PROTEOMICS

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An *in vivo* protein-labeling strategy could enable more comprehensive surveys of organelle proteomic contents.

Many proteins have a preferred address in a specific organelle, and the composition of these protein 'neighborhoods' can reveal valuable functional information. Unfortunately, strategies for selective organelle purification are often too crude or contamination-prone to yield a comprehensive census.

With a minimally disruptive technique for *in vivo*, region-specific protein labeling, Alice Ting of the Massachusetts Institute of Technology and her colleagues aim to simplify this process. Her team had previously devised an engineered variant of ascorbate peroxidase (APEX) that can be genetically targeted for expression in virtually any region of the cell.

She and her colleagues have now adapted APEX to selectively tag proteins that localize to the mitochondrial matrix in living cells. APEX processes phenol-containing compounds in a hydrogen peroxide–dependent manner to yield highly reactive products that covalently link to certain amino acids. These reactive products have a lifespan of less than a millisecond and label molecules only in their immediate proximity—typically a radius of under 20 nanometers. In principle, this should prevent protein labeling outside the region of interest.

As a demonstration, Ting and colleagues transfected human cells with a DNA construct encoding APEX with a short tag targeting the enzyme to the mitochondrial matrix. After treating these cells with hydrogen peroxide and phenol-biotin, they efficiently isolated the biotin-labeled proteins from the mitochondrial interior and then analyzed them by mass spectrometry.

They identified 464 known mitochondrial proteins as well as 31 that were not previously known to localize to this organelle. The method proved highly reproducible, consistently isolating 80%–90% of a reference set of known mitochondrial matrix proteins; absent proteins likely lacked suitable amino acids for labeling or were buried within complexes, the authors propose. Using microscopy, they verified the localization of a random subset of 5 of the 31 'mitochondrial orphans', demonstrating the usefulness of this method as a discovery tool. They also note that the submitochondrial localization of more than half of the matrix-specific proteins they identified was previously unknown. This method thus seems to offer an efficient means for exploring proteomic organization in the milieu of the living cell.

Michael Eisenstein

RESEARCH PAPERS

Rhee, H.-W. *et al.* Proteomic mapping of mitochondria in living cells via spatially-restricted enzymatic tagging. *Science* **339**, 1328–1331 (2013).