

Plant Tropisms

Edited by

SIMON GILROY

PATRICK H. MASSON



Plant Tropisms

Plant Tropisms

Edited by

SIMON GILROY

PATRICK H. MASSON



Simon Gilroy, Ph.D., is Professor of Biology at Pennsylvania State University.
Patrick H. Masson, Ph.D., is Professor of Genetics at the University of Wisconsin.

© 2008 Blackwell Publishing
All rights reserved

Blackwell Publishing Professional
2121 State Avenue, Ames, Iowa 50014, USA

Orders: 1-800-862-6657
Office: 1-515-292-0140
Fax: 1-515-292-3348
Web site: www.blackwellprofessional.com

Blackwell Publishing Ltd
9600 Garsington Road, Oxford OX4 2DQ, UK
Tel.: +44 (0)1865 776868

Blackwell Publishing Asia
550 Swanston Street, Carlton, Victoria 3053, Australia
Tel.: +61 (0)3 8359 1011

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Blackwell Publishing, provided that the base fee is paid directly to the Copyright Clearance Center, 222 Rosewood Drive, Danvers, MA 01923. For those organizations that have been granted a photocopy license by CCC, a separate system of payments has been arranged. The fee codes for users of the Transactional Reporting Service are ISBN-13: 978-0-8138-2323-2/2007.

First edition, 2008

Library of Congress Cataloging-in-Publication Data
Plant Tropisms/edited by Simon Gilroy, Patrick H. Masson.-1st ed.
p. cm.

Includes bibliographical references.

ISBN 13:978-0-8138-2323-2 (alk. paper)

ISBN 10:0-8138-2323-4

1. Tropisms. 2. Growth (Plants) I. Gilroy, Simon. II. Masson, Patrick H.

QK745.P59 2007

571.8'2—dc22

2007013116

The last digit is the print number: 9 8 7 6 5 4 3 2 1

Contents

List of Contributors	ix
Preface	xiii
Chapter 1: Mechanisms of Gravity Perception in Higher Plants	3
ALINE H. VALSTER AND ELISON B. BLANCAFLOR	
1.1 Introduction	3
1.2 Identification and characterization of gravity perception sites in plant organs	4
1.2.1 Roots	4
1.2.2 Hypocotyls and inflorescence stems (dicotyledons)	6
1.2.3 Cereal pulvini (monocotyledons)	8
1.3 The starch-statolith hypothesis	9
1.3.1 A variety of plant organs utilize sedimenting amyloplasts to sense gravity	9
1.3.2 Amyloplast sedimentation is influenced by the environment and developmental stage of the plant	11
1.4 The gravitational pressure model for gravity sensing	11
1.5 The cytoskeleton in gravity perception	12
1.6 Concluding remarks and future prospects	14
1.7 Acknowledgment	15
1.8 Literature cited	15
Chapter 2: Signal Transduction in Gravitropism	21
BENJAMIN R. HARRISON, MIYO T. MORITA, PATRICK H. MASSON, AND MASAO TASAKA	
2.1 Introduction	21
2.2 Gravity signal transduction in roots and aboveground organs	22
2.2.1 Do mechano-sensitive ion channels function as gravity receptors?	24
2.2.2 Inositol 1,4,5-trisphosphate seems to function in gravity signal transduction	26
2.2.3 Do pH changes contribute to gravity signal transduction?	27
2.2.4 Proteins implicated in gravity signal transduction	28
2.2.5 Global '-omic' approaches to the study of root gravitropism	32
2.2.6 Relocalization of auxin transport facilitators or activity regulation?	37
2.2.7 Could cytokinin also contribute to the gravitropic signal?	38
2.3 Gravity signal transduction in organs that do not grow vertically	39
2.4 Acknowledgments	40
2.5 Literature cited	40
Chapter 3: Auxin Transport and the Integration of Gravitropic Growth	47
GLORIA K. MUDAY AND ABIDUR RAHMAN	
3.1 Introduction to auxins	47
3.2 Auxin transport and its role in plant gravity response	47
3.3 Approaches to identify proteins that mediate IAA efflux	51
3.4 Proteins that mediate IAA efflux	51
3.5 IAA influx carriers and their role in gravitropism	53

