PROTEOMICS

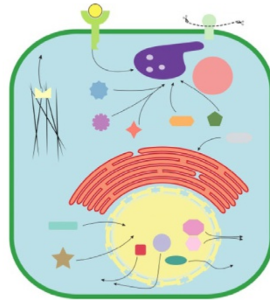
Tracking the proteome

A combination of quantitative mass spectrometry, subcellular fractionation and stringent statistical analyses allows the description of protein translocation events at the proteome scale.

Eukaryotic cells are characterized by a high degree of compartmentalization, and trafficking between specific subcellular compartments often serves to regulate the biological functions of proteins. Whereas the localization of specific proteins is routinely determined with microscopy-based methods, a true understanding of proteome dynamics requires the ability to simultaneously establish the subcellular locations of a large number of proteins.

With the goal of enabling a proteome-level view of subcellular dynamics, a group led by Georg Borner of the Max Plank Institute of Biochemistry in Martinsried recently reported the development of a method they call Dynamic Organellar Maps (Itzhak *et al.*, 2016). In this approach, cells are first metabolically labeled for downstream quantitation, then lysed under mild conditions before organelles and the nuclear and cytosolic fractions are separated by differential centrifugation. A reference fraction labeled with heavy isotope is then mixed with light-isotope-labeled samples from each organelle-containing fraction to generate SILAC ratios by mass spectrometry. Label-free quantitation is also carried out in parallel for the nuclear, cytosolic and combined organellar fractions. After data acquisition, Itzhak *et al.* used principal-component analysis to cluster the data, which revealed the localization of each identified protein through overlay with known organellar markers. Precise assignment to organellar clusters was further aided by a support vector machine supervised learning approach. In this basic iteration of Organellar Maps, the authors obtained quantitative cellular localization data for close to 9,000 HeLa cell proteins, with highly reproducible organellar profiles for more than 5,000.

To demonstrate the ability of this approach to track proteome-wide translocation events, Itzhak *et al.* analyzed the spatial proteome of HeLa cells after treatment with epidermal growth factor (EGF). In addition to the expected translocation of EGFR from the plasma membrane to the endosome, they could document several other protein



Dynamic Organellar Maps enables quantitative mapping of subcellular translocation events. Adapted from Itzhak *et al.* (2016) (https://creativecommons.org/licenses/by/4.0/legalcode).

translocation events with confidence. Combined with estimates of protein abundances, these results enabled Borner's group to obtain a quantitative description of global protein movement in the cell after EGF exposure.

A testament to the long-standing interest in tackling the spatial proteome, the approach used by Borner's team is not the first to be implemented in this area. Improving on their LOPIT (localization of organelle proteins by isotope tagging) method, a group led by Kathryn Lilley at the University of Cambridge recently reported a spatial map of the mouse pluripotent stem cell proteome (Christoforou *et al.*, 2016). LOPIT also relies on a fractionation step but uses tandem mass tagging after cell lysis for multiplex labeling. With their approach, Lilley's team was able to pinpoint the localization of more than 5,000 proteins with high spatial resolution. A direct comparison of the localization data obtained by the two groups showed an exceptionally high degree of agreement in predicted localization, with 92% overlap across different species and cell types.

Because proteome-wide approaches such as Dynamic Organellar Maps and LOPIT do not require a priori knowledge of the key players involved in the cellular process being investigated, they should allow deep system-level interrogations that are likely to significantly affect how cell biology is approached.

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RESEARCH PAPERS

Itzhak, D.N., Tyanova, S., Cox, J. & Borner, G.H.H. Global, quantitative and dynamic mapping of protein subcellular localization. *eLife* **5**, e16950 (2016).

Christoforou, A. *et al.* A draft map of the mouse pluripotent stem cell spatial proteome. *Nat. Commun.* **7**, 9992 (2016).

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