

The Effects of Gene Regulation on Aging in *Caenorhabditis elegans* (2003)

In 2003, molecular biology and genetics researchers Coleen T. Murphy, Steven A. McCarroll, Cornelia I. Bargmann, Andrew Fraser, Ravi S. Kamath, Julie Ahringer, Hao Li, and Cynthia Kenyon conducted an experiment that investigated the cellular aging in, *Caenorhabditis elegans* (*C. elegans*) nematodes. The researchers investigated the interactions between the transcription factor DAF-16 and the genes that regulate the production of an insulin-like growth factor 1 (IGF-1-like) protein related to the development, reproduction, and aging in *C. elegans*. Transcription factors, like DAF-16, are proteins that regulate the transcription of deoxyribonucleic acid (DNA) into messenger ribonucleic acid (mRNA), which later determines which proteins the cell produces. The research team's experiment suggested that an increase in the activity of the DAF-16 protein decreases the transcription of the genes that regulate the production of IGF-1-like proteins, increasing lifespan in nematodes. The team published their results in the article "Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans* " in *Nature* in June 2003. By comparing the regulation of gene expression in *C. elegans* with similar genes and pathways in humans, Murphy's research team sought to better understand cellular function and aging in humans.

The experimental efforts spanned across two continents. At the time of publication, the primary investigator of the experiment, Coleen Murphy, worked on molecular biology at the Lewis-Singler Institute for Integrative Genomics at Princeton University in Princeton, New Jersey and was the director of Paul F. Glenn Laboratories for Aging Research also at Princeton University. Steven A. McCarroll, who studied genetics at Harvard Medical School in Boston, Massachusetts, and Cornelia Bargmann, who studied neurobiology at the Howard Hughes Medical Institute in Chevy Chase, Maryland, assisted Murphy in the experimental analyses. Julie Ahringer, Hao Li, and Cynthia Kenyon also contributed to the experiments as part of the Department of Biochemistry and Biophysics at the University of Southern California in San Francisco, California. In Europe, researchers Andrew Fraser and Ravi S. Kamath at the Wellcome CRC Institute and Department of Genetics at the University of Cambridge in Cambridge, England, helped in the molecular genetics aspects of the experiment.

Using the nematode *Caenorhabditis elegans* (*C. elegans*) as their model organism, Murphy and her collaborators conducted their experiments primarily at the University of California and at the University of Cambridge. In those laboratories, the scientists raised and studied a total of 30,000 to 50,000 nematodes. The researchers divided the nematodes into two groups and fed each group a different diet. They fed one group of nematodes the bacteria *Escherichia coli* (*E. coli*), their normal food source, and they fed the other group *E. coli* that had been designed to inhibit particular gene expression when the nematodes ingest them through a process called ribonucleic acid interference (RNAi). RNAi is a method of inhibiting gene expression by destroying specific sequences of messenger ribonucleic acid (mRNA). In cells, mRNA transfers the information of a DNA sequence out of the cell nucleus into the cytoplasm where proteins are assembled. By destroying particular mRNA molecules, RNAi prevents the production of the proteins encoded by that mRNA. In *C. elegans*, DAF-16 is a protein responsible for increasing transcription rates of genes that are associated with slowing the rate of aging. The function of DAF-16 can be inhibited via RNAi interference.

Researchers then harvested the nematodes at specified times of development to observe gene expression and lifespan in both groups. The team determined extent gene expression using a DNA microarray, which attaches fluorescent markers to the proteins that the expression of particular genes produce. The fluorescence enabled the researchers to image the products of gene expres-

sion and determine the level of gene expression. The experimenters also measured lifespan from the first day of adulthood to identify changes in gene expression that occur in different stages of development.

Murphy and her collaborators specifically studied cellular pathways that *C. elegans* shares with humans. In humans, the insulin growth factor 1 (IGF-1) gene produces the IGF-1 protein, which plays a role in cellular metabolism and aging. In *C. elegans*, there is a similar gene called *ins-7*. The *ins-7* gene produces an IGF-1-like protein, which functions in cellular pathways analogous to those of IGF-1 in humans. The *ins-7* pathway provided Murphy and the other researchers with a method for investigating potential causes of cellular aging in humans by comparison with a similar cellular feedback system in *C. elegans*. They could more easily observe the impact of gene regulation in an organism such as *C. elegans* than one generally can in humans because nematodes have less complex biological systems. By comparing the *ins-7* gene and IGF-1-like protein interactions in *C. elegans* with similar genes and pathways in humans, Murphy's research team sought to better understand cellular function and aging in humans.