3.6	Regulation of IAA efflux protein location and activity during gravity response	55
3.6.1	Mechanisms that may control localization of IAA efflux carriers	56
3.6.2	Regulation of IAA efflux by synthesis and degradation of efflux carriers	58
3.6.3	Regulation of auxin transport by reversible protein phosphorylation	59
3.6.4	Regulation of auxin transport by flavonoids	61
3.6.5	Regulation of auxin transport by other signaling pathways	61
3.6.6	Regulation of gravity response by ethylene	64
3.7	Overview of the mechanisms of auxin-induced growth	65
3.8	Conclusions	67
3.9	Acknowledgements	68
3.10	Literature cited	68
Chapter 4:	Phototropism and Its Relationship to Gravitropism	79
	JACK L. MULLEN AND JOHN Z. KISS	
4.1	Phototropism: general description and distribution	79
4.2	Light perception	80
4.3	Signal transduction and growth response	82
4.4	Interactions with gravitropism	83
4.5	Importance to plant form and function	84
4.6	Conclusions and outlook	85
4.7	Literature cited	86
Chapter 5:	Touch Sensing and Thigmotropism	91
	GABRIELE B. MONSHAUSEN, SARAH J. SWANSON, AND SIMON GILROY	
5.1	Introduction	91
5.2	Plant mechanoresponses	91
5.2.1	Specialized touch responses	92
5.2.2	Thigmomorphogenesis and thigmotropism	94
5.3	General principles of touch perception	95
5.3.1	Gating through membrane tension: the mechanoreceptor for hypo-osmotic stress in bacteria, MscL	98
5.3.2	Gating through tethers: the mechanoreceptor for gentle touch in <i>Caenorhabditis elegans</i>	99
5.3.3	Evidence for mechanically gated ion channels in plants	101
5.4	Signal transduction in touch and gravity perception	103
5.4.1	Ionic signaling	103
5.4.2	Ca ²⁺ signaling in the touch and gravity response	103
5.5	Insights from transcriptional profiling	107
5.6	Interaction of touch and gravity signaling/response	110
5.7	Conclusion and Perspectives	113
5.8	Acknowledgements	114
5.9	Literature cited	114
Chapter 6:	Other Tropisms and their Relationship to Gravitropism	123
	GLADYS I. CASSAB	
6.1	Introduction	123
6.2	Hydrotropism	123
6.2.1	Early studies of hydrotropism	124
6.2.2	Genetic analysis of hydrotropism	125
6.2.3	Perception of moisture gradients and gravity stimuli by the root cap and the curvature response	126

- 6.2.4 ABA and the hydrotropic response 128
- 6.2.5 Future experiments 129
- 6.3 Electrotropism 129
- 6.4 Chemotropism 131
- 6.5 Thermotropism and oxytropism 132
- 6.6 Traumatropism 134
- 6.7 Overview 135
- 6.8 Acknowledgments 135
- 6.9 Literature cited 135

Chapter 7: Single-Cell Gravitropism and Gravitaxis **141**

MARKUS BRAUN AND RUTH HEMMERSBACH

- 7.1 Introduction 141
- 7.2 Definitions of responses to environmental stimuli that optimize the ecological fitness of single-cell organisms 141
- 7.3 Occurrence and significance of gravitaxis in single-cell systems 142
- 7.4 Significance of gravitropism in single-cell systems 143
- 7.5 What makes a cell a biological gravity sensor? 144
- 7.6 Gravity susception—the initial physical step of gravity sensing 145
- 7.7 Susception in the statolith-based systems of *Chara* 145
- 7.8 Susception in the statolith-based system *Loxodes* 149
- 7.9 Susception in the protoplast-based systems of *Euglena* and *Paramecium* 150
- 7.10 Gravi-perception in the statolith-based systems of *Chara* 150
- 7.11 Gravi-perception in the statolith-based system *Loxodes* 151
- 7.12 Gravi-perception in the protoplast-based systems *Paramecium* and *Euglena* 151
- 7.13 Signal transduction pathways and graviresponse mechanisms in the statolith-based systems of *Chara* 153
- 7.14 Signal transduction pathways and graviresponse mechanisms in *Euglena* and *Paramecium* 154
- 7.15 Conclusions 155
- 7.16 Acknowledgements 156
- 7.17 Literature cited 156

