

METHODS IN MOLECULAR BIOLOGY

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Plant Endosomes

Methods and Protocols

Edited by

Marisa S. Otegui

Departments of Botany and Genetics, University of Wisconsin-Madison, Madison, WI, USA

 **Humana Press**

Editor

Marisa S. Otegui
Departments of Botany and Genetics
University of Wisconsin-Madison
Madison, WI, USA

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Preface

The composition of the plasma membrane is tightly controlled by cells through vesicular trafficking. Cells can internalize plasma membrane transporters, enzymes, receptors, and other key signaling molecules through the formation of vesicles in a process called endocytosis. Endocytosed material is delivered to early endosomes where it can be recycled back to the plasma membrane or be further sorted into endosomal intraluminal vesicles for degradation in vacuoles/lysosomes. In plant cells, the *Trans* Golgi Network (TGN) and TGN-derived compartments have been shown to act as early endosomes whereas multivesicular bodies (MVBs) sort proteins for degradation. Both TGN and MVBs also traffic cargo material that has been synthesized in the endoplasmic reticulum and Golgi and is destined to the vacuole. In recent years, many significant contributions have dramatically changed our understanding of the plant endosomal system. The identification of key components in the regulation of plasma membrane endocytosis, recycling, and degradation supports both the unique and conserved aspects of the plant vesicular trafficking machinery. In addition, plant endocytic and endosomal trafficking plays a central role in mediating responses to biotic and abiotic stimuli and in general plant development and cell differentiation. However, the analysis of plant endosomal trafficking pathways can be difficult and entails several challenges: (1) The endosomal system comprises a dynamic set of organelles that are in continuous flux. (2) Not all plasma membrane cargoes are trafficked equally. (3) Posttranslational modifications regulate the fate of plasma membrane proteins. (4) Endosomes act as platforms for the traffic of cargo from different pathways. (5) Key endosomal subdomains, such as intraluminal vesicles of MVBs, are too small to be visualized directly by light microscopy techniques.

This book contains a collection of protocols and techniques to analyze *in vivo* trafficking of endocytic/endosomal cargo, including lipids, fluids, proteins, and ligands, ultrastructural features of endosomes by high-pressure freezing/freeze-substitution and electron tomography, protein-protein interactions in the endosomal and endomembrane system, sorting defects in the transport of vacuolar storage proteins, function conservation of plant endosomal proteins, endosomal trafficking during plant responses to pathogens, protein composition of endosomes and endocytic vesicles, ubiquitination of endosomal cargo proteins, and identification of novel endosomal components by chemical genomics and proteomics. I hope that these contributions from many leading and emerging plant membrane trafficking researchers from all over the world will promote and facilitate novel studies and ideas in this field. Finally, I would like to thank all the authors and colleagues that have contributed chapters and ideas to this book and the National Science Foundation for supporting research on endosomal trafficking in my laboratory.

Madison, WI, USA

Marisa S. Otegui

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Contributors

- BEN AUGUST • *Electron Microscope Facility, School of Public Health, University of Wisconsin-Madison, Madison, WI, USA*
- VERA BANDMANN • *Plant Cell Biology, Department of Biology, INM-Leibniz-Institute for New Materials, Germany*
- SEBASTIAN Y. BEDNAREK • *Department of Biochemistry, University of Wisconsin-Madison, Madison, WI, USA*
- STANLEY W. BOTCHWAY • *Rutherford Appleton Laboratory, Central Laser Facility, Research Complex at Harwell, Science and Technology Facilities Council, Didcot, UK*
- YOHANN BOUTTÉ • *Membrane Biogenesis Laboratory, UMR 5200, CNRS-Université Bordeaux Segalen, INRA Bordeaux Aquitaine, Villenave d'Ornon Cédex, France*
- YI CAI • *School of Life Sciences, Centre for Cell and Developmental Biology and State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, China*
- ALEXANDRA CHANOCA • *Department of Botany, University of Wisconsin, Madison, WI, USA*
- GEORGIA DRAKAKAKI • *Department of Plant Sciences, University of California Davis, CA, USA*
- TORU FUJIWARA • *Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan*
- CAIJI GAO • *School of Life Sciences, Centre for Cell and Developmental Biology and State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, China; CUHK Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen, China*
- MARKUS GREBE • *Department of Plant Physiology, Umeå Plant Science Centre (UPSC), Umeå University, Umeå, Sweden; Institut für Biochemie und Biologie, Pflanzenphysiologie, Universität Potsdam, Potsdam-Golm, Germany*
- IKUKO HARA-NISHIMURA • *Department of Botany, Graduate School of Science, Kyoto University, Kyoto, Japan*
- PETER HAUB • *DIPsystems.de, Altlußheim, Germany*
- PING HE • *Department of Biochemistry and Biophysics, Institute for Plant Genomics and Biotechnology, Texas A&M University, College Station, TX, USA*
- ANTJE HEESE • *Division of Biochemistry, Interdisciplinary Plant Group (IPG), University of Missouri-Columbia, Columbia, MO, USA*
- GLENN R. HICKS • *Department of Botany and Plant Sciences, Center for Plant Cell Biology, University of California, Riverside, CA, USA*
- NILOUFER G. IRANI • *Department of Plant Systems Biology, VIB, Ghent, Belgium; Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, Belgium; Department of Plant Sciences, University of Oxford, Oxford, UK*
- ERIKA ISONO • *Department of Plant Systems Biology, Technische Universität München, Freising, Germany*
- EMI ITO • *Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan*
- ADRIANA JELÍNKOVÁ • *Institute of Experimental Botany, ASCR, Praha, Czech Republic*

