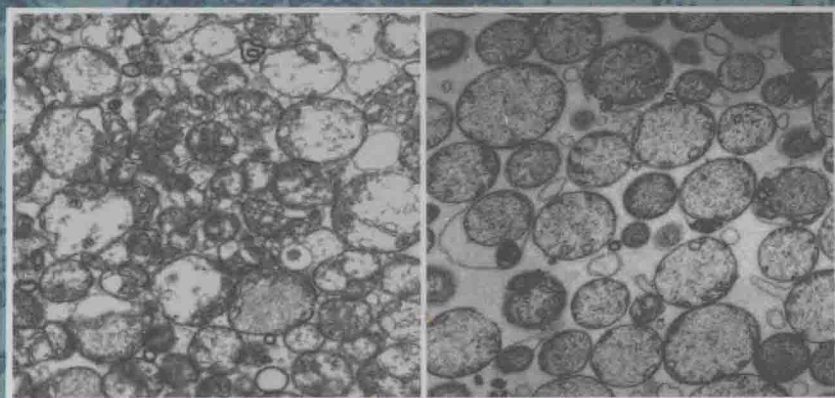


Organelle Proteomics

Edited by

Delphine Pflieger
Jean Rossier



METHODS IN MOLECULAR BIOLOGY™

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Preface

Human genome sequencing has identified about 25,000 genes, most being of unknown function. Localization of the final gene products—that is, the proteins—in a specific organelle is a key to deciphering the proteins' roles within the cell. Over the past 20 years, proteomic analyses have progressively proved to be an invaluable tool to obtain high-throughput protein identification from low-abundance, complex biological samples. These analyses boomed thanks to dramatic technological progresses in mass spectrometry instrumentation, optimization of its coupling to capillary liquid chromatography, and the development of software enabling processing of the vast amount of generated data. In the context of organelle study, such analyses have allowed greater depth in the characterization of the proteins constitutive of, or transiently present in, these large functional modules. For example, in 2002, we published the analysis of a total yeast mitochondrial protein extract by the coupling between capillary liquid chromatography and tandem mass spectrometry, known as liquid chromatography-tandem mass spectrometry (LC-MS/MS) (1). We were then able to identify 179 proteins (<http://mitochondria.cgm.cnrs-gif.fr/>) out of about 500 expected mitochondrial constituents. Among these, 132 were already recorded as mitochondrial in the Yeast Protein Database, YPD (2), 28 were described to be of unknown localization and function, and 19 were described to belong to other subcellular compartments. Among the 28 identified proteins that were uncharacterized in early 2002, eight were further functionally studied by other groups and demonstrated to play a role in mitochondria. For example, Ykr065cp was shown to be involved in the import of mitochondrial matrix proteins (3), Ylr201cp in ubiquinone biosynthesis (4), and Ynl177cp was proved to be a mitochondrial ribosomal protein (5). Another 17 were identified in more recent proteomic analyses of yeast mitochondria (such as reference [6]) confirming our results. Little to no data are currently available in yeast databases to support mitochondrial localization of the three remaining proteins. This study, among many others, illustrates that proteomic analysis of a carefully prepared organelle sample reliably reveals new proteins constitutive of the cellular compartment and paves the way for their detailed functional characterization.

Cell proteomics is faced with the extraordinary chemical diversity of expressed proteins and the very large dynamic range of their cellular concentrations. In any given human cell, the most abundant protein is usually actin, present at above 10^8 molecules per cell, whereas cellular receptors, signaling

proteins, and transcription factors may exist at a few hundreds of copies or even less. Yet, the understanding of the diverse cellular processes requires the study of the expression of those proteins—needles in the cellular haystack. To achieve that goal, it is indispensable to enrich such target proteins within a subcellular sample of reduced complexity. To characterize proteins of minor abundance in an organelle, this subcellular compartment can be divided into fractions, such as soluble and insoluble membrane proteins. In one last step of fractionation, the proteomic analysis can be focused on a given protein machinery, such as a single complex. *Organelle Proteomics* focuses on these three levels of subcellular organization by describing the preparation of samples and their proteomic analysis.