Color Section

Chapter 8: Space-Based Research on Plant Tropisms **161**

MELANIE J. CORRELL AND JOHN Z. KISS

- 8.1 Introduction—the variety of plant movements 161
- 8.2 The microgravity environment 162
- 8.3 Ground-based studies: mitigating the effects of gravity 165
- 8.4 Gravitropism 166
 - 8.4.1 Gravitropism: gravity perception 166
 - 8.4.2 Gravitropism: signal transduction 168
 - 8.4.3 Gravitropism: the curving response 169
- 8.5 Phototropism 171
- 8.6 Hydrotropism, autotropism, and oxytropism 172
- 8.7 Studies of other plant movements in microgravity 174
- 8.8 Space flight hardware used to study tropisms 175
- 8.9 Future outlook and prospects 177
- 8.10 Literature cited 177

Chapter 9: Plan(t)s for Space Exploration	183
CHRISTOPHER S. BROWN, HEIKE WINTER SEDEROFF, ERIC DAVIES, ROBERT J. FERL, AND BRATISLAV STANKOVIC	
9.1 Introduction	183
9.2 Human missions to space	184
9.3 Life support	184
9.4 Genomics and space exploration	185
9.5 Nanotechnology	187
9.6 Sensors, biosensors, and intelligent machines	187
9.7 Plan(t)s for space exploration	188
9.8 Imagine . . .	192
9.9 Literature cited	192
Index	197

List of Contributors

Elison B. Blancaflor

Plant Biology Division
The Samuel Roberts Noble Foundation
2510 Sam Noble Parkway
Ardmore, OK 73401
USA
Tel: (580) 224-6687
Fax: (580) 224-6692
E-mail: eblancaflor@noble.org

Markus Braun

Gravitationsbiologie
Institut für Molekulare Physiologie und
Biotechnologie der Pflanzen Universität
Bonn
53115 Bonn
Germany
Tel: (49) 228-73-2686
Fax: (49) 228-732677
E-mail: mbraun@uni-bonn.de

Christopher S. Brown

Kenan Institute for Engineering, Technology
& Science
North Carolina State University
Raleigh, NC 27695
USA
Tel: (919) 513-2457
Fax: (919) 515-5831
E-mail: cbrown@gw.fis.ncsu.edu

Gladys I. Cassab

Department of Plant Molecular Biology
Institute of Biotechnology
National Autonomous University of Mexico
P.O. Box 510-3
Cuernavaca, Mor. 62250
Mexico
Tel: (52) 5556-22-7660
Fax (52) 7773-13-9988
E-mail: gladys@ibt.unam.mx

Melanie J. Correll

Department of Agricultural and Biological
Engineering
University of Florida
209 Frazier Rogers Hall
P.O. Box 110570
Gainesville, FL 32611-0570
USA
Tel: (352) 392-1864
Fax: (352) 392-4092
E-mail: Correllm@ufl.edu

Eric Davies

Department of Plant Biology
North Carolina State University
1231 Gardner Hall
Box 7612
Raleigh, NC 27695
USA
Tel: (919) 513-1901
Fax: (919) 515-3436
E-mail: eric_davies@ncsu.edu

Robert J. Ferl

Department of Horticulture
University of Florida
Gainesville, FL 32611
USA
Tel: (352) 392-1928
Fax: (352) 392-4072
E-mail: robferl@ufl.edu

Simon Gilroy

Biology Department
The Pennsylvania State University
208 Mueller Laboratory
University Park, PA 16802
USA
Tel: (814) 863-9626
Fax: (814) 865-9131
E-mail: sxg12@psu.edu

Benjamin R. Harrison

Laboratory of Genetics (Room 3262)
University of Wisconsin–Madison
425G Henry Mall
Madison, WI 53706
USA
Tel: (608) 265-8632
Fax: (608) 262-2976
E-mail: brharrison@wisc.edu

Ruth Hemmersbach

Institute of Aerospace Medicine
DLR (German Aerospace Research
Establishment)
51140 Köln
Under Höhe
Germany
Email: ruth.hemmersbach@dlr.de

John Z. Kiss

Department of Botany
Pearson Hall
Miami University
Oxford, OH 45056
USA
Phone: (513) 529-5428
Fax: (513) 529-4243
E-mail: kissjz@muohio.edu

Patrick H. Masson

Laboratory of Genetics (Room 3262)
University of Wisconsin–Madison
425G Henry Mall
Madison, WI 53706
USA
Tel: (608) 265-2312
Fax: (608) 262-2976
E-mail: phmasson@wisc.edu

