Study identifies proteins that modulate life span in worms

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Researchers at the Stanford University School of Medicine have identified a new group of proteins involved in determining the life span of laboratory roundworms. Blocking the expression of one member of the group can extend the worm's life span by up to 30 percent. Because the proteins work in the worms' reproductive systems, the research represents yet another intriguing link between longevity and fertility.

In particular, the researchers showed that the proteins are involved in epigenetics - a phenomenon in which chemical modifications to DNA and the proteins around it affect how it is packaged and expressed in a cell. Although an organism can't change the DNA sequence of the genes it has inherited, epigenetic changes allow it to silence or tweak their expression in response to environmental or other external cues.

"We've shown here that an epigenetic change can affect the life span of an organism," said Anne Brunet, PhD, assistant professor of genetics, "but only within the context of an intact reproductive system." Brunet is the senior author of the study, which will be published online June 16 in *Nature*.

Roundworms, also known as Caenorhabditis elegans, are a popular laboratory animal. They are easy to care for, and their approximately four-week life span makes them good models for longevity studies. For technical reasons, though, most longevity researchers have conducted their experiments on sterile worms.

Brunet and graduate student Eric Greer wanted to explore the effect of epigenetic changes on longevity. But they wondered if using fertile worms might be more appropriate for their studies. After all, other studies of the worms have suggested that fertility is at least indirectly linked to longevity.

Greer, who is the lead author of the study, used a technique called RNA interference in fertile worms to methodically block the expression - one by one - of genes known to affect a cell's epigenetic status. He identified a number of genes that, when inhibited, caused the worms to live up to 30 percent longer than normal.

The gene with the most pronounced effect, Ash-2, makes a protein that functions as a methyltransferase - meaning it works together with other proteins to add a chemical tag called a methyl group to a component of a cell's DNA packaging machinery, which is known as a histone. The presence or absence of this tag affects whether the DNA remains wound up tightly like thread on a spool, or unfurls to allow its genes to be expressed.

Inhibiting Ash-2 activity reduces the number of methyl tags on the histone, which keeps the DNA inaccessible and somehow extends the animal's life by as much as 30 percent. Conversely, the researchers found, blocking the expression of a protein called Rbr-2 taxed with removing the tag - a demethylase - shortened the worm's life span by about 15 to 25 percent. Worms in which the expression of both proteins were blocked had slightly shortened lives.

Clearly the levels of methylation on that particular spot on the histone are important to longevity. But why? And how are they calibrated?

The researchers found that Ash-2 is highly expressed in the germline, or reproductive cells, as well as in newly formed eggs. These cells also had

high levels of the methyl tag. When Greer blocked the expression of Ash-2 in worms that lacked normal reproductive cells, he found that this no longer extended worm life span, suggesting that an intact germline is necessary for Ash-2 to regulate longevity.

Further investigation showed that Ash-2 activity affects the expression of several genes specific to germline cells, including a group previously shown to affect adult life span. Blocking Ash-2 expression only in germline cells, but not in the rest of the worm's body, still extended its life span, as did expressing excess amounts of the tag-removal protein Rbr-2 in the germline. Finally, another series of experiments showed that the presence of mature eggs is required for Ash-2 knockdown to have an effect.

"We still don't know exactly how this works mechanistically," said Brunet, "but we've shown that the presence of the germline is absolutely essential for this longevity extension to happen."

In the future the researchers plan to monitor the methylation status of the histone during the animal's life span. Because epigenetic changes are reversible, it's likely they'll see a natural ebb and flow as the worm ages. They'd also like to examine the effect of environmental situations known to affect longevity, such as calorie restriction, on the tagged histone.

"Aging is a very plastic process," said Brunet, who cautioned that it's possible that Ash-2 also works elsewhere in the worm. "This tagging doesn't affect reproduction directly, but it somehow talks to the rest of the body to affect the whole organism." Perhaps, they speculate, the genes activated by the loss of Ash-2 work together with other factors produced by mature eggs to lengthen the animal's life.

"It makes a sort of sense that the reproductive system would be involved in life span, since that is really the only 'immortal' part of an organism,"

said Brunet. "In that context, the body is just the mortal envelope."

Provided by Stanford University