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Plant MAP Kinases

Methods and Protocols

Edited by

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Cover illustration: The cover image is a collage showing comparison of whole seedlings of *Arabidopsis thaliana* Columbia wild type (left image) and a mitogen activated protein kinase 6-2 mutant (*mpk6-2*; middle image). Contrasting of the roots with toluidine blue histochemical stain allows better visualization and documentation of differences in root patterning between wild type and mutant using binocular optics. The image on the right illustrates a part of living *Arabidopsis thaliana* root stably overexpressing the heterologous SIMKK of *Medicago sativa* tagged with yellow fluorescent protein (YFP; pseudocolored dark red). The root was counterstained with FM4-64 (pseudocolored blue) to delineate root cell borders and visualized with confocal laser scanning microscope. In this way two successive and juxtaposed lateral root primordia are visible, reflecting the impact of SIMKK-YFP overexpression on lateral root formation. All images were kindly provided by Dr. Miroslav Ovečka (Department of Cell Biology, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University Olomouc, Olomouc, Czech Republic).

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Preface

The highly expanded family of mitogen-activated protein kinases (MAPKs) allows remarkable versatility in the adaptation and tolerance of plants to abiotic and biotic stresses when compared to other eukaryotes. Furthermore, MAPK signaling modules in model plants such as *Arabidopsis thaliana* are densely interconnected, often function redundantly, while different MAPK pathways may cross talk during a single signaling event. The above complexity as well as the central role of MAPK signaling in the reaction and adaptation to often unfavorable abiotic and biotic conditions have prompted the vigorous research necessary to uncover MAPK signaling networks, not only in the model plant Arabidopsis but also in important crops. Ever since the first description of plant MAPKs, the number of publications related to the topic increases in an exponential manner. For the above reasons, we believe that the time is ripe to provide a central source of proofread and exhaustively troubleshot protocols that will encompass the entire array of experimental resources necessary for either the novice or the expert researcher.

The present book entitled "Plant MAP Kinases: Methods and Protocols" from the Methods in Molecular Biology™ series addresses the complexity of conditional and developmentally important plant MAPKs at many levels. Technically, the contents cover a wide array of techniques and methods used in MAPK research as these were contributed by experts of each method described.

The experimental survey of the plant MAPK world in the Part 1 is devoted to the collection of protocols aiming to interrogate MAPK function, and it starts with a robust transient expression system using Arabidopsis mesophyll protoplasts (Chapter 1). The following chapter (Chapter 2) addresses the MAPK transcriptional regulation during abiotic and biotic stresses with quantitative real-time PCR explained to thorough detail. Next three chapters (Chapters 3–5) are dedicated to the assessment of MAPK phosphorylation/activation and function by nonradioactive means. Chapter 3 demonstrates the efficient use of phospho-specific antibodies that were originally raised against mammalian MAPKs, in order to follow temporal and dose-dependent activation of MAPKs. The other two chapters employ two different electrophoretic approaches which allow the efficient discrimination of phosphorylated and non-phosphorylated protein forms in one dimension. This can be achieved by either one-dimensional isoelectric focusing (Chapter 4) or phospho-affinity-based denaturing SDS-PAGE (Chapter 5). Part 2 encompasses protocols discovering function of MAPK signaling by genetic tools including the engineering of constitutively active MAPKs (Chapter 6), the silencing of MAPKs by either virus-induced silencing (Chapter 7) or RNA interference (Chapter 8). In Part 3 effort is made to put MAPK signaling at the cellular context. Thus MAPKs are immunolocalized in either root whole-mount samples (Chapter 9) or Steedman wax sections (Chapter 10), while strategies for their in vivo imaging as well as for the subcellular visualization of their interactions are presented in following respective chapters (Chapters 11 and 12). Part IV surveys approaches to identify and study MAPK substrates. Thus, Chapter 13 shows the design of experimental work necessary to identify phosphorylation sites in putative MAPK substrates using as an example the microtubule-associated protein MAP65-1. Next, a strategy to generate phospho-specific

antibodies against verified substrates such as the WRKY transcription factors is presented (Chapter 14). Finally, a mutational approach towards the identification of MAPK substrates is aimed to uncover previously unknown targets of MAPK signaling (Chapter 15). The last part of the book tops up MAPK research and brings into light large-scale protocols. These will provide strategies for high-throughput screening of MAPK interactors by yeast two-hybrid technique (Chapter 16) or by protein microarrays (Chapter 17). Chapter 18 provides the protocol for tandem affinity purification of MAPK complexes. Finally, Chapter 19 describes iTRAQ for the enrichment of phosphoproteins which will allow the mass identification of MAPK targets.

On the side of the "Plant MAP Kinases: Methods and Protocols" the reader will find classical protocols that accompany MAPK research, including immunocomplex and in-gel kinase assays as well as redundant information of thoroughly described workflows including plant handling, work with transgenes and standard biochemical techniques such as co-immunoprecipitation, polyacrylamide gel electrophoresis, and western blotting to name a few. We trust therefore that all individual chapters are autonomous and can be used as a bench-side aid to researchers irrespective of the level of experience.

We are grateful to all 54 authors who contributed to the content of the present volume. Each author disclosed his/her experience in each respective chapter, but also provided critical troubleshooting—Notes—representing important sections for the novice reader. After all even the most ambitious experiment may fail due to the tiniest detail. We warmly acknowledge Professor John M. Walker, series editor, who apart from honoring us with the invitation to host the present volume also provided enthusiastic support throughout the entire editing procedure. Finally, we extend our thanks to the members of the Methods in Molecular Biology™ Springer editorial team for guiding us through the assembly of a useful book.

Olomouc, Czech Republic

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