Gabriele B. Monshausen

Biology Department
The Pennsylvania State University
208 Mueller Laboratory
University Park, PA 16802
USA
Tel: (814) 863-9625
Fax: (814) 865-9131
E-mail: gbm10@psu.edu

Miyo T. Morita

Graduate School of Biological Sciences
Nara Institute of Science and Technology
8916-5 Takayama
Ikoma, Nara 630-0101
Japan
Phone: (81) 743-72-5487
Fax: (81) 743-72-5487
E-mail: mimorita@bs.naist.jp

Gloria K. Muday

Department of Biology
Wake Forest University
Winston-Salem, NC 27109-7325
USA
Tel: (336) 758-5316
Fax: (336) 758-6008
E-mail: muday@wfu.edu

Jack L. Mullen

Department of Bioagricultural Sciences and
Pest Management
Plant Science Building, Room C 129
Colorado State University
Fort Collins, CO 80523-1177
USA
Tel: (970) 491-5261
E-mail: jamullen@lamar.colostate.edu

Abidur Rahman

Biology Department
University of Massachusetts
611 North Pleasant St.
106 Morrill 3
Amherst, MA 01003
USA
Phone: (413) 545-2776
Fax: (413) 545-3243
E-mail: abidur@bio.umass.edu

Heike Winter Sederoff

Department of Plant Biology
North Carolina State University
Raleigh, NC 27695
USA
Phone: (919) 513-0076
Fax: (919) 515-3436
E-mail: heike_winter@ncsu.edu

Bratislav Stankovic

Brinks Hofer Gilson & Lione
455 N. Cityfront Plaza Drive, Suite 3600
Chicago, IL 60611-5599
USA

Sarah J. Swanson

Biology Department
The Pennsylvania State University
208 Mueller Laboratory
University Park, PA 16802
USA
Tel: (814) 863-9625
Fax: (814) 865-9131
E-mail: sjs31@psu.edu

Masao Tasaka

Graduate School of Biological Sciences
Nara Institute of Science and Technology
8916-5 Takayama
Ikoma, Nara 630-0101
Japan
Phone: (81) 793-72-5480
Fax: (81) 793-72-5489
E-mail m-tasaka@bs.naist.jp

Aline H. Valster

Plant Biology Division
The Samuel Roberts Noble Foundation
2510 Sam Noble Parkway
Ardmore, OK 73401
USA
Tel (580) 224-6756
Fax (580) 224-6692
E-mail: ahvalster@noble.org

Preface

As sessile organisms, plants spend their entire lives at the site of seed germination. Consequently, they require a suite of strategies to survive very diverse environmental stresses. Part of this plasticity relies on the ability of most plant organs to grow in directions that are dictated by specific cues from the environment, seeking out better conditions to fulfill their primary functions. Typical guidance for the growth of plant organs is provided by gravity, light, touch, gradients in humidity, ions, oxygen, and temperature. Such directional growth, defined by vectorial stimuli, is called a tropism and is believed to significantly contribute to plant survival.

The concept of tropism was introduced 200 years ago, when Knight (1806) postulated that a plant's perception of gravity might modulate its ability to direct shoots to grow upward and guide roots downward. Eighty years later, Darwin (1880) made seminal contributions to the field by documenting a wide array of tropic responses and identifying regions of the root and shoot specialized for the perception of light and gravity. He also predicted the existence of auxin by proposing the presence of a plant growth regulator (hormone) whose gravity-induced redistribution across the tip of an organ might signal differential growth.

Since these discoveries, our analysis of tropic growth has expanded to include measurements of responses to light, touch, and gradients in humidity, ions, chemicals, and oxygen. However, only recently have the data converged to provide a picture of the physiological, molecular, and cell biological processes that underlie plant tropisms. Thus, the last few years have witnessed a true renaissance in the analysis of tropic response, mainly driven by the marrying of modern tools and strategies in the fields of forward and reverse genetics, biochemistry, cell biology, expression profiling, and proteomics, to a very careful analysis of the growth process itself.

