

JUNE 24, 2009

## Eat Less, Live Longer: Unraveling the Connection

For those hoping to extend their life by fine-tuning their diet, the available advice can be confusing and often contradictory. Scientists can agree on at least one thing, however: radically slashing calories is a surefire strategy to increase lifespan – and it appears to work in many different types of animals. Now, researchers have identified two proteins that begin to provide a new molecular explanation for how eating less leads to living longer.

Howard Hughes Medical Institute investigator Andrew Dillin and colleagues at The Salk Institute for Biological Studies have identified two proteins, WWP-1 and UBC-18, that link dietary restriction to longevity in roundworms. The proteins could represent the first elements of a genetic pathway that may be conserved throughout the animal kingdom. In the June 24, 2009, issue of *Nature*, Dillin, postdoctoral fellow Andrea Carrano, Zheng Liu, and senior author Tony Hunter, report that the two proteins are part of cells' ubiquitination pathways, which normally tag unnecessary proteins for disposal. The scientists say understanding how these molecules influence longevity could have implications for developing new treatments for age-related diseases.

---

"If we could get small molecules to influence the activity of these ubiquitin pathway players, I would expect that to have a very significant effect on human diseases of aging."

— Andrew Dillin

---

Worms on a near-starvation diet—about a 40 percent reduction in calories compared to what worms typically eat—live twice as long as normal worms. Dillin and his colleagues have now shown that even worms that are allowed to eat as much as they want can still lead a longer than average life when researchers artificially increase the amount of WWP-1 protein they produce.

The results of the studies are important because they begin to provide an explanation for the “life extension” effect seen in laboratory animals when food is restricted. So the studies could offer new clues about the molecular mechanisms that living organisms employ when food is scarce.

Experiments done in the 1930s showed that severely restricting food intake leads to an increase in longevity in some animals. Since then, the same effect has been shown in many species, and further research has demonstrated that a dramatic reduction in food intake also delays the onset of many age-related diseases. Even though the longevity phenomenon is well documented in laboratory animals, researchers have been unsure how it works.

Dillin points out that caloric restriction is not the only way to lengthen animals' lives. Manipulating insulin signaling or the activity of the cellular power plants known as mitochondria can have similar effects. "But the diet restriction pathway seems to be easier to manipulate. It is the only environmental influence that we can place on the longevity program," said Dillin.

Two years ago, Dillin's group found an intriguing clue into what causes starved cells to live longer. They observed worms live longer on a restricted diet only if they have a gene called *pha-4* (in worms) or *Foxa* (in mice). The gene was the first genetic regulator uncovered that specifically links dietary restriction to increased lifespan. Since that discovery, Dillin's lab has been working to tease out more details about how cells sense a scarcity of calories and translate it into a longer life.

When Carrano, a postdoctoral fellow in Tony Hunter's lab at the Salk Institute, teamed up with Dillin's lab in 2003, she proposed a research project to explore how ubiquitination might influence stress resistance. Ubiquitination is a process whereby proteins are tagged with a small molecule known as ubiquitin. Its best known role is in tagging unwanted proteins for destruction. Ubiquitin ligases are enzymes that link ubiquitin to the target proteins. Dillin explained that one class of ligase, known as E3, "had been known to regulate transcription factors that play key roles in diseases and stress resistance. So they had the right pedigrees to suggest they may be involved in longevity."

Carrano used genetic techniques to create worms that either lacked the E3 ligase WWP-1 or produced too much of the enzyme. The team found that worms without the enzyme lived an average of 20 days – regardless of how much food they ate.

An excess of WWP-1, on the other hand, extended the worms' lives to 25 days, even when they were given unlimited access to food. Dillin noted that for a human in the United States, whose life expectancy is about 78 years, a 20 percent increase in longevity would mean living to 96.

In further experiments, Carrano discovered that WWP-1 is a regulator of the PHA-4 pathway. She also identified another ubiquitin ligase, UBC-18, that works with WWP-1.

Now that they know these enzymes contribute to longevity, Dillin says, the team has some new clues about how the process might be manipulated with drugs or other molecules. "It would be great to be able to trick the system" to

trigger the extended-lifespan process in animals – and eventually people – without subjecting them to extreme dietary deprivation, he said. “If we could get small molecules to influence the activity of these ubiquitin pathway players, I would expect that to have a very significant effect on human diseases of aging.”

Dillin said his group now plans to widen its search to look for other control points in the circuit connecting dietary restriction and extended lifespan. “We want to find the receptor that senses the signal of a restricted diet – that’s the Holy Grail,” said Dillin. “And we want to look downstream to see what the transcription factor [PHA-4] is actually regulating” to produce a longer life.

“The fact that the pathway [including wwp-1, UBC-18 and PHA-4] is specific to dietary restriction – as opposed to insulin signaling or mitochondrial function – is interesting,” said Tony Hunter of the Salk Institute. “Now we need to find out how the dietary restriction signal leads to WWP-1 and what the targets of WWP-1 are.”