
Preface

Eukaryotic cells are surrounded by membranes consisting of various lipids, including sterols, sphingolipids, glycolipids, and phospholipids. Besides structural functions, membranes also contain lipids with regulatory and signaling roles. Such lipids include polyphosphoinositides, the low-abundant derivatives of phosphatidylinositol, phosphatidic acid, certain sphingolipids, *N*-acyl ethanolamines, and oxylipins, oxidized derivatives of C18-fatty acids; all these are emerging as important players regulating various physiological processes in plants.

As we are beginning to understand the complexity of lipid signaling and its roles in plant biology, there is an increasing interest in their analysis. However, due to the low abundance and transient nature of some of these hydrophobic compounds, this is not always easy. The difficulty in analyzing signaling lipids arises from some unique analytical challenges. For instance, the structural similarity of polyphosphoinositide species and of derived soluble inositol polyphosphates must be taken into account when using “specific” *in vivo* markers or when assaying relevant enzyme activities. Furthermore, plants exhibit greater metabolic complexity in comparison to animals or yeast, and the detection of low levels of signaling molecules requires specialized technology. Together, these considerations have created a need for novel means to identify and quantify signaling lipids and have raised new questions regarding the biochemical properties of enzymes involved in their conversion.

This book is dedicated to the various experimental approaches by which plant signaling lipids can be studied. Experts in the field have contributed their knowledge and extensive experience to provide a collection of 27 chapters on the analysis of plant signaling lipids, including detailed protocols to detect various relevant compounds by targeted or nontargeted approaches; to assay relevant enzyme activities in biological material or using recombinant enzymes; to test for specific binding of signaling lipids to protein partners; or to visualize signaling lipids or lipid-derived signals in living plant cells. On the way, a broad spectrum of methods is detailed, such as radiolabeling, diverse chromatographic methods (TLC, HPLC, GC), mass spectrometry, nuclear magnetic resonance spectroscopy, and confocal imaging. By looking left and right at signaling events related to lipids, key protocols for the analysis of other important players are also covered, such as diacylglycerol, galactolipids, and Ca^{2+} . All methods described have been developed or optimized in particular for the use in plants and are described including proper controls and notes for meaningful interpretation to ensure that tools and assays are used correctly and to their full potential. Tools presented often provide alternative options for relevant analyses, either requiring extensive instrumentation or being achievable with the most basic of laboratory equipment, thus helping interested researchers to select methods suitable for their questions and equipment available.

Research groups around the world are pursuing an increasing number of questions about functions of plant lipid signals, including adaptation to environmental stresses, guard cell functioning, vesicle trafficking, or control in development, such as cell polarity and pattern formation. Paying tribute to its emerging complexity, we are convinced that plant lipid

signaling is best approached by multiple parallel methods, combining key biochemical tests and cell biological analyses described in this book. It is therefore timely and important to present plant researchers with this full set of tools needed to elucidate the particular roles of signaling lipids in plants. We hope that this collection of current protocols will aid plant researchers in their endeavor to elucidate the roles of lipid signals, and we are eager to see these methods put to use towards exciting new discoveries.

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