'Living fossil' may upend basic tenet of evolutionary theory

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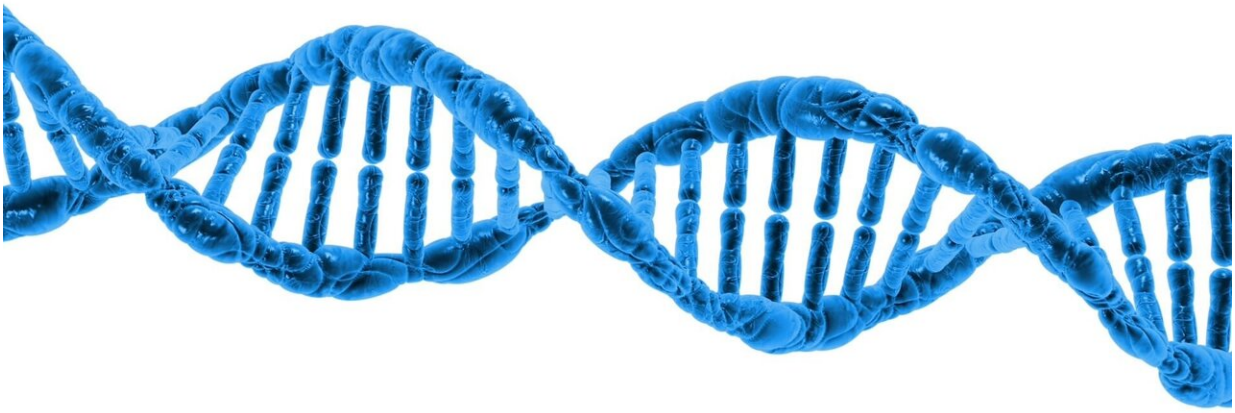

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The field of evolutionary biology has seen its share of spirited debates. But if there's one principle that virtually every expert in the field agrees on, it's that natural selection occurs at the level of the genome.

But now, a UC San Francisco-led research team has discovered the first conclusive evidence that selection may also occur at the level of the

epigenome—a term that refers to an assortment of chemical "annotations" to the genome that determine whether, when and to what extent genes are activated—and has done so for tens of millions of years. This unprecedented finding subverts the widely accepted notion that over geologic timescales, natural selection acts exclusively on variation in the genome sequence itself.

In a study published Jan. 16, 2020 in the journal *Cell*, the researchers show that *Cryptococcus neoformans*—a pathogenic yeast that infects people with weakened immune systems and is responsible for about 20 percent of all HIV/AIDS-related deaths—contains a particular epigenetic "mark" on its DNA sequence, which, based on their lab experiments and statistical models, should have disappeared from the species sometime during the age of the dinosaurs.

But the study shows that this methylation mark—so named because it's created through a process that attaches a molecular tag called a methyl group to the genome—has managed to stick around for at least 50 million years—maybe as long as 150 million years—past its predicted expiration date. This amazing feat of evolutionary tenacity is made possible by an unusual enzyme and a hefty dose of natural selection.

"What we've seen is that methylation can undergo natural variation and can be selected for over million-year time scales to drive evolution," explained Hiten Madhani, MD, Ph.D., professor of biochemistry and biophysics at UCSF and senior author of the new study. "This is a previously unappreciated mode of evolution that's not based on changes in the organism's DNA sequence."

Though not seen in all life forms, DNA methylation isn't uncommon either. It's found in all vertebrates and plants, as well as many fungi and insects. In some species, however, methylation is nowhere to be found.

"Methylation has a patchy evolutionary presence," said Madhani, who is also a member of the UCSF Helen Diller Family Comprehensive Cancer Center and a Chan-Zuckerberg Biohub investigator. "Depending on what branch of the evolutionary tree you look at, different epigenetic mechanisms have been maintained or not maintained."

