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Autophagy

Methods and Protocols

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Preface

The process of self-eating as a way to survive periods of starvation has been known for over 150 years, and this response was from the beginning designated as "autophagy." Following early progress in establishing the morphological and some biochemical parameters of the autophagic response in the 1950s and 1960s, the field of autophagy was transformed when Yoshinori Ohsumi and others applied a genetic approach to isolate a near complete set of autophagy genes in yeast, which allowed the characterization of equivalent genes and processes in other organisms including mammals.

We now know that autophagy is a highly conserved catabolic and quality control pathway across all eukaryotes. Its dysregulation can underpin disease and developmental problems, frequently in ways not immediately predictable. Despite tremendous progress in researching the autophagic response in a variety of experimental settings, much remains to be discovered, from the basic mechanisms to the physiological functions.

This volume hopes to be a comprehensive and extensive set of protocols for the study of autophagy in vitro and in vivo, primarily in mammals but also in various model organisms including yeast, Drosophila, and *C. elegans*. Protocols are also presented for the study of autophagy-related processes outside of the canonical autophagy pathways.

We first present 11 chapters describing protocols for studying autophagy in vitro, i.e., using pure or semi-pure components; these studies have contributed a great deal toward understanding autophagy in molecular terms, and it is likely that they will continue to do so in the future. The next 8 chapters describe protocols for the study of autophagy (canonical and noncanonical) by imaging methods, either using light microscopy or EM. Being able to follow the formation of autophagosomes and to study their unique architecture has traditionally been a productive area of research, and it is likely that novel insights from such studies will continue to be derived in the future. In the last few years, much effort has been devoted to the establishment of assays and screening platforms for large-scale discovery efforts to identify genes and chemicals affecting autophagy; here we have included 10 chapters describing a set of up-to-date protocols for such studies. The next 9 chapters deal with the study of autophagy not in tissue culture but in whole organisms. We believe that work along those lines is likely to come to dominate the autophagy field in the future, and it is therefore very useful to have a set of protocols mapping out these useful approaches. The final 10 chapters describe assays and techniques for studying selective autophagy, i.e., the induction of autophagy for the elimination of specific cargoes. Although autophagy is sometimes solely considered a starvation response, it can be argued that its function as a specific eliminator of unwanted substances is a major physiological function and therefore likely to continue to be studied intensely.

We wish to thank all authors who took time off from their own work in order to provide the protocols included in this book.

Cambridge, UK

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