When such analyses have been coupled with the utilization of model systems such as *Arabidopsis thaliana* and rice, where their entire genome has been sequenced, these strategies have provided an unprecedented power of resolution in our analysis of growth behaviors. Consequently, our conception of tropisms has evolved from their being considered as simple laboratory curiosities to becoming important tools/phenotypes with which to decipher basic cell biological processes that are essential to plant growth and development. Thus, current insight into tropisms is intimately involved in our understanding of auxin transport and response; cytoskeleton organization and its involvement in the control of anisotropic cell expansion; the perception and transduction of stimuli such as light, touch, humidity, ions, or oxygen; the biogenesis and function of organelles such as plastids and vacuoles; and even the control of vesicular trafficking, to name but a few (Blancaflor and Masson 2003).

Of the tropic stimuli, our understanding of the mechanisms behind gravitropic and phototropic response has shown extremely rapid advances in the last few years and, in

Chapter 1, Valster and Blancaflor describe our current models of gravitropic sensing in plants, a theme further developed in Chapter 2, where Harrison and colleagues discuss the molecular mechanisms behind transduction of the gravity signal. In Chapter 7, Braun and Hemmersbach further explore sensing and signaling in plants by comparison to the wealth of data on how single-celled organisms detect and respond to gravity. Similarly, in Chapter 4, Mullen and Kiss describe the remarkably detailed knowledge we now have of the mechanisms whereby plants perceive light and translate that cue into a phototropic growth.

Despite Darwin's prediction of the action of auxin in tropic response as early as 1880, only recently have the mechanisms behind auxin transport and action been defined to the molecular level. For example, we now understand that the relocalization of auxin transporters is a central component regulating tropic response pathways and critical components of the auxin transport pathway have been defined with molecular precision. In Chapter 3, Muday and Rahman provide an overview of this extremely rapidly evolving field.

Although individual tropic stimuli are often studied in a controlled laboratory setting, nature provides a harsh environment where multiple vectorial stimuli often signal conflicting information for a plant organ. An important step in our conceptualization of plant responses to such a complex environment has been the realization that organs not only perceive and respond to each one of these parameters, but they also have to integrate and interpret the corresponding environmental information into global "decisions" that manifest themselves into complex growth behaviors.

The integration of other tropic stimuli with the gravitropic response has recently received intense analysis and, in Chapters 5 and 6, Monshausen and colleagues and Gladys Cassab describe the wealth of tropic responses in plants and specifically how responses to touch and moisture alter gravitropic response. Such integrated responses to combined environmental cues appear to involve complex intra- and intercellular communications. Recent analyses have uncovered some of these fascinating signaling events (Fasano et al. 2002), opening the possibility of, one day, being able to engineer plants that are capable of using a defined set of directional cues for growth guidance while being oblivious to other cues. Such engineering accomplishments could find applications in agriculture and in more futuristic endeavors such as space exploration.

Indeed, spaceflight has offered researchers a unique opportunity to dissect tropic response in the absence of the effects of gravity. However, in space, in addition to exposure to microgravity, organisms also suffer from a lack of convection, growth-space limitations, lower light exposures, and increased radiation levels. Hence, the spaceflight environment appears quite unfavorable to plant success, and tropic responses are likely to be altered accordingly. Because plants have been identified as an ideal choice for utilization in bioregenerative life-support systems during long-term space exploration missions, there is a definite need for a better understanding of their growth behavior and sustainability during long-term exploration travels in order to prevent or overcome potential catastrophic system breakdowns in the midst of a mission.

Recognition of this need recently fueled efforts at developing orbit-based experiments on plant growth behavior and gravitropic sensitivity, eventually leading to the design and building of the International Space Station where such studies can be carried out. Space

experiments have added new information on plant growth responses to directional cues such as gravity, light, or oxygen gradients and, in Chapter 8, Correll and Kiss describe the opportunities that spaceflight has provided to understand how a range of such tropic responses operate.

However, spaceflight experimentation has also been plagued by a variety of constraints that have diminished their potential scientific value. Hence, a combined approach, including both ground- and orbit-based research, is necessary to gain a better understanding of the behavior of plants and their organs under micro- or hypergravity environments in the hope of being able to, one day, engineer cultivars that are better-adapted to the conditions likely to be encountered during space exploration missions.

Thus, the field of plant tropisms has received considerable attention in the last few years for its impact on both basic understanding of plant growth and development and applied aspects, such as crop response or application to spaceflight. We hope this book will provide a comprehensive yet integrated coverage of our current state of knowledge on the molecular and cell biological processes that govern plant tropisms, with major emphasis on gravitropism (one of the most extensively studied plant tropisms). Our understanding of tropic responses is rapidly increasing and, with each new insight, the potential to engineer new traits into plants moves closer. Therefore, for the last chapter of the book we asked Chris Brown and colleagues to present a vision for how our increasingly detailed understanding of these plant growth responses might translate into designing plants to sustain human endeavors in perhaps the most inhospitable environment for life imaginable—space.

