Special News Feature

PERSISTENT EPIGENETIC MEMORY

Nematodes transmit environmental information, such as temperature or food availability, across generations. Amber Dance looks at how this is possible.

Credit: Columbia University Medical Center

In the lab, *Caenorhabditis elegans* embryos develop quickly under a stable temperature, reaching adulthood within a few days. In the wild, however, life is not so stable, with temperatures going up and down every sunrise and nightfall. What could help those nascent worms in the wild would be having some information on temperatures from the last few weeks so that they could adjust their metabolism accordingly, says Adam Klosin, a postdoctoral fellow at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, Germany. And surprisingly, they actually do.

Reporting in the journal *Science*, Klosin and his colleagues describe how the nematodes's germline can “remember” temperatures experienced by recent ancestors, thanks to the inheritance of specific epigenetic marks. In one experiment, an artificial transgene array influenced by high temperatures maintained its heat-influenced high expression levels for 14 generations (1).

In people, the effects of famine or abundance echo in offspring; babies born during the Dutch Famine of World War II were smaller than those born the year before, and this effect persisted in their children and grandchildren. And in Sweden, an abundance of food experienced by a boy was linked to diabetes in his grandchildren (2).

In nematodes, researchers have observed gene silencing due to high temperatures, dietary restriction or starvation, viruses, or transposons. Scientists are just now beginning to work out the mechanisms underlying this phenomenon; Klosin found that gene silencing through histone methylation was crucial in his system, while other researchers are pursuing pathways mediated by small RNAs.

Afterglow

Klosin defines “epigenetic inheritance” as any persistent trait that occurs in the absence of genetic changes. *C. elegans*, long a favorite organism of geneticists, offers an apt model for exploring epigenetic transmission. Its 3-day generation time makes this worm a

convenient model for multigenerational studies, and thanks to the animal's hermaphroditism, it can self-fertilize, yielding hundreds of offspring genetically identical to the homozygous parent line. That way, any changes in gene expression should be due to epigenetics.

At first, though, Klosin wasn't looking at transgenerational inheritance. His work focused on regulation of the chaperone protein DAF-21, also known as heat shock protein 90, using an mCherry reporter construct tied to the *daf-21* promoter.

While 20°C is the standard temperature of a nematode incubator, worm geneticists often use different temperatures to activate temperature-sensitive mutations or simply to manage the animals' growth speed. Going away for the weekend? Slow things down at 16°C. Eager to produce a large population? Speed up development at 25°C.

When Klosin kept his worms at 25°C, they glowed red because the heat shock promoter was activated. When he returned the worms and their offspring to 20°C, the effect didn't completely disappear—the offspring of worms that spent time at 25°C glowed a bit more dully than their parents, but brighter than worms from a line kept consistently at 20°C. “We accidentally observed that they have this large expression difference that's inherited,” explains Klosin.

When he tried the same experiment with a larger array of mCherry-tagged genes, the effect was even more pronounced. “The longer you grow the worms at 25[°C], the higher the expression,” says Klosin. “This means there is also a ‘memory’ of low temperature.” The effect maxes out after 4 or 5 generations grown at 25°C.

If a worm with the array lives at 25°C—for just 1 generation—its progeny will have heightened mCherry expression for 7 generations after a return to 20°C. And after 5 generations at 25°C, it took 14 generations at 20°C for the fluorescent gene expression to dwindle to normal levels.

Based on past research of epigenetic inheritance, Klosin and his colleagues suspected that small RNAs might be transferring tem-

perature information from one generation to the next. To find out if their hunch was correct, they crossed worms with high mCherry expression to those with low levels. All of the resulting worms had similar mCherry levels, as would be expected if the small RNAs from sperm and egg mixed together in a zygote. Then, the researchers crossed their F1 generation with wild-type hermaphrodites, expecting to again see similar average mCherry levels due to the small RNAs transmitted in the sperm. Instead, 50% of the worms had high levels of mCherry expression, and 50% had low mCherry expression.

“The trait segregates with the chromosome,” Klosin concludes. That is, the genes from the high-expresser P generation kept expressing high mCherry levels, and the ones from the low-expresser grandparents maintained their mCherry levels, too.

