Tools For Spatiotemporally Specific Proteomic Analysis In Multicellular Organisms

by

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To Dave and Paul

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Abstract

The emergence of mass spectrometry-based proteomics has revolutionized the study of proteins and their abundances, functions, interactions, and modifications. However, in a multicellular organism, it is difficult to monitor dynamic changes in protein synthesis in a specific cell type within its native environment. In this thesis, we describe methods that enable the metabolic labeling, purification, and analysis of proteins in specific cell types and during defined periods in live animals. We first engineered an eukaryotic phenylalanyl-tRNA synthetase (PheRS) to selectively recognize the unnatural L-phenylalanine analog *p*-azido-L-phenylalanine (Azf). Using *Caenorhabditis elegans*, we expressed the engineered PheRS in a cell type of choice (i.e. body wall muscles, intestinal epithelial cells, neurons, pharyngeal muscles), permitting proteins in those cells – and only those cells – to be labeled with azides. Labeled proteins are therefore subject to "click" conjugation to cyclooctyne-functionalized affinity probes, separation from the rest of the protein pool and identification by mass spectrometry. By coupling our methodology with heavy isotopic labeling, we successfully identified proteins – including proteins with previously unknown expression patterns – expressed in targeted subsets of cells. While cell types like body wall or pharyngeal muscles can be targeted with a single promoter, many cells cannot; spatiotemporal selectivity typically results from the combinatorial action of multiple regulators. To enhance spatiotemporal selectivity, we next developed a two-component system to drive overlapping – but not identical – patterns of expression of engineered PheRS, restricting labeling to cells that express both elements. Specifically, we developed a split-intein-based split-PheRS system for highly efficient PheRS-reconstitution through protein splicing. Together, these tools represent a powerful approach for unbiased discovery of proteins uniquely expressed in a subset of cells at specific developmental stages.

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