C. elegans progresses through a series of larval stages before becoming a reproductive adult. The average lifespan of *C. elegans* falls within two to three weeks after hatching from an egg. The DAF-2 protein regulates progression through larval development in the nematode. Activity of the DAF-2 protein impedes nematode development and reduces their overall lifespan. Murphy and her research team corroborated their hypothesis by observing that mutations that make DAF-2 not function properly are associated with increased lifespan. DAF-16 activity, however, extends the lifespan of *C. elegans* by decreasing the activity of the DAF-2 protein pathway. When DAF-16 is active, it inhibits the expression of the *ins-7* gene. When the *ins-7* gene is inhibited, less of the corresponding IGF-1-like protein is produced. In *C. elegans*, the IGF-1-like protein activates the DAF-2 protein pathway, which Murphy and her collaborators linked to decreased lifespan. Therefore, the more DAF-16 is present and active in *C. elegans*, the less activity there is in the DAF-2 pathway and the nematodes tend to have longer lifespans. Furthermore, when active, the DAF-2 protein inhibits the function of DAF-16. In turn, the activity of DAF-16 inhibits the activity of the DAF-2 protein. Thus, the interactions of DAF-2 and DAF-16 create a feedback loop in which initial decreases in the activity of DAF-2 cascades to further decreases in DAF-2 activity, further extending lifespans in *C. elegans*.

To identify the genes in which expression was increased or decreased, Murphy and the team performed a DNA microarray analysis, a technique in which different fluorescent colors glow in intensities in relation to gene expression. The stronger the fluorescence, the stronger the gene expression.

In the experiment, Murphy and the research team used RNAi to inhibit gene expression of *daf-2* and *daf-16*, which produce the proteins DAF-2 and DAF-16 respectively. Using DNA microarrays, the team observed that inhibiting *daf-2* expression, led to doubling of nematode lifespan. In contrast, Murphy and her collaborators noted that inhibiting *daf-16* expression led to a decrease in nematode lifespan. Researchers concluded that reducing the level of IGF-1-like signaling in the DAF-2 pathway during early adulthood increased the lifespan of the nematode. Furthermore, Murphy and her collaborators concluded that the expression of *daf-2* and *daf-16* genes directly affects the lifespan of *C. elegans*.

Murphy and her collaborators' study showed the impact that gene expression and regulation have on the lifespan of *C. elegans*. Moreover, their experiment provided a model for comparison for similar human cellular signaling pathways, particularly in early stages of development.

Sources

1. Howard Hughes Medical Institute. "Cornelia I. Bargmann, PhD." Howard Hughes Medical Institute. <http://www.hhmi.org/scientists/cornelia-i-bargmann> (Accessed April 14, 2016).
2. Laboratory of Neural Circuits and Behavior. "What Do We Work On?" The Rockefeller University. <http://lab.rockefeller.edu/bargmann/> (Accessed April 14, 2016).
3. Murphy, Coleen T., Steven A. McCarroll, Cornelia I. Bargmann, Andrew Fraser, Ravi S. Kamath, Julie Ahringer, Hao Li, and Cynthia Kenyon. "Genes That Act Downstream of DAF-16 to

- Influence the Lifespan of *Caenorhabditis elegans* .” *Nature* , 2003, 277-83. <http://mcb.berkeley.edu/courses/mcb240/Meyer/nature01789.pdf> (Accessed May 6, 2016).
4. President and Fellows of Harvard College. “Steven McCarroll, Ph.D.” Harvard Medical School Department of Genetics. <http://genetics.med.harvard.edu/faculty/mccarroll> (Accessed April 14, 2016).
 5. Princeton University. “Molecular Biology Faculty: Coleen T. Murphy.” Department of Molecular Biology. <http://molbio.princeton.edu/faculty/molbio-faculty/120-murphy> (Accessed April 14, 2016).
 6. The Rockefeller University. “Cori Bargmann Awarded 2015 Benjamin Franklin Medal.” Newswire. <http://newswire.rockefeller.edu/2014/11/04/cori-bargmann-awarded-2015-benjamin-franklin-medal/> (Accessed April 14, 2016).
 7. The Rockefeller University. “Cori Bargmann, Ph.D.” The Rockefeller University. <http://www.rockefeller.edu> (Accessed April 14, 2016).