Bringing new energy to mitochondria research

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Credit: Steven Lee/Whitehead Institute

Tiny mitochondria in our cells turn oxygen and nutrients into usable energy in a process called respiration. This process is essential for powering our cells, and yet in spite of its importance many of the finer

details of how it happens remain unknown. One long-standing mystery is how a molecule called nicotinamide adenine dinucleotide (NAD), which plays a big part in respiration and metabolism, gets into the mitochondria in humans and other animals. Mitochondria use NAD in order to produce adenosine triphosphate (ATP), the energy supply molecules used throughout the cell. Researchers knew the identities of the molecules that transport NAD from the wider cell into the mitochondria of yeast and plants, but had not found the animal equivalent—in fact, there was some debate over whether one even existed or whether animal cells used other methods altogether.

Now, research from postdoctoral researcher Nora Kory in Whitehead Institute Member David Sabatini's lab may end the debate. In a paper published in *Science Advances* on September 9, the researchers show that the missing human NAD transporter is likely the protein MCART1. This discovery not only answers a longstanding question about a vital cellular process, but may contribute to research on aging—during which cells' NAD levels drop—as well as research on diseases that involve certain mitochondrial dysfunctions, for which cells with broken NAD transporters could be an experimental model.

"I find it striking that mitochondria play such an important role in metabolism in the cell, which in turn plays a huge role in health and disease, but we still don't understand how all of the molecules involved get in and out of mitochondria. It was exciting to fill in a piece of that puzzle." Kory says.

An unexpected discovery

Kory did not set out to find the long sought-after transport molecule. Rather, she was trying to better understand mitochondrial respiration by mapping the genes involved. She was comparing gene essentiality profiles, which show how important a gene is to different processes in a

cell—the more co-essential two genes are, the more likely they are to be involved in the same cellular process—and one gene stood out: MCART1, also known as SLC25A51. It was highly correlated to other genes involved in mitochondrial respiration, and belonged to a family of genes known to code for transporters, yet its function was unknown. The protein coded for by MCART1 clearly played an important role, so Kory decided to figure out what that was; as her research progressed, she realized she had found the missing NAD transporter.

Kory and colleagues applied a common approach to determine MCART1's function: inactivate the gene in cells, and see what breaks down in its absence. This approach is like troubleshooting a machine; if you cut a wire in your car and the headlights stop working, but everything else is fine, then that wire was probably linked to the headlights. When the researchers removed MCART1, the cells exhibited much lower oxygen consumption, reduced respiration and ATP production, and reliance on other, far less efficient means of ATP production—exactly what you'd expect to see if the inactivated gene was needed for respiration. Moreover, the biggest change that the researchers observed in cells without MCART1 was reduced levels of NAD in the mitochondria, while NAD levels in the wider cell remained the same, which they quantified using experiments previously developed in the lab. The researchers confirmed that MCART1 is essential for NAD transport into isolated mitochondria and overabundance of MCART1 caused an increased uptake.

"It's very satisfying when our lab returns to the techniques that we have developed in order to make new findings such as identifying this important protein," says Sabatini, who is also a professor of biology at Massachusetts Institute of Technology and an investigator with the Howard Hughes Medical Institute.

The evidence supports that the protein MCART1 is itself the transport

channel. However, it is possible that the protein may play some other essential contributing role to transportation, or that it combines with other molecules to do its job. To strengthen the case for MCART1 as the transporter, the researchers showed that MCART1 and the known yeast NAD transport could be switched out for each other in both human and yeast cells, suggesting an equivalent function. Still, further experiments are needed to determine the precise mechanism of transport.

A serendipitous case of synchronous discovery reinforces Kory's findings. A paper by other researchers published on the same day in the journal *Nature* also put forth that MCART1 is the missing NAD transporter, based on a completely different set of evidence. Combined, the papers provide an even more compelling case.

"It was nice to see how our different approaches complemented each other, and led to the same conclusion," Kory says.

Understanding how NAD gets into the mitochondria opens up new questions about the details of mitochondrial respiration.

More information: Nora Kory et al. MCART1/SLC25A51 is required for mitochondrial NAD transport, *Science Advances* (2020). DOI: 10.1126/sciadv.abe5310

Timothy S. Luongo et al. SLC25A51 is a mammalian mitochondrial NAD+ transporter, *Nature* (2020). DOI: 10.1038/s41586-020-2741-7

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