# METHODS IN MOLECULAR BIOLOGY

*Series Editor*
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# Plant Cell Morphogenesis

## Methods and Protocols

### Second Edition

Edited by

**Fatima Cvrčková and Viktor Žárský**

*Department of Experimental Plant Biology, Charles University, Prague, Czech Republic*

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*Editors*
Fatima Cvrčková
Department of Experimental
Plant Biology
Charles University
Prague, Czech Republic

Viktor Žárský
Department of Experimental
Plant Biology
Charles University
Prague, Czech Republic

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Cover Illustration Caption: Growing epidermal cells color-coded according to several growth parameters (from Chapter 18)

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# Preface

Five years after the publication of the first edition of this volume, the focus of post-genomic land plant biology is definitely leaving the confines of a handful of major models, such as *Arabidopsis thaliana* or *Physcomitrella patens*, thus opening new spaces for defining and solving major questions of basic plant biology. Collecting wisdom and skills accumulated mostly from work on the founding molecular biology models can undoubtedly guide applications in other species, including crop plants. As editors of this volume, we strive to reflect this development in order to inspire future research in cellular aspects of land plant morphogenesis.

Studying the dynamics of plant shapes, starting from the cellular level and advancing via tissues to organs and onward to the whole plant, is a truly fascinating perspective that we share with the nineteenth- and twentieth-century founders of our field. Here in Prague, we acknowledge continuous inspiration by Jan Evangelista Purkyně and, in our field especially, his disciple Julius Sachs, who started his career in the German-speaking part of the Charles University in Prague and became the father of modern plant physiology (including pioneering studies of the processes of plant morphogenesis). We have worked on this volume in a building constructed in 1898 for the German Plant Physiology department, directed in those years by Professor Hans Molisch, author of *Mikrochemie der Pflanzen* (published after his move to Vienna in 1909). Several of the Czech contributors to this volume consider themselves "academic grandchildren" of Professor Bohumil Němec, one of the fathers of experimental plant cell biology. When Němec discovered the decisive role of starch statoliths in root columella for root gravitropism (1900), he immediately understood that, to function in graviperception, columellar cells need to be not only internally dynamically polarized but also connected in a communicative (i.e., signaling) network with other root cells. This indicated an intricate internal cellular structure and intercellular communication, beyond the imagination of scientists of those times. Němec taught us, via his students (our teachers) and his impressive published volumes on plant biology, to understand tissues and cells as products, not mere constituents or "bricks," of a living plant body as a whole.

As in the first edition of this book, the first eight chapters of this volume (Chapters 1–8) focus on the visualization of plant cell structures, since seeing the objects of interest is an obvious prerequisite of understanding the processes that brought them into being. Chapters 1 and 2 present a contemporary take on light microscopy, the classical approach that was instrumental in establishing the plant cell biology field, These chapters are directly linked to a classic plant histochemistry methods book published by Bohumil Němec—*Botanical microtechnique* ("Botanická mikrotechnika" in Czech, Prague 1962).

While light microscopy remains a central visualization method in plant cell biology, electron microscopy provides exciting insights into cellular ultrastructure. Chapters 3 and 4 describe a collection of useful electron microscopy techniques, including immunogold localization procedures. Chapters 5 and 6 then present techniques for in situ qualitative analysis of plant cell wall composition.

Modern cell biology is dominated by digital data. This is reflected by the recurrent inclusion of digital image analysis protocols in several of the following chapters. Chapters 7 and 8 provide generally applicable techniques for quantitative image analysis, as well as for the only seemingly mundane task of presenting image data *lege artis*.

The second part (Chapters 9–13) is devoted to qualitative and quantitative detection of the organization and dynamics of individual intracellular structures responsible for the generation of cell shape, in particular the two cytoskeletal systems, as well as endomembranes, whose structure and behavior can be studied using in vivo fluorescent markers.

The third section (Chapters 14–19) is devoted to exciting new possibilities of manipulating intracellular structures by means of optical tweezers, and probing their mechanical features by Cellular Force Microscopy. It also covers detailed monitoring and quantifying structural dynamics of meristems and developing organs on the cellular level.