Simon Gilroy
Patrick H. Masson

Literature Cited

- Blancaflor EB and Masson P. 2003. Update on Plant gravitropism. Unraveling the ups and downs of a complex process. *Plant Physiology* 133: 1677–1690.
- Darwin C. 1880. *The Power of Movement in Plants*. London: John Murray.
- Fasano JM, Massa GD and Gilroy S. 2002. Ionic signaling in plant responses to gravity and touch. *Journal of Plant Growth Regulation* 21: 71–88.
- Knight T. 1806. On the direction of the radicle and germen during the vegetation of seeds. *Philosophical Transactions of the Royal Society* 99: 108–120.

Plant Tropisms

1 Mechanisms of Gravity Perception in Higher Plants

Aline H. Valster and Elison B. Blancaflor*

1.1 Introduction

Plant growth and development is influenced by a multitude of exogenous and endogenous signals. Among the signals a plant encounters during its lifetime, gravity is one that remains constant throughout development. Since the plant needs to orient its organs to position itself within available environmental resources such as light and soil nutrients, the gravity stimulus is significant for its survival. From the moment the seed germinates, the seedling orients its emerging root such that it grows downward, toward the gravity vector, whereas it directs its shoot to grow upward, opposite the gravity vector. This phenomenon, referred to as gravitropism (geotropism in the older literature) requires the coordinated response and interaction of different cell types. Furthermore, an array of cellular structures and endogenous molecules, which in turn are modulated by a variety of environmental stimuli including light, moisture, oxygen, and touch, eventually determine the final manifestation of the gravity response (Blancaflor and Masson 2003; Morita and Tasaka 2004; Perrin et al. 2005; Esmon et al. 2005).

Gravitropism has traditionally been divided into a series of events: gravity perception, signal transduction, and the growth response (Sack 1991; Kiss 2000). In higher plants, these events appear to take place in spatially distinct regions of the organ, in contrast to tip-growing cells such as rhizoids of the green algae *Chara* and protonemata of moss and *Chara* where, as discussed in Chapter 7, all phases of gravitropism occur within the same cell (Sievers et al. 1996; Schwuchow et al. 2002). Since gravity must ultimately work on a mass to exert its effect on a given biological system, it has been widely accepted that plants sense gravity through falling organelles (statoliths) within specialized cells (statoctes). Through the years, this model of plant gravity perception has been refined and alternative hypotheses have been proposed, including the possibility that the settling of the whole cell protoplast rather than sedimenting organelles is responsible for gravity sensing (Staves 1997). A number of excellent articles which provide a historical perspective on gravity perception in plants include Sack (1991, 1997) and Kiss (2000). The reader is referred to these articles for an in-depth discussion and critical analysis of the experimental data that have led to current models on how plants sense gravity.

In this chapter, we revisit the topic of gravity perception mechanisms, focusing primarily on roots and shoots of higher plants. Although we occasionally refer to some of the older literature, this chapter will highlight recent findings that are leading to new, testable models explaining how plants sense gravity.

*Corresponding author

1.2 Identification and Characterization of Gravity Perception Sites in Plant Organs

Gravity has been shown to regulate the orientation of different plant organs such as roots, shoots (Fukaki et al. 1998; Morita and Tasaka 2004; Perrin et al. 2005), leaves (Mano et al. 2006), inflorescence stems (Weise et al. 2000), cereal pulvini (Perera et al. 2001), and peanut gynophores (Moctezuma and Feldman 1999). The response to gravity in the majority of these plant organs is manifested as differential cell growth between opposite flanks of the organ, leading to upward or downward bending. Since not all cells within the organ undergo differential growth (Sack et al. 1990), an important question in gravitropism research is how the different cell and tissue types within the organ contribute to the gravity response. A more specific question is whether the machinery for sensing gravity occurs in the same sites as the responding tissues.