As in humans and other animals, methylation of histone 3 lysine number 9 (H3K9) is a common gene-silencing epigenetic marker in *C. elegans*. When analyzing the mCherry array at the molecular level, Klosin and his colleagues found that the grandchildren of 25°C worms had fewer repressive H3K9 methyl marks than those that descended from 16°C grandparents. But when they inactivated *set-25*, which encodes the putative histone methyltransferase, the temperature effect disappeared.

This led the authors to propose that high temperatures inhibit the SET-25 protein, causing some of those histone markers to disappear from the germline. Subsequent generations express the mCherry array more fully, until, eventually, low temperatures allow SET-25 to replace the silencing marks. Therefore, Klosin cautions *C. elegans* aficionados working with artificial transgenes to be wary when they switch their worms between temperatures; the effects might not fade as quickly as they think.

He also found that some endogenous genes, especially transposons, relied on the effects of SET-25 repression to raise their expression at high temperatures, with the effects lasting as long as 5 generations after a return to 20°C.

RNAs in the germline

Although Klosin ultimately found a role for histone methylation in his *Science* study, he still can't fully discount the involvement of small interfering RNAs in the transmission of temperature memory.

“We suspect they do have some role,” he says. And Oded Rechavi, a researcher from Tel Aviv University in Israel working on transgenerational gene regulation, agrees.

Researchers first discovered RNA interference (RNAi) in nematodes and plants, and they are now finding similar small RNAs at work in worm germline epigenetic inheritance, according to Rechavi. When nematodes are exposed to double-stranded RNA—perhaps from a virus—they chop it up into primary small

RNAs. These bits base-pair with mRNAs and recruit protein machinery to synthesize secondary small RNAs. These secondary RNA snippets enter the nucleus and recruit regulators, such as chromatin modifiers, to silence transcription.

In 2011, Rechavi reported that small RNAs against foreign viral genes are transmitted across generations (3). “These small RNAs vaccinate the next generation from additional rounds of viral replication,” he says.

Then in 2014, he found that the same transmission could occur for endogenous genes too. Starving the worms led to small RNA transmission through the next three generations. The RNAs targeted genes involved in nutrition and gave the F3-generation worms an enhanced lifespan (4).

“We wanted to understand the rules of this inheritance,” says Rechavi. One of his first questions was how the RNA-based memory fades after a few generations.

In 2016, Rechavi reported on a molecular timer for transgenerational gene silencing (5). The initiation of a new RNAi response competes with and down-regulates production of other, endogenous small RNAs that are specific inhibitors of the RNA inheritance machinery. That allows the inheritance machinery, consisting of proteins that transport the small RNAs across the germline, to function. But after three to

five generations, the endogenous small RNAs win out, shutting off the inheritance system.

“It's like a transgenerational clock,” says Rechavi. By mutating genes involved in the clock, he could make inheritance of small RNAs and their effects last forever (5,6).

Rechavi suspects that despite differences between human and nematode epigenetic systems, the findings from his lab and others might turn out to have a counterpart in human biology. “Many things that were, in the beginning, thought to be peculiarities of worms were later found to be true in humans,” he notes, citing RNAi itself. “The details might change, but it could be relevant.”

***Caenorhabditis elegans* is a useful model for transgenerational inheritance.** Credit: Bob Goldstein, University of North Carolina, Chapel Hill.

References

1. Klosin, A., et al. 2017. Transgenerational transmission of environmental information in *C. elegans*. *Science*. 356:320-323.
2. Wei, Y., et al. 2015. Environmental epigenetic inheritance through gametes and implications for human reproduction. *Hum Reprod Update*. 21:194-208.
3. Rechavi, O., et al. 2011. Transgenerational inheritance of an acquired small RNA-based antiviral response in *C. elegans*. *Cell*. 147:1248-1256.
4. Rechavi, O., et al. 2014. Starvation-induced transgenerational inheritance of small RNAs in *C. elegans*. *Cell*. 158:277-287.
5. Houri-Ze'evi, L., et al. 2016. A tunable mechanism determines the duration of the transgenerational small RNA inheritance in *C. elegans*. *Cell*. 65:88-99.
6. Lev I., et al. 2017. MET-2-dependent H3K9 methylation suppresses transgenerational small RNA inheritance. *Curr Biol*. 27:1138-1147.

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