The choice of the experimental model is, as a rule, tightly linked with the choice of questions that can be studied. It is hard to find a field where this would be more obvious than the study of cell morphogenesis. In the final six chapters (Chapters 20–25), we present specific techniques for studying model cell types such as filaments of the moss *Physcomitrella patens*, *Arabidopsis* root hairs, pavement cells, developing xylem vessels, pollen tubes, or plant cell lines.

We decided to leave out several topics addressed in the first edition of this book. Several previously included methods received excellent coverage in recent volumes of the *Methods in Molecular Biology* series. This is, in particular, the case for additional electron microscopy techniques, such as cryofixation with freze substitution and subsequent immunodetection [1], electron microscopy tomography and 3D reconstruction [2, 3], or analyses of nuclear morphology [4]. Omission of other topics, such as automated microscopy application in forward genetics screens, use of laser microdissection to study gene expression, microfluidics applications, general techniques for transient gene expression in plant systems, or heterologous expression in yeast, was in effort to keep the present volume focused and its size manageable.

We approached our task in editing this collection of protocols with the hope that this volume may become a source of inspiration for further research into the morphogenesis of plant cells, tissues, and organs. We are especially grateful to many colleagues—the best experts in their fields from all over the world—who accepted our invitation and contributed chapters to this volume, for making it more likely that our hope may be fulfilled.

*Prague, Czech Republic* *Fatima Cvrčková*
*Viktor Žárský*

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# Contents

# Contributors


Jana Albrechtová • *Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic*

Tobias I. Baskin • *Biology Department, University of Massachusetts, Amherst, MA, USA*

Radek Bezvoda • *Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic*

Jeffrey P. Bibeau • *Department of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA, USA*

Dorota Borowska-Wykręt • *Department of Biophysics and Morphogenesis of Plants, University of Silesia in Katowice, Katowice, Poland*

Katharina Bürstenbinder • *Department of Molecular Signal Processing, Leibniz Institute of Plant Biochemistry (IPB), Halle (Saale), Germany*

Agata Burian • *Department of Biophysics and Morphogenesis of Plants, University of Silesia in Katowice, Katowice, Poland*

Vojtěch Čermák • *Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic*

Fatima Cvrčková • *Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic*

Pradeep Das • *Laboratoire de Reproduction et Développement des Plantes, Université de Lyon, UCB Lyon 1, ENS de Lyon, INRA, CNRS, Lyon, France*

Norbert de Ruijter • *Laboratory of Cell Biology, Wageningen University, Wageningen, The Netherlands*

Mateusz Dulski • *Institute of Material Science, University of Silesia in Katowice, Chorzów, Poland; Silesian Center for Education and Interdisciplinary Research, Chorzów, Poland*

Lukáš Fischer • *Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic*

Ilse Foissner • *Department of Biosciences, University of Salzburg, Salzburg, Austria*

Giulia Galotto • *Department of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA, USA*

Olivier Hamant • *Laboratoire de Reproduction et Développement des Plantes, Université de Lyon, UCB Lyon 1, ENS de Lyon, INRA, CNRS, Lyon, France*

Margit Hoeftberger • *Department of Biosciences, University of Salzburg, Salzburg, Austria*

Ján Jásik • *Plant Science and Biodiversity Center, Institute of Botany, Slovak Academy of Sciences, Bratislava, Slovakia*

Yvon Jaillais • *Laboratoire Reproduction et Développement des Plantes, Université de Lyon, ENS de Lyon, CNRS, INRA, Lyon, France*

Jiří Janáček • *Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic*

Adriana Jelínková • *Institute of Experimental Botany of the Czech Academy of Sciences, Prague, Czech Republic*

Tijs Ketelaar • *Laboratory of Cell Biology, Wageningen University, Wageningen, The Netherlands*