To address these questions, research spanning two centuries has focused on elucidating the spatial regulation of gravitropism. For example, work that began with Charles Darwin in the late 1800s and followed-up by several other investigators in the 1900s identified the cap as the major gravity perception site in roots (reviewed by Konings 1995; Boonsirichai et al. 2002). These early experiments showed that surgical removal of the root cap tissue inhibited the gravitropic curvature without affecting overall root growth. In this section, we briefly review experimental evidence that has further reinforced the existence of specific gravity-sensing sites, distinct from the responding tissues, in the best-studied multicellular plant organs, namely roots, dicot stems, and grass pulvini.

1.2.1 Roots

As noted earlier, gravity must work on a mass to elicit a specific biological response. Therefore, cells that would be prime candidates for perceiving gravity are those which exhibit a distinct structural polarity with respect to the gravity vector. Indeed, detailed ultrastructural studies of the cap of vertically growing roots in a variety of plant species revealed that the central region of the cap (called the columella) contains cells with organelles that are consistently positioned at the bottom of the cell (reviewed in Sack 1991, 1997). These organelles, later identified as starch-containing plastids called amyloplasts (Figure 1.1A and Color Section), would rapidly change position (i.e., sediment) when the root was reoriented. The sedimentation of amyloplasts is the most widely accepted explanation for how plant organs sense gravity, a model currently known as the starch-statolith hypothesis (refer to The Starch-Statolith Hypothesis section later in this chapter).

The identification of the cap, particularly the columella, as a major gravity-sensing site in roots led many researchers to utilize roots as a model system for studying mechanisms underlying plant gravitropic responses. This is because the apparent physical separation of the gravity-sensing cells in the cap from the responding cells in the elongation zone in angiosperm primary roots could, in theory, facilitate the analysis of individual phases of gravitropism. For instance, more than a century after Darwin first reported on the necessity of the cap for root gravitropism, laser ablation of *Arabidopsis* root cap cells allowed a more detailed spatial analysis of specific cells within the columella region that contributed most to the gravity response (Blancaflor et al. 1998). In this study, ablation of the most centrally located root cap cells, namely the second story (S2) columella cells

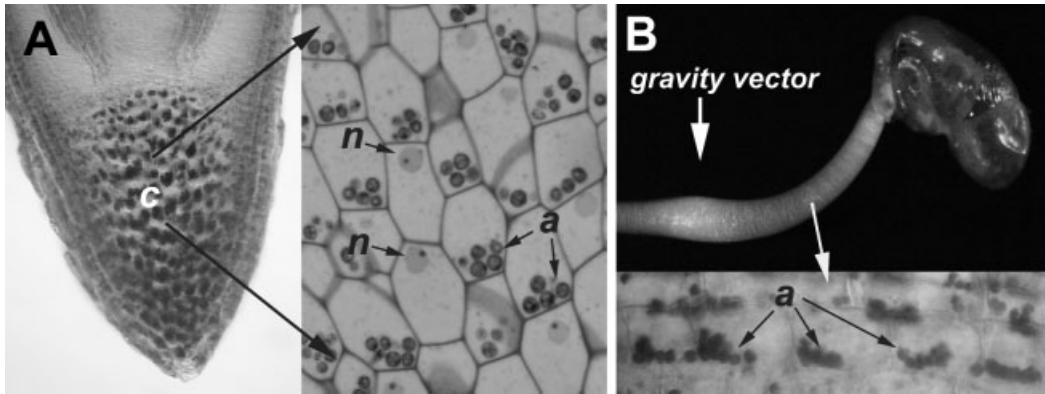


Figure 1.1 (also see Color Section). A. Longitudinal section of the root cap of *Medicago truncatula* showing the centrally located columella cells (*c*) containing starch-filled plastids (*a*, amyloplasts). Note that the columella cells are highly polarized with the nucleus (*n*) located at the upper side of the cell and amyloplasts (*a*) sedimented on the bottom side. B. Hypocotyl of a *Medicago truncatula* seedling bends upward when positioned horizontally. Longitudinal section of the reoriented hypocotyl shows amyloplasts (*a*) sedimented to the new bottom side of the cell. White arrow indicates the direction of gravity.