This book starts with a chapter by Dr. Edwin Romijn and Prof. John R. Yates III, who introduce the different analytical strategies developed and successfully utilized to study organelle proteomes and detail the use of multidimensional liquid chromatography coupled to tandem mass spectrometry for peptide sample analysis. This book is further composed of two main sections. First, detailed protocols are provided to perform the purification of the various organelles present in eukaryotic cells, as well as to prepare certain subfractions of organelles (Chapters 2–22). In all cases, the samples are aimed to be analyzed by a mass spectrometry technique. Although an exhaustive list of chapters covering all the proteomic analyses of organelles and organelle fractions was not conceivable, we nevertheless wanted to provide analysis examples reflecting the trend toward more specific purifications of organelle subfractions, which will allow reaching the more comprehensive and accurate characterization of the organelle. Most of the chapters cover the whole analytical procedure of organelle characterization, from its purification starting with whole cells up to protein identification using mass spectrometry. In some cases, the chapter may provide a detailed description of the purification process wherein less classical techniques appear, which are implemented by a minority of laboratories (e.g., free flow electrophoresis). Second, however optimized the organelle purification protocol—and skilled the operator—the sample of interest will never consist of the pure targeted organelle. Therefore, among the proteins identified, one has to separate the true from the intruders. The actual subcellular localization of some individual proteins newly attributed to the studied organelle can be evaluated by orthogonal assays, such as microscopy, by expressing the GFP-tagged version of the protein candidates. Yet, this approach is labor-intensive and is usually restricted to a few selected proteins. We devoted the second section of this book to methods enabling a global estimate of the reliability of the protein list assigned to an organelle. An average ratio of proteins wrongly attributed to the organelle of interest is provided by assessing sample purity (Chapter 23). To determine whether every identified protein is

an actual component of the purified organelle, quantitative mass spectrometry methods can be employed (Chapters 24–26). In Chapter 26, Dr. Wei Yan et al. more specifically demonstrate the utility of quantitative approaches to scrutinize protein shuttling between organelles. The examples of quantitative mass spectrometry analysis of organelle fractions presented use a few commercially available isotope-tagged reagents, but many other chemicals, either commercial or prepared in-house, can be utilized. A larger variety of the existing polypeptide-labeling strategies can be found in another volume of this series entitled *Quantitative Proteomics*, edited by Dr. Salvatore Sechi. Finally, the last chapter of this book, by Dr. Wallace F. Marshall, addresses the use of transcriptomic data to identify genes potentially encoding organelle proteomes.

One should keep in mind that some, if not the majority, of peptide sequences assigned by software tools to raw mass spectra must be rejected. The degree of false-positive protein identification can be estimated by statistical interpretation of mass spectrometry results. This aspect, while of greatest importance to generate interlaboratory databases of proteins organized by localization and function, is beyond the scope of this book. It is dealt with in an alternative volume of this series, *Mass Spectrometry Data Analysis in Proteomics*, edited by Dr. Rune Matthiesen.

In terms of chapter format, each chapter begins by introducing the protocol to be described, with its goals and possible advantages over other techniques. In the Materials section, all the equipment and reagents necessary for performing the protocol are listed. The Methods section details the different steps of the protocol while the Notes collect remarks, tricks, and troubleshooting that are likely to help dealing with difficulties that might be encountered during the protocol.

Delphine Pflieger
Jean Rossier

References

1. Pflieger, D., Le Caer, J. P., Lemaire, C., Bernard, B. A., Dujardin, G., Rossier, J. (2002) Systematic identification of mitochondrial proteins by LC-MS/MS. *Anal. Chem.* **74**, 2400–2406.
2. Hodges, P. E., Payne, W. E., Garrels, J. I. (1997) The Yeast Protein Database (YPD): a curated proteome database for *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **26**, 68–72.
3. van der Laan M., Chacinska, A., Lind, M., Perschil, I., Sickmann, A., Meyer, H., et al. (2005) Pam17 is required for architecture and translocation activity of the mitochondrial protein import motor. *Mol. Cell. Biol.* **25**, 7449–7458.

4. Johnson, A., Gin, P., Marbois, B. N., Hsieh, E. J., Wu, M., Barros, M. H., et al. (2005) COQ9, a new gene required for the biosynthesis of coenzyme Q in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **280**, 31397–31404.
5. Gan X., Kitakawa, M., Yoshino, K., Oshiro, N., Yonezawa, K., Isono, K. (2002) Tag-mediated isolation of yeast mitochondrial ribosome and mass spectrometric identification of its new components. *Eur. J. Biochem.* **269**, 5203–5214.
6. Sickmann, A., Reinders, J., Wagner, Y., Joppich, C., Zahedi, R., Meyer, H. E., et al. (2003) The proteome of *Saccharomyces cerevisiae* mitochondria. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 13207–13212.

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