Methods in ENZYMOLOGY

Volume 452

Autophagy in Mammalian Systems, Part B

Edited by

Daniel J. Klionsky

VOLUME FOUR HUNDRED AND FIFTY-TWO

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PREFACE

Research into the topic of autophagy started in the late 1950s. At that time, and for the following several decades, there were few methods available for studying this process. The initial methodology relied primarily on electron microscopy, sometimes coupled with subcellular fractionation, and electron microscopy remains one of the principal methods of analysis. Additional techniques were eventually added, which included sequestration and protein degradation assays, and all of these are described in this volume of *Methods in Enzymology*. Overall, however, the methods for examining autophagy in mammalian cells have been relatively limited.

In the 1990s, the autophagy-related (*ATG*) genes were identified in various fungi, which in part opened a new era, allowing an understanding of the molecular mechanism of autophagy. In addition, the identification of homologues to the fungal genes in higher eukaryotes provided evidence for the role of autophagy in a growing number of processes, in pathophysiology and also in development.

Although there is tremendous conservation among the autophagy-related genes across species, the relative difficulty of using molecular genetic approaches in mammalian cell culture and, in some cases, the need to monitor autophagy in intact organisms or tissue samples, has limited the development of techniques for assessing autophagy in mammalian systems. By far, the most versatile methodology among the molecular approaches relies on detection of the Atg8 homologue microtubule-associated protein 1 light chain 3, or LC3. The primary reasons for the utility of this protein are that it is often upregulated following autophagy induction, it undergoes posttranslational modifications that can be used to monitor certain aspects of autophagy, and it is presently the only Atg protein that is reliably associated with the completed autophagosome membrane. The LC3 protein can be followed by western blot, fluorescence and immunoelectron microscopy. Accordingly, many of the chapters in this volume describe different ways to use LC3 for following autophagy, and one of the main benefits of these chapters is that they describe specific variations that are applicable to particular systems and/or questions.

Despite the overall robustness of LC3-dependent assays, the entrance of many researchers into this field has also led to the development of new techniques, as well as modifications of earlier approaches. Accordingly, the chapters in this volume describe the use of dyes including DQ-BSA that can be used to monitor amphisome fusion with the lysosome, alternative

sequestration substrates such as GST-BHMT, the analysis of p62/SQSTM1 that links LC3 with ubiquitin, the use of WIPI-1/Atg18 as a phagophore marker, and methods to monitor selective peroxisome and mitochondrial degradation.

Finally, the chapters in this volume are concerned not just with methodology, but also provide the background that allows the reader to appreciate the importance of monitoring autophagy with regard to the particular questions being asked. This second volume concludes with several chapters that are focused on the analysis of autophagy in connection with microbial pathogenesis and the immune response, as well as autophagy in tissues and intact organisms; these chapters set the stage for the third volume that will be devoted to connections with disease and clinical applications. Indeed, there is growing interest in manipulating autophagy for therapeutic purposes. One hope is that this and the companion volumes of *Methods in Enzymology* will stimulate researchers to pursue ongoing and new lines of investigation into autophagy so that we may continue to understand, and ultimately manipulate to our advantage, this complex and ubiquitous process.

DANIEL J. KLIONSKY

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