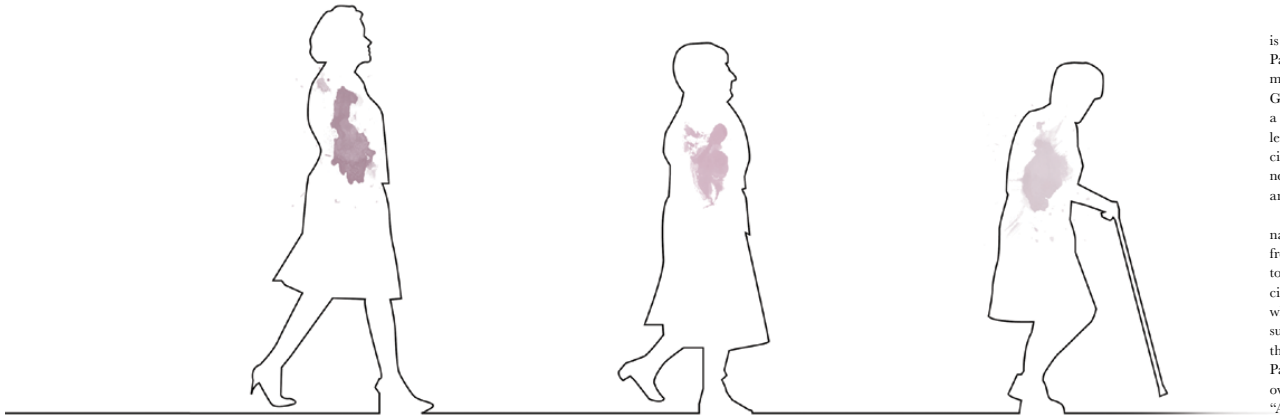
This, says Chan, is a real problem. "We've found that if you simultaneously have more mutations in the mtDNA and a loss of mitochondrial fusion due to a mutation in the mitofusins, there is a synergistic effect and you get much more severe symptoms in the mouse," he says. After all, if mutations in mtDNA genes result in missing essential proteins—and the mitochondrion can't replace these proteins by fusing with a "healthy" mitochondrion—the effects of the mutations will be compounded. "So when there are lots of mutations, mitochondrial fusion seems to be important to mitigate the effects of these mutations," Chan says. "That's how fusion ties into aging."

Chan has also been investigating the role of mitochondrial fusion in Parkinson's disease—a degenerative motor and nervous disorder associated with aging—due to the increased presence of Mfn2 not only in muscle cells, but in the neurons of the brain, nerves, and spinal cord.

Usually affecting people over the age of 50, Parkinson's disease is characterized by a loss of brain neurons that produce dopamine, a neurotransmitter responsible for relaying signals to other nerve cells. As a result, people with Parkinson's often experience symptoms such as trembling hands, slowed movements, emotional changes, and dementia.

The exact causes of Parkinson's disease are not clearly defined, but scientists have found evidence that links the disorder to defects in mitochondrial function, Chan says. For one thing, some inherited forms of Parkinson's disease result from mutations of genes that have a function in the mitochondria. In addition, Chan points to case studies of "young people—some in their 20s—who injected synthetic opiates and developed chronic Parkinsonian-type symptoms. The drugs were later found to be contaminated with a mitochondrial poison," he notes.

With this evidence in hand, Chan and his colleagues have been investigating the role of Mfn2 in the dopaminergic neurons—the main nerve cells affected by Parkinson's—in order to better understand the relationship between mitochondrial fusion and Parkinson's disease. In one part of the study, published in the journal *Human Molecular Genetics* in 2012, the researchers observed both normal mice and mice lacking Mfn2, tracing the spontaneous walking patterns of the mice as they traversed an open space. At



four weeks of age, the Mfn2-less mice traveled only about 68 percent of the distance that normal mice walked; by about 12 weeks, the mice without Mfn2 traveled only approximately a third of the normal distance. These mice also had trouble rearing back on their hind legs and moved more slowly than their normal counterparts.

In other words, says Chan, "when we knock out a gene that's involved in the fusion of mitochondria in the dopaminergic neurons, we get a mouse that has some characteristics of Parkinson's disease. It seems like those neurons are very sensitive to changes in mitochondrial dynamics." Restoring the mitochondria's ability to fuse and divide in damaged cells, then, could be an important aspect of future treatments for Parkinson's disease, he notes.

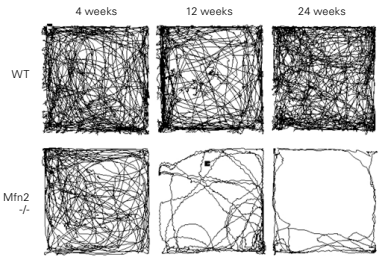
CIRCUIT LIGHTS

Neuroscientist Viviana Gradinaru (BS '05) is seeking to solve the puzzle of Parkinson's disease and the aging brain in a different way: by looking deeply at the brain's neural circuitry.

"Parkinson's is a disease of aging," Gradinaru says. "So if we want to think about increasing longevity, we need to understand what kind of use and abuse the circuits in our aging brains can take, and how we can make them last longer."

