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Plant Endosomes

Methods and Protocols

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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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