Preface

Signal transduction is the fundamental mechanism for regulation of cellular activities by environmental cues and regulatory signals. Signal transduction is particularly important for plants, whose survival requires proper physiological and developmental responses to the environmental changes. Genome sequencing has revealed expansion of gene families encoding signal transduction proteins in plants compared to animals. Genetic studies in the last two decades have identified receptors and key signal transduction components of many signaling pathways in plants, mostly in the model system Arabidopsis. However, plant signaling systems are complex and require diverse approaches and techniques to dissect. Conceptually, signal transduction involves signal perception by receptors and activation of receptor activity, intracellular signal relay, which often involves protein–protein interaction and posttranslational protein modifications such as phosphorylation, glycosylation, ubiquitination, and oxidation, and regulation of gene expression. Much progress has been made recently in the plant signal transduction research field thanks to the development of diverse techniques, including proteomics and mass spectrometry methods for studying protein modification, biochemical and cell biological tools for studying protein–protein interactions, genomic techniques for dissecting protein–DNA interaction and transcription networks, and computation methods that integrate molecular networks into plant developmental processes. *Plant Signaling Networks* describes many of these advanced research methods.

Chapters 1–3 describe mass spectrometry methods for studying protein phosphorylation and glycosylation. One of the most important classes of plant receptors is the receptor-like kinases localized on the cell surface. Methods for analysis of receptor kinase phosphorylation using mass spectrometry are provided in Chap. 1. These methods have yielded insights into the molecular details of receptor kinase activation by autophosphorylation and transphosphorylation in receptor kinase complexes. Chapter 2 describes quantitative measurement of protein phosphorylation in complex samples, which is useful in identifying phosphorylated components in signal transduction pathways. Chapter 3 describes enrichment and mass spectrometry analysis of O-GlcNAc modification of proteins, which is an important protein modification for signaling. Chapters 4–6 describe advanced two-dimensional electrophoresis methods for quantitative proteomic analysis of proteins localized on the plasma membrane, or modified by phosphorylation or redox.

Genetic approaches are powerful for identifying essential signaling components, but have limitations due to genetic redundancy. Several elaborate strategies have been shown to be effective in overcoming genetic redundancy. Chapters 7 and 8 describe chemical genetics—use of small molecule chemicals, to dissect signaling pathways, and Chapter 9 describes an improved tool for generating overexpression mutants.

G-proteins are important components of many signal transduction pathways. Chapters 10 and 11 describe biochemical and cell biological methods for analyzing G-protein activation. Ubiquitination is another universal mechanism used widely in all cellular regulatory processes. Specific interactions between E3 ubiquitin ligases and substrate proteins are key for regulating degradation/accumulation of signaling proteins. Chapters 12 and 13 describe in vivo and in vitro methods for analyzing E3–substrate interaction and ubiquitination.

Most signal transduction pathways regulate development and physiology by controlling gene expression. Identification of all target genes of a signaling pathway is key for understanding not only the functions of the pathway but also the regulatory network that integrates multiple pathways. Chapter 14 describes the use of chromatin immunoprecipitation followed by microarray (ChIP-chip) or high-throughput sequencing (ChIP-Seq) for identifying target genes of transcription factors in both *Arabidopsis* and rice. Quantitative analysis of gene expression as output of signal transduction provides effective assays for functions of signaling components. Chapter 15 describes a smart pooling approach that improves the efficiency of RNA profiling experiments. Chapter 16 describes the powerful cell-based transient gene expression assay for testing functions of and delineating relationships among signaling components. Chapter 17 describes a method for profiling un-capped RNA, which can reveal posttranscriptional regulation of RNA abundance. Finally, Chap. 18 provides brief account of recently developed imaging and computation methods for analyzing both local and global patterns of gene expression and growth in *Arabidopsis* shoot apical meristems (SAMs).

Plant Signalling Networks provides detailed protocols for a wide range of research approaches including genetics, proteomics, biochemical, cell biological, and computational approaches. These are powerful methods for understanding various aspects of signaling networks in plants. We hope that this timely overview of diverse approaches for studying signal transduction systems provides a guide for researchers to gain comprehensive understanding of complex signaling networks in plants.

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