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Chemical proteomics for subcellular proteome analysis

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Protein functions are tightly regulated by their subcellular localization and dynamic alteration. Chemical proteomics offers convenience and efficiency for profiling protein features in a native context. In this review, we summarize the recent progress of subcellular-compartment-focused chemical proteomics which do not rely on organelle fractionation. Organelle-specific activity-based protein profiling (ABPP) and engineered ascorbate peroxidase (APEX) have been developed for proteome analysis within organelles and even sub-organelles. In parallel, our lab designed organelle-localizable reactive molecules (ORMs) to selectively tag nuclear and mitochondrial proteins. ORMs-based proteomics is applicable to primary neurons and brain slices, as well as cultured cell lines. In addition, we invented a conditional proteomics approach to elucidate zinc homeostasis by labeling and identifying proteins localized in Zn^{2+}-rich space of live cells.

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Introduction

Protein functions are closely associated with its subcellular distribution in live cells. Each subcellular compartment, or organelle, contains varied protein compositions that underlie the diversity of biochemical reactions in a single cell. Protein localization and expression level are dynamically varied in response to environmental changes. The spatiotemporal changes of proteomes reflect the biological states of resided organelles. Organelle-focused proteomics relying on subcellular fractionation have been used to identify the components of cellular organelles [1–3]. However, the conventional methods [1] often suffer from the limited specificity and the low coverage. Moreover, they can neither accurately report temporal dynamics of proteins, because of the time-consuming biochemical purification processes, nor readily access the sub-organelle proteomes.

Chemical proteomics is now a powerful strategy for the focused protein profiling [4–6]. The proteome of interest is covalently tagged with chemical reagents in live cells, followed by standard enrichment and mass spectrometry (MS) analysis, which allows for fixing protein information before cell lysis and, thus is able to obtain a snapshot of dynamically altered subcellular proteomes that cannot be addressed by the organelle fractionation. Organelle-focused chemical proteomics exploits spatially limited reactions by directing labeling reagents or enzymes to specific subcellular compartments. These may provide organelle and even sub-organelle proteome mapping with high spatiotemporal resolutions.

In addition to subcellular localization, cellular microenvironments, such as pH, concentrations of metals, hypoxia/hyperoxia and redox states, tightly regulate local protein structures and activities. Such environmental conditions are spatially heterogeneous and dynamically fluctuated in live cells and tissues. Useful methods to precisely address the local proteomes are enormously desirable for comprehensive elucidation of proteome dynamics. We firstly proposed a strategy termed 'conditional proteomics' [7••] as a powerful approach to selectively label and identify the conditional proteomes and profile their dynamics.

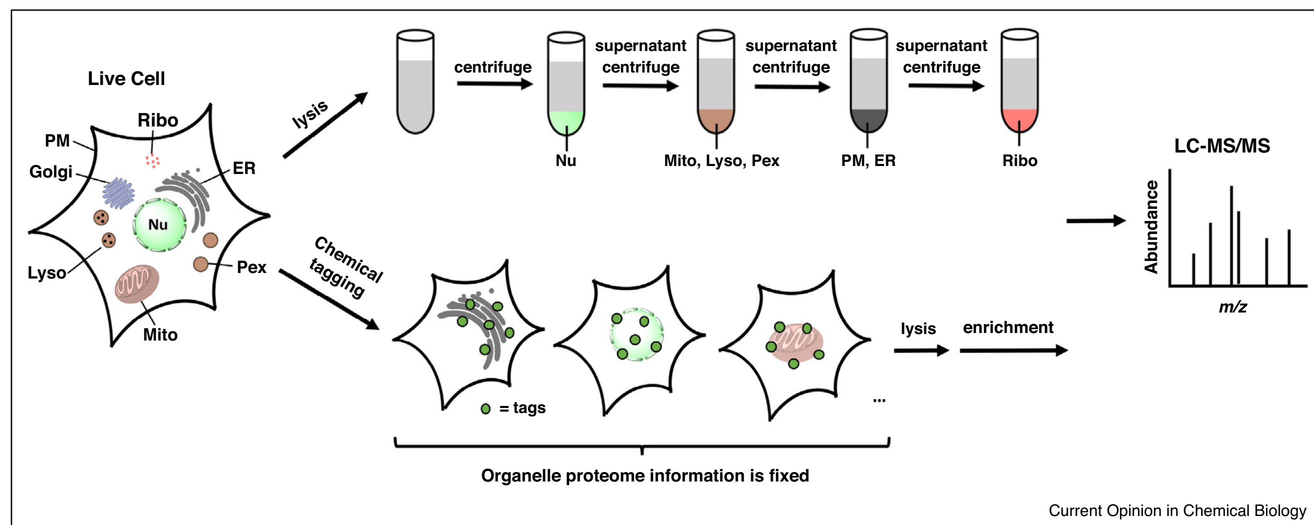
In this review, we introduce the recent progress of chemical proteomics that focus on subcellular compartments, including organelle-focused proteomics and conditional proteomics (Figure 1).

