Novel method enables absolute quantification of mitochondrial metabolites

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Whitehead Institute scientists have developed a method to quickly isolate and systematically measure metabolite concentrations within the cellular organelles known as mitochondria, often referred to as the "powerhouses of the cell." Prior attempts at such measurements have yielded unreliable results, either by taking too long to isolate mitochondria or by contaminating mitochondrial metabolites with contents from other cellular components.

"The advantage of this new method is that it offers a combination of both increased speed and specificity," say Whitehead Member David Sabatini, who is also a Howard Hughes Medical Institute investigator and a professor of biology at MIT. "We are quite excited about applying this workflow in vivo and to other organelles such as lysosomes."

Through precisely controlled chemical reactions, the mitochondria produce energy in the form of ATP and play a critical role in cellular homeostasis. Mitochondrial dysfunction is found in several disorders, including Parkinson's disease, cardiovascular disease, and mitochondrial diseases. Until now, peering into the inner metabolic workings of these vital organelles has been challenging at worst and inaccurate at best.

One conventional method of profiling mitochondrial metabolites involves purifying mitochondria using several rounds of centrifugation, a process that can take more than an hour to complete. According to Walter Chen, a graduate student in Whitehead Member David Sabatini's lab, time is a significant issue when studying metabolites.

"Even if you keep your sample at 4 degrees or 0 degrees Celsius to slow down any reactions, you're still gradually getting distortion of the mitochondrial metabolite profile because the enzymes are still going and so are the transporters," says Chen, who is also a third-year medical student at Massachusetts General Hospital. "As time goes on, the mitochondria are getting less happy outside the cell."

The other commonly used method for profiling mitochondrial metabolites relies on abbreviated forms of centrifugation to isolate mitochondria. Although faster, this protocol also brings down non-mitochondrial material and other organelles, thereby distorting the true mitochondrial signal with metabolites from extra-mitochondrial sources.

To reduce the time needed to isolate mitochondria and increase the accuracy of the metabolite analysis, Chen took a completely different tack— rapid immunopurification. He coated the exterior of mitochondria with epitope tags and added tiny beads covered in antibodies specific for the tags. By locking onto the tags, the antibodies link the mitochondria to the beads, allowing Chen to isolate the mitochondria easily, break them open, and stop all enzymatic activity within 10 minutes. According to his analysis, this quicker method yields results that better reflect the actual mitochondrial metabolite levels found within a living cell. Chen's work is described in the journal *Cell*.

"From the data we have so far, profiling mitochondria with this method definitely gives you greater resolution than what you would obtain using traditional methods to profile whole cells," says Chen, who is a co-author of the *Cell* paper. "On the in vivo front, I think this is going to be quite powerful, and that's what I'm most excited about. But I can already see that this can lead people in new and interesting directions."

Chen says the method is potentially very versatile and could be adapted to analyze the metabolite contents of other organelles and to compare

mitochondria in cells affected by mitochondrial dysfunction—such as neurons damaged by Parkinson's disease—with normal cells or other cell types seemingly unaffected by disease.

More information: Walter W. Chen et al, Absolute Quantification of Matrix Metabolites Reveals the Dynamics of Mitochondrial Metabolism, *Cell* (2016). DOI: 10.1016/j.cell.2016.07.040

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