Many model organisms that are staples of the modern molecular biology lab—including the baker's yeast *S. cerevisiae*, the roundworm *C. elegans*, and the fruit fly *D. melanogaster*—lack DNA methylation entirely. These species are descended from ancient ancestors that lost enzymes that were, until this study was published, thought to be essential for propagating methylation for generation upon generation. How *C. neoformans* managed to avoid the same fate was a mystery up to now.

In the new study, Madhani and his collaborators show that hundreds of millions of years ago, the ancestor of *C. neoformans* had two enzymes that controlled DNA methylation. One was what's known as a "de novo methyltransferase," which was responsible for adding methylation marks to "naked" DNA that had none. The other was a "maintenance methyltransferase" that functioned a bit like a molecular Xerox. This enzyme copied existing methylation marks, which had been put in place by the de novo methyltransferase, onto unmethylated DNA during DNA replication. And like every other species with an epigenome that includes methylation, the ancestor of *C. neoformans* had both types of methyltransferase.

But then, sometime during the age of the dinosaurs, the ancestor of *C. neoformans* lost its de novo enzyme. Its descendants have been living without one since then, making *C. neoformans* and its closest relatives the only species alive today known to have DNA methylation without a de novo methyltransferase. "We didn't understand how methylation could still be in place since the Cretaceous period without a de novo enzyme," said Madhani.

Though the maintenance methyltransferase was still available to copy any existing methylation marks—and the new study clearly demonstrates that this enzyme is unique among such enzymes for a number of reasons, including its ability to propagate existing methylation marks with exceptionally high fidelity—the study also shows that unless natural selection were acting to preserve methylation, the ancient loss of the de novo methyltransferase should have resulted in the rapid demise and eventual disappearance of DNA methylation in *C. neoformans*.

That's because methylation marks can be randomly lost, which means that no matter how exquisitely a maintenance methyltransferase copies existing marks onto new strands of DNA, the accumulated loss of methylation would eventually leave the maintenance enzyme with no template to work from. Though it's conceivable that these loss events might occur at a sluggish pace, experimental observations allowed the researchers to determine that each methylation mark in *C. neoformans* was likely to disappear from half of the population after just 7500 generations. Even assuming that for some reason *C. neoformans* might reproduce 100 times more slowly in the wild than in the lab, this would still be the equivalent of only 130 years.

The rare and random acquisition of new methylation marks can't account for the persistence of methylation in *C. neoformans* either. The researchers' lab experiments demonstrated that new methylation marks arise by chance at a rate 20 times slower than methylation losses. Over evolutionary timescales, the losses would clearly predominate, and without a de novo enzyme to compensate, methylation would have vanished from *C. neoformans* around the time when dinosaurs disappeared had it not been for selection pressures favoring the marks.

In fact, when the researchers compared a variety of *C. neoformans* strains that were known to have diverged from one another nearly 5 million years ago, they found that not only did all the strains still have

DNA methylation, but the methylation marks were coating analogous regions of the genome, a finding which suggests that methylation marks at specific genomic sites confer some sort of survival advantage that's being selected for.

"Natural selection is maintaining methylation at much higher levels than would be expected from a neutral process of random gains and losses. This is the epigenetic equivalent of Darwinian evolution," said Madhani.

Asked why evolution would select for these particular marks, Madhani explained that "one of methylation's major functions is genome defense. In this case we think it's for silencing transposons."

Transposons, also known as jumping genes, are stretches of DNA that are able to extract themselves from one part of the genome and insert themselves into another. If a transposon were to insert itself into the middle of a gene needed for survival, that gene may no longer function and the cell would die. Therefore, transposon-silencing methylation provides an obvious survival advantage, which is exactly what's needed to drive evolution.

However, it remains to be seen how common this unappreciated form of natural selection is in other species.

"Previously, there was no evidence of this kind of selection happening over these time scales. This is an entirely novel concept," Madhani said. "But now the big question is 'Is this happening outside of this exceptional circumstance, and if so, how do we find it?'"

More information: Sandra Catania et al. Evolutionary Persistence of DNA Methylation for Millions of Years after Ancient Loss of a De Novo Methyltransferase, *Cell* (2020). DOI: 10.1016/j.cell.2019.12.012