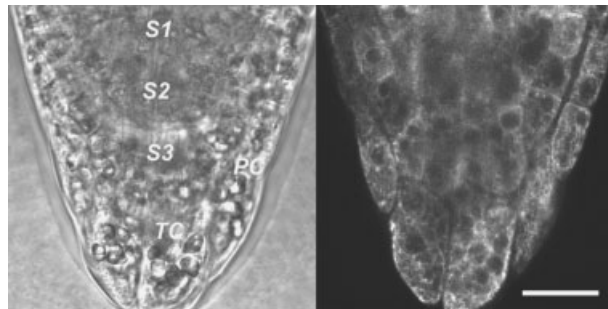


Figure 1.2. Brightfield and corresponding fluorescence micrograph of an *Arabidopsis* root cap expressing the actin binding domain (ABD-2) of Fimbrin. Actin filaments in the centrally located columella cells, stories 2 and 3 (S2, S3) appear to have a finer structure than the peripheral cap (PC) and tip cells (TC). Bar = 20 μm .

(Figure 1.2), had the strongest inhibitory effect on the gravity response—identifying those specific cells of the cap as the most important for gravity sensing. Destroying the lower part of the cap in horizontally positioned roots with heavy-ion microbeams also inhibited gravitropism, possibly by interfering with the cap tissue responsible for transmission of the gravity signal from the columella (Tanaka et al. 2002).

Another set of studies implicating the root cap in the gravitropic response employed a genetic approach to remove root cap cells. A protein synthesis inhibitor (diphtheria toxin A) was expressed under a root cap specific promoter in *Arabidopsis*, killing the expressing cells (Tsugeki and Fedoroff 1999). In addition to having altered morphology, the re-

sulting roots were agravitropic, providing further evidence that the cap is the primary site of gravity perception in roots.

Despite overwhelming evidence supporting the cap as a major gravity-sensing site in roots, there are sparse reports demonstrating that the root cap might not be the only tissue that is able to perceive gravity. Early experiments employing centrifugation methods suggested that the elongation zone might also be involved (reviewed in Boonsirichai et al. 2002). However, these experiments are difficult to interpret because the centrifugation technique itself possibly introduces mechanical effects that could contribute to the bending response of the root. More recently, Wolverton et al. (2002a) devised a method (named ROTATO) that allowed different regions of the root outside the cap to be maintained at a defined angle to the vertical (continuously gravistimulated). If a section of the elongation zone of the root was kept at a defined angle, curvature of the root persisted even after the root cap had reached its normal vertical position. From these experiments it was concluded that the elongation zone can contribute to gravitropic sensing, although to a lesser extent than the root cap. It was estimated that 20% of the total rate of curvature originates from the distal elongation zone or the apical portion of the central elongation zone.

The finding that the elongation zone contributes to root gravitropic sensing might explain why roots sometimes curve past the vertical and why starchless mutants of *Arabidopsis* still have a residual gravitropic response (Wolverton et al. 2002a, b). In support of the notion that other tissues outside the cap can sense gravity was the recent observation that gravitropic curvature in decapped roots of maize can be restored by myosin and actin inhibitors. This indicates the existence of a mechanism for gravity sensing outside the cap that relies on a dynamic cytoskeleton (Mancuso et al. 2006; see The Cytoskeleton in Gravity Perception section). Although these new findings continue to support the conclusion made more than a century ago that the root cap is a major site for gravity perception, it appears that it may not be the sole site. The availability of techniques such as ROTATO should allow more detailed investigations into alternative gravity-sensing sites in roots.

1.2.2 Hypocotyls and Inflorescence Stems (Dicotyledons)

In contrast to roots, shoots exhibit negative gravitropism, meaning that they grow upward. In shoots of dicots, sedimented amyloplasts were often observed in endodermal cells adjacent to the vasculature, leading to the proposal that the endodermis might be the primary gravity-sensing tissue in shoots (reviewed in Kiss 2000; Morita and Tasaka 2004; see Figure 1.1B). However, it was not until the late 1990s that a better appreciation of the importance of the endodermis for shoot gravitropism was realized. This was due to the fact that, unlike the cap in roots, which is easy to microsurgically remove, the endodermal cells in shoots are physically difficult to manipulate because of their internal location within the organ. The isolation of a series of *Arabidopsis* mutants with defects in shoot gravitropism (*sgr*) facilitated the genetic analysis of gravity-sensing and signaling mechanisms in shoots (Morita and Tasaka 2004). Of particular importance was the identification of two *Arabidopsis* mutants (*sgr1* and *sgr7*) that were allelic to the radial pattern mutants *scarecrow* (*scr*) and *short-root* (*shr*), respectively. *sgr1* and *sgr7* were shown