Daniel Kierzkowski • *Max Planck Institute for Plant Breeding Research, Cologne, Germany; Institut de Recherche en Biologie Végétale, University of Montréal, Montreal, QB, Canada*
Sandra Klemm • *Department of Molecular Signal Processing, Leibniz Institute of Plant Biochemistry (IPB), Halle (Saale), Germany*
Petr Klíma • *Institute of Experimental Botany of the Czech Academy of Sciences, Prague, Czech Republic*
Miroslav Krausko • *Plant Science and Biodiversity Center, Institute of Botany of the Czech Academy of Sciences, Slovak Academy of Sciences, Bratislava, Slovakia*
Zuzana Kubínová • *Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic*
Zuzana Kusá • *Plant Science and Biodiversity Center, Institute of Botany, Slovak Academy of Sciences, Bratislava, Slovakia*
Dorota Kwiatkowska • *Department of Biophysics and Morphogenesis of Plants, University of Silesia in Katowice, Katowice, Poland*
Mária Labajová • *Plant Science and Biodiversity Center, Institute of Botany, Slovak Academy of Sciences, Bratislava, Slovakia*
Alexandra Lešková • *Plant Science and Biodiversity Center, Institute of Botany, Slovak Academy of Sciences, Bratislava, Slovakia*
Mateusz Majda • *Max Planck Institute for Plant Breeding Research, Cologne, Germany*
Kateřina Malínská • *Institute of Experimental Botany of the Czech Academy of Sciences, Prague, Czech Republic*
Birgit Möller • *Institute of Computer Science, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany*
Karel Müller • *Institute of Experimental Botany of the Czech Academy of Sciences, Prague, Czech Republic*
Sandra Natonik-Białoń • *Department of Biophysics and Morphogenesis of Plants, University of Silesia in Katowice, Katowice, Poland*
Jana Nebesářová • *Biology Centre of CAS, Institute of Parasitology, České Budějovice, Czech Republic; Faculty of Science, Charles University, Prague, Czech Republic*
Stefan Niehren • *Molecular Machines & Industries GmbH, Eching, Germany*
Lise C. Noack • *Laboratoire Reproduction et Développement des Plantes, Université de Lyon, ENS de Lyon, CNRS, INRA, Lyon, France*
Yoshihisa Oda • *Center for Frontier Research, National Institute of Genetics, Mishima, Shizuoka, Japan; Department of Genetics, SOKENDAI (Graduate University for Advanced Studies), Mishima, Shizuoka, Japan*
Zdeněk Opatrný • *Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic*
Denisa Oulehlová • *Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic; Institute of Experimental Botany of the Czech Academy of Sciences, Prague, Czech Republic*
Gabriel Paës • *Fractionation of AgroResources and Environment (FARE) Laboratory, INRA, University of Reims Champagne-Ardenne, Reims, France*
Přemysl Pejchar • *Institute of Experimental Botany, Czech Academy of Sciences, Prague, Czech Republic; Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic*

Jan Petrášek • *Institute of Experimental Botany of the Czech Academy of Sciences, Prague, Czech Republic; Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic*
Yvonne Poeschl • *Institute of Computer Science, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany; iDiv, German Integrative Research Center for Biodiversity, Leipzig, Germany*
Martin Potocký • *Institute of Experimental Botany, Czech Academy of Sciences, Prague, Czech Republic; Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic*
Amparo Rosero • *Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic; Coordinación de Innovación Regional, C.I. Turipaná, Montería, Córdoba, Colombia*
Anne-Lise Routier-Kierzkowska • *Max Planck Institute for Plant Breeding Research, Cologne, Germany; Institut de Recherche en Biologie Végétale, University of Montréal, Montreal, QC, Canada*
Aleksandra Sapala • *Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland; Max Planck Institute for Plant Breeding Research, Cologne, Germany*
Takema Sasaki • *Center for Frontier Research, National Institute of Genetics, Mishima, Shizuoka, Japan*
Juraj Sekereš • *Institute of Experimental Botany, Czech Academy of Sciences, Prague, Czech Republic; Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic*
Richard S. Smith • *Max Planck Institute for Plant Breeding Research, Cologne, Germany*
Josef Šonka • *Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic*
Aleš Soukup • *Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic*
Miroslav Srba • *Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic*
Soeren Strauss • *Max Planck Institute for Plant Breeding Research, Cologne, Germany*
Christine Terryn • *PICT Platform, University of Reims Champagne-Ardenne, Reims, France*
Edita Tylová • *Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic*
Luis Vidali • *Department of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA, USA*
Viktor Žárský • *Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic; Institute of Experimental Botany of the Czech Academy of Sciences, Prague, Czech Republic*
Ellen Zelinsky • *Biology Department, University of Massachusetts, Amherst, MA, USA*
Luise Zergiebel • *Department of Molecular Signal Processing, Leibniz Institute of Plant Biochemistry (IPB), Halle (Saale), Germany*