CHEMICAL BIOLOGY

Proteome labels à la carte

A method enables labeling and detection of newly synthesized proteins in an animal in a tissue- and time-specific manner.

Replacing natural amino acids with novel chemical moieties during synthesis of a protein can facilitate its labeling and detection, helping to address many fundamental biological questions. Such efforts may seem like a losing battle; protein synthesis is an essential mechanism not easily fooled into incorporating chemical entities that do not belong in the protein. Nonetheless, Jason Chin's group at the Medical Research Council Laboratory of Molecular Biology has been working to manipulate the cell's natural translational machinery to do just that—reprogram the genetic code to incorporate unnatural amino acids. Now, his laboratory has advanced a modular approach to tag newly synthesized proteins in cells and in an animal in a tissue-specific and temporally controlled way (Elliott *et al.*, 2014).

In the past, approaches to label the proteome have relied on the ability of endogenous aminoacyl-tRNA synthetases to introduce amino acid analogs bearing reactive chemical handles. But the rate at which cells incorporate these analogs is low, so these experiments are typically done under starvation conditions, which limits their physiological relevance. An alternative is to use genetic reprogramming methods that rely on orthogonal aminoacyl-tRNA synthetases.

In earlier work, Chin's group showed that unnatural amino acids could be incorporated site specifically into individual proteins of interest; for the latest work, to detect newly synthesized proteins, they wanted to put an unnatural amino acid at a low frequency throughout the proteome. To do this, they used an orthogonal synthetase-tRNA pair that can be modified to recognize a variety of unnatural amino acids but no natural amino acids. This synthetase-tRNA pair is not normally present in cells, so a promoter of choice can drive its expression and starvation conditions are unnecessary. As proteins are synthesized, the orthogonal system stochastically incorporates cyclopropene-modified amino acids, small chemical entities presenting a reactive chemical handle for labeling that is not bulky enough to disrupt protein structure when present at low incorporation rates.

Relying on what Chin calls "pretty amazing" chemistry—the inverse electron demand Diels-Alder reaction, which occurs nonenzymatically under physiological conditions and with high rate constants—the unnatural cyclopropene-modified amino acids can then be covalently labeled with tetrazine-based fluorescent probes.

With the technology ironed out in *Escherichia coli* and human cells, the researchers moved on to apply the tools to *Drosophila melanogaster*. Using a germline-specific driver, they expressed the orthogonal synthetase-tRNA pair during a specific stage in oogenesis in a subset of cells in the ovary. When the flies were fed their normal diet supplemented with the cyclopropene-modified amino acid, the newly synthesized proteome of the ovary cells was specifically labeled. The researchers used two-dimensional gel electrophoresis and mass spectrometry to identify proteins that are synthesized in those cells and at that developmental stage.

This approach has various applications, such as studying protein synthesis in long-term memory and lineage tracing during cell differentiation, says Chin. Other tools could be coupled to the proteome-wide approach as well. "One of the things we imagine as an extension of this is to put photo-cross-linkers into proteins in specific cells, at specific times," says Chin. "Then, when you shine light, you create covalent bonds between proteins that are in complexes." These tissue-specific protein interactions could then be resolved using downstream assays.

Chin's group recently advanced another method for manipulating the genetic code: they incorporated two distinct unnatural amino acids at specific sites in the protein calmodulin and then labeled them with different fluorescent probes (Wang *et al.*, 2014). Chin explains that with this method, they could "watch the protein change conformation as it binds each of four calcium ions sequentially."

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RESEARCH PAPERS

Elliott, T.S. *et al.* Proteome labeling and protein identification in specific tissues and at specific developmental stages in an animal. *Nat. Biotechnol.* **32**, 465–472 (2014).

Wang, K. *et al.* Optimized orthogonal translation of unnatural amino acids enables spontaneous protein double-labelling and FRET. *Nat. Chem.* **6**, 393–403 (2014).