PROTEOMICS

Cartography of an organelle

Peptide counting in mass spectrometry allows researchers to draw a quantitative proteomic map of the ER and Golgi.

The characterization of organelles—their structure, function and composition—dates back to the dawn of cell biology, and subcellular fractionation has been an inextricable part of this process. Indeed, the discovery in 1955 of the lysosome by Christian de Duve and colleagues came out of studies on the subcellular distribution of enzyme activity. Today, coupled with the sophisticated tools of quantitative proteomics, this effort carries on with renewed fervor. "As cell biologists, we've got to know the identities of the organelles we study. We've got to put it all together," says John Bergeron of McGill University, one principal author of a recent paper describing the use of tandem mass spectrometry (LC-MS/MS) to generate a proteomic map of the rough and smooth endoplasmic reticulum (ER) and the Golgi apparatus.

In a collaborative effort, researchers on both sides of the Atlantic used a method known as redundant peptide counting to determine the relative abundance of proteins in highly purified organelle preparations. Peptide counting is based on the simple idea that, when normalized for protein size, an abundant protein within a sample will generate more peptides than a relatively rare one. "It isn't in principle so different from the sort of thing I'm more used to doing," says Bergeron, "which is to quantify gold particles bound to antibodies in an electron micrograph and estimate relative levels that way". They validated the method for the complex ER-Golgi system by comparing their results to data from quantitative western blotting and standard enzyme assays. Moreover, data collection that approached heroic proportions and took "multiple biological replicates and more than 5,000 hours of mass spec time," according to Bergeron, countered the underrepresentation of low-abundance proteins that arises as an inherent consequence of random peptide sampling in LC-MS/MS.

The researchers made measurements of relative abundance for established organellar markers to estimate cross-contamination between samples, a notorious problem in studies involving subcellular fractionation. Using their quantitative estimates as benchmarks, the authors predicted that ERp44, thought to be ER-localized, is in fact a predominantly Golgi-localized protein. They validated this by immunofluorescence staining (**Fig. 1**)—one instance, as Bergeron emphasizes, "[of how] quantitative proteomics is becoming a predictive tool, not just one for generating a catalog of proteins." In fact, of the 345 previously unidentified proteins assigned to the ER-Golgi proteome in this work, the subcellular location of 280 was predicted and will undoubtedly be the basis of many further experiments.

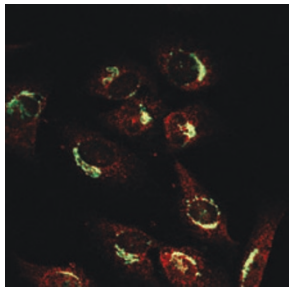

Figure 1 | Indirect immunofluorescence of ERp44 shows dual Golgi and ER localization based on colocalization with the Golgi marker GM130. Reprinted with permission from Elsevier.

Bergeron and colleagues assigned the proteins to one of 23 different functional categories; they observed that proteins coclustered in defined categories across smooth and rough ER as well as Golgi. As organelles are to some extent functional units, this was not unexpected. More remarkably, hierarchical cluster analysis of suborganellar ER fractions revealed that 77% of all assigned peptides were present in six clusters, each representing a distinct functional category. In the Golgi, secreted cargo was enriched in the cisternae, whereas resident Golgi proteins were predominantly in COP1-coated vesicles, which provides support for the cisternal maturation model of biosynthetic protein transport.

With this extension into the suborganellar realm, quantitative proteomics seems poised to serve as a form of 'biochemical imaging', furthering the cell biologist's half-century quest for a complete and dynamic understanding of the organelle.

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RESEARCH PAPERS

Gilchrist, A. *et al.* Quantitative proteomics analysis of the secretory pathway. *Cell* **127**, 1265–1281 (2006).