- LIWEN JIANG • *School of Life Sciences, Centre for Cell and Developmental Biology and State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, China; CUHK Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen, China*
- KAMILA KALINOWSKA • *Department of Plant Systems Biology, Technische Universität München, Freising, Germany*
- KOJI KASAI • *Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan*
- YASUKO KOUMOTO • *Department of Botany, Graduate School of Science, Kyoto University, Kyoto, Japan*
- JOHANNES LEITNER • *Department of Applied Genetics and Cell Biology, BOKU, University of Natural Resources and Life Sciences, Wien, Austria*
- MICHELLE E. LESLIE • *Division of Biochemistry, Interdisciplinary Plant Group (IPG), University of Missouri-Columbia, Columbia, MO, USA*
- IRENE LICHTSCHEIDL • *Core Facility of Cell Imaging and Ultrastructure Research, University of Vienna, Vienna, Austria*
- CHRISTIAN LUSCHNIG • *Department of Applied Genetics and Cell Biology, BOKU, University of Natural Resources and Life Sciences, Wien, Austria*
- KATEŘINA MALÍNSKÁ • *Institute of Experimental Botany, ASCR, Praha, Czech Republic*
- TOBIAS MECKEL • *Membrane Dynamics, Department of Biology, Technische Universität, Darmstadt, Germany*
- LORENA NORAMBUENA • *Plant Molecular Biology Laboratory, Department of Biology, Faculty of Sciences, University of Chile, Santiago, Chile*
- MARISA S. OTEGUI • *Departments of Botany and Genetics, University of Wisconsin, Madison, WI, USA*
- MIROSLAV OVEČKA • *Department of Cell Biology, Faculty of Science, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University Olomouc, Olomouc, Czech Republic*
- EUNSOOK PARK • *Department of Plant Sciences, University of California Davis, CA, USA*
- TIBOR PECHAN • *Institute for Genomics, Biocomputing and Biotechnology, Mississippi State University, Mississippi State, MS, USA*
- JAN PETRÁŠEK • *Institute of Experimental Botany, ASCR, Praha, Czech Republic*
- FRANCISCA C. REYES • *Centro de Biotecnología Vegetal, Facultad Ciencias Biológicas, Universidad Andrés Bello, Santiago, Chile*
- GREGORY D. REYNOLDS • *Department of Biochemistry, University of Wisconsin-Madison, Madison, WI, USA*
- SIMONE DI RUBBO • *Department of Plant Systems Biology, VIB, Ghent, Belgium; Department of Biology, University of Washington-HHMI, University of Washington, Seattle, WA, USA*
- CARLOS RUBILAR-HERNÁNDEZ • *Plant Molecular Biology Laboratory, Department of Biology, Faculty of Sciences, University of Chile, Santiago, Chile*
- EUGENIA RUSSINOVA • *Department of Plant Systems Biology, VIB, Ghent, Belgium; Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, Belgium*
- JOZEF ŠAMAJ • *Department of Cell Biology, Faculty of Science, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University Olomouc, Olomouc, Czech Republic*
- OLGA ŠAMAJOVÁ • *Department of Cell Biology, Faculty of Science, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University Olomouc, Olomouc, Czech Republic*

- JENNIFER SCHOBERER • *Department of Applied Genetics and Cell Biology, BOKU, University of Natural Resources and Life Sciences, Vienna, Austria*
- LIBO SHAN • *Department of Plant Pathology and Microbiology, Institute for Plant Genomics and Biotechnology, Texas A&M University, College Station, TX, USA*
- TOMOO SHIMADA • *Department of Botany, Graduate School of Science, Kyoto University, Kyoto, Japan*
- THOMAS STANISLAS • *Department of Plant Physiology, Umeå Plant Science Centre (UPSC), Umeå University, Umeå, Sweden*
- TOMÁŠ TAKÁČ • *Department of Cell Biology, Faculty of Science, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University Olomouc, Olomouc, Czech Republic*
- JUNPEI TAKANO • *Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan*
- TAKASHI UEDA • *Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan; Japan Science and Technology Agency (JST), PRESTO, Saitama, Japan*
- JINGGENG ZHOU • *Department of Biochemistry and Biophysics, Institute for Plant Genomics and Biotechnology, Texas A&M University, College Station, TX, USA*
- XIAOHONG ZHUANG • *School of Life Sciences, Centre for Cell and Developmental Biology and State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, China*