Organelle-focused proteomics

Organelle-specific ABPP

Activity-based protein profiling (ABPP) represents one of the most successful examples of chemical proteomics, which was invented by Cravatt's group [8,9]. Proteins exhibiting an enzymatic activity-of-interest are selectively labeled by activity-based probes (ABPs) through a bioorthogonal reaction. For organelle-specific ABPP, Wright and co-workers developed a lysosome-targeting ABP by conjugating a weakly basic amine (DAMP) to a cathepsin-reactive warhead [10•]. Liquid chromatography (LC)–MS/MS analysis of labeled macrophages showed an increased enzyme activity of cathepsins B and Z during starvation-induced autophagy.

Figure 1



Organelle-focused proteomics workflow. Top: conventional methods isolate cell organelles by subcellular fractionation after cell lysis. Bottom: chemical proteomics can fix organelle proteome information by chemical tagging before cell lysis. *Abbreviations*: Nu, nucleus; Mito, mitochondria; Lyso, lysosomes; Pex, peroxisomes; PM, plasma membrane; ER, endoplasmic reticulum; Ribo, ribosomes.

Proximity proteomics

In the past few years, several groups have independently developed a class of methods termed 'proximity labeling' for protein network mapping [11–14]. The basic strategy exploits engineered peroxidase or biotin ligase to generate highly reactive and short-lived reagents that covalently tag proteins with biotin in the vicinity (within nanometers) of the enzyme. For organelle-focused proteomics, the enzyme is directed to specific cellular compartments, using genetically encodable organelle localization signals. Ting and co-workers explored the engineered ascorbate peroxidase (APEX or APEX2) in proteome profiling of various organelles and even organelle subcompartments, such as sub-mitochondrial spaces, ER lumen and membranes [15•,16–19]. It now turns out that APEX-based organelle proteomics is powerful to uncover novel components of organelles and determine the subcellular localization of unknown proteins. Recently, the same group used the engineered horseradish peroxidase in live neurons and characterized the proteomes of excitatory and inhibitory synaptic clefts [20], many of which are membrane-unbounded and biochemically unpurifiable. The resultant proteome lists are highly specific to synaptic clefts with high coverages, where new synaptic proteins were discovered and subtype localizations of some of synaptic proteins were revealed. In addition, APEX-based organelle proteomics can serve as spatially specific references, for example, endosome and plasma membrane, to reveal protein interaction networks that necessitate higher spatial resolution [21] (Figure 2).

Organelle-localizable reactive molecules

In parallel, we developed a new method for organelle-focused proteomics that does not require genetic manipulation and is not limited to active enzymes as targets. The organelle-localizable reactive molecules (ORMs) composed of an organelle-localizing moiety and a chemically reactive moiety were designed in this technology. ORMs can penetrate biomembranes and spontaneously accumulate in target organelles of live cells, where the protein labeling is facilitated by the condensation effect [22•,23••]. In the case of mitochondria-localizable reactive molecules (MRMs), for example, the concentrations of inside cells are 4 to 21-fold higher than those extracellularly added [23••] (Figure 3).

For nucleus-focused proteomics, nucleus-localizable reactive molecules (NRMs) were firstly prepared [22•], in which the Hoechst acts as a molecular vehicle to the nucleus [24,25] and chloroacetyl is employed as a reactive handle that shows potential reactivity to cysteine. Incubation of HeLa cells with NRMs allowed for their selective localization in the cellular nucleus as observed by CLSM imaging, and plenty of labeled proteins were detected by SDS–PAGE and Western blotting (WB) analysis. The labeled proteins were enriched by immunoprecipitation using anti-Hoechst antibody, and characterized by mass proteomics. 67 proteins were identified in total, among which 58 proteins (87%) were assigned as nucleus-localized proteins. This first report successfully demonstrated the feasibility of our strategy for organelle-focused proteomics by ORMs.

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