One way that Parkinson's researchers currently study these circuits in living human brains is via a therapeutic technique called deep brain stimulation, or DBS, in which physicians or scientists send electrical signals into the brain's motor centers via electrodes. This stimulation has been shown to counteract abnormal brain signals, alleviating the motor symptoms most often associated with Parkinson's disease, such as shaking and hand tremors.

Although used as a therapy for humans, DBS can also be used as a research tool in rodents—allowing researchers to analyze the animal's behavior after stimulating neurons in a certain region of the brain. It's an imperfect tool, however. Because the brain's neurons are so tightly networked and interconnected, the electrodes can "wind up stimulating neurons they're not intended to stimulate," says Gradinaru. This can cause side effects such as mood alterations in human patients and also make it difficult for researchers to be sure they're hitting the specific group of cells they're interested in studying in rodents.



Above: The traced walking patterns of both normal, wildtype mice (WT) and mice lacking Mfn2, a gene necessary for mitochondrial fusion: as they aged, mice without Mfn2 moved more slowly than their aging wildtype counterparts and traveled only a fraction of their walking distance.

All of which is why Gradinaru instead uses a more targeted technique called optogenetics to study how circuits in the brain associate with one another. With this technique, researchers genetically engineer neurons in the brains of rodents to produce a class of proteins, called opsins, which respond to light; neurons producing opsins can then be specifically excited by shining light on them.

By engineering different neurons to produce different opsins, each of which can only be activated by a specific color, or wavelength, of light, Gradinaru is able to use different colors of lasers to activate individual neurons—or networks of neurons—in a living brain in real time. In this way, by stimulating only the targeted neurons and observing their resultant behavior, optogenetics researchers are increasingly able to parse out the specific neurons responsible for specific symptoms in Parkinson's.

Of course, the technique can be applied to other conditions associated with an aging brain. "Optogenetics is being used to study Alzheimer's, Parkinson's, depression, addiction, memory, cognition, and sleep," says Gradinaru. "It can be used with such a wide range of processes because it lets you really focus on any of these circuits, stimulate a small population of neurons, and see what the behavioral and mechanistic outcome is."

In September of last year, Gradinaru received a New Innovator Award from the National Institutes of Health to support her work in the study of circuits involved in Parkinson's—and why the aging brain becomes more susceptible to the disease. "One of the goals of the award is to look at the Parkinson's-related dopaminergic cells over their lifetime," Gradinaru says. "A healthy individual starts with a population of dopaminergic cells that mature and function. But over time, as that individual ages, some of those cells will either die or just won't function as a dopaminergic cell anymore. We want to know how aging contributes to these changes."

One way Gradinaru and her colleagues plan to do this is by using optogenetics to stimulate the same set of dopaminergic neurons in a young brain and in an aging brain, and observing the differences in the animals' behavior. But to be certain they know what's going on, the researchers need to also look at the physical changes that occur in the brain as it ages. They'll be able to do that thanks to a visualization technique Gradinaru helped develop while a research associate at Stanford.

The technique, dubbed CLARITY, renders brain-tissue samples nearly transparent, allowing researchers to visualize those hard-to-see neurons and their connections from deep inside the brain. "The hope is that we can use CLARITY to observe and compare across aging brains and young brains, diseased brains and healthy brains, and get very detailed maps of neuron connectivity," Gradinaru says.

This is important because, as more humans live to very ripe old ages, conditions of aging are being diagnosed at an unprecedented rate—for instance, some researchers believe that over the next two decades the number of Parkinson's diagnoses will double. "The clock is ticking to find a solution," she says.

Although tools like optogenetics and CLARITY help us better understand the aging brain, they are not therapies that can be used in humans now, Gradinaru notes. Still, she says, "while this kind of basic, fundamental research won't directly provide a cure for Parkinson's or a way to halt aging, it's a necessary part of finding better treatments. We can gain a greater understanding of how aging changes specific cells and circuits in the brain."

We aging humans try to hide from the passage of time, but our cells and their genes don't lie—and that honesty provides important information for researchers like Viviana Gradinaru, David Chan, and Judith Campbell.

"Sure, you might be able to develop a cream that makes your wrinkles go away," Campbell says. "But what we're doing through the study of aging is unlocking some of the basic mechanisms of human health. And that's much more important." E&S

Judith Campbell is a professor of chemistry and biology. Her work on telomeres and aging is supported by the National Institutes of Health (NIH) and the Ellison Medical Foundation.

David Chan is a professor of biology at Caltech and an investigator with the Howard Hughes Medical Institute (HHMI). His work on mitochondrial dynamics is funded by NIH and HHMI.

Viviana Gradinaru is an assistant professor of biology. Her work on optogenetics is supported by the Human Frontiers in Science Program, the Beckman Institute, the Mallinckrodt Foundation, the Gordon and Betty Moore Foundation, the Pew Charitable Trust, the CIT-GIST program, the Michael J. Fox Foundation, and the NIH/NINDS New Innovator Award.