Protoplasma (2006) 229: 175–181
DOI 10.1007/s00709-006-0216-9

PROTOPLASMA
Printed in Austria

Auxin transport and gravitational research: perspectives

K. Palme*, **A. Dovzhenko,** and **F. A. Ditengou**

Institute of Biology II, University of Freiburg, Freiburg

Received December 2, 2005; accepted February 1, 2006; published online December 16, 2006
© Springer-Verlag 2006

Summary. Gravity is a fundamental factor which affects all living organisms. Plant development is well adapted to gravity by directing roots downward and shoots upwards. For more than a century, plant biologists have been fascinated to describe the molecular mechanisms underlying the gravitropic response of plants. Important progress towards signal perception, transduction, and response has been made, but new tools are beginning to uncover the regulatory networks for gravitropic control. We summarise recent progress in study of gravitropism and discuss strategies to identify the molecular basis of the gravity response in *Arabidopsis thaliana*. This will put us on a road towards the molecular systems biology of the Arabidopsis gravitropic response.

Keywords: *Arabidopsis thaliana*; Root; Gravitropism; Auxin transport.

Introduction

Gravitational force is one of the few constants in the evolution of life on Earth which played an important role in the development of flora and fauna. Although there is a huge and obvious difference between animals and plants, they share a broad basis of genetic repertoire related to developmental and physiological processes. This makes plants the perfect research object for the effects of gravity on living beings: as sessile organisms they cannot move like animals, but instead respond by growth and development. Hence the effect of gravity can be more easily monitored. Studies on plants and their responses to gravity have focussed over many years on the responses elicited by the direction of the gravity vector and how plants then change their pattern of growth and development. A well studied topic was, for example, how plant stems and roots alter their growth direction to maintain a set angle with the gravity vector (gravitropism). An important research topic was also the question whether growth and development of plants are impaired by the absence of gravity and whether metabolic processes are altered under conditions of reduced gravity. Another important question in this context was to define whether gravity is indeed essential for exertion of metabolic processes, regardless of the direction of the gravity vector. Finally, over the past few years, space agencies were pushing work aiming to grow plants successfully in space either for experimental purposes or for human consumption on long-term missions in case lunar or mars colonisation are ever set up. Conditions have been assessed that achieve optimal crop yields, the best plants have been defined to grow in space and we have to learn about the problems that are inherent to plants growing in a gravity-free and totally enclosed system. Despite numerous relevant accomplishments in all these different areas, we need further research as many problems and questions are still unanswered. Though it is clear that the gravitational force has a crucial influence on a number of biological processes, there is only little known yet about the molecular mechanisms with which higher plants perceive gravity and about the influence of gravity on the substances that transmit the information within these organisms.

Focus on model species

Major progress has been made in the past few years using *Arabidospis thaliana* as a model for gravity research (Perrin et al. 2005). This plant has many advantages for large-scale molecular research. The short generation time, large number of offspring, mutants in literally every gene, and a small, fully sequenced genome make it an ideal research object (Somerville and Meyerowitz 2001). Molecular ex-

---

* Correspondence and reprints: Institute of Biology II, University of Freiburg, Schänzlestrasse 1, 79104 Freiburg, Federal Republic of Germany.

ploration of signal transduction pathways is now starting to shed light on the major pathways that affect gravity signal transduction, particularly on how the plant hormone auxin controls the curving of plants in response to light and gravity. Several excellent recent reviews have been published over the past few years (Boonsirichai et al. 2002, Blancaflor and Masson 2003, Teale et al. 2005).

Therefore, we will only discuss recent progress in understanding gravitropism in Arabidopsis and then outline where future research activities should be focused in order to understand the gravity response in a Biosystems context (Kitano 2005). Particularly, we need to understand how plants like Arabidopsis monitor a wide range of external and internal signals and compute appropriate developmental responses. How do Arabidopsis plants translate these myriad signals to an appropriate response integrating these signals to reach a finely balanced physiological reaction? As they respond by irreversible growth to many of these signals, their signal transduction systems must compute each developmental decision with care. The huge number of receptor kinases encoded by their genome may indicate that these processes are regulated by complex networks. Good research on the long way to understand gravity perception and transduction have been recent studies focusing on questions like the following. What is the role of auxin in gravity-mediated growth processes (reviewed by Wolverton et al. 2002)? Does gravity drive the expression of particular classes of genes? Does microgravity inhibit or induce particular classes of genes? Does gravity influence the expression of the same genes in different organs, like roots and shoots? Does gravity lead to changes in protein localisation and/or delivery, and if so, what is the role for molecules involved in auxin transport and/or signalling? *Arabidopsis thaliana* has served as an ideal model and will provide a basis for Biosystems approaches and mathematical modelling of gravity responses.

Auxin transport

Since the early days of Darwin and later Cholodny and Went, auxin transport has been thought to be a central component of the gravitropic signalling mechanism. The polar auxin transport stream mediates basipetal, long-distance movement of auxin from its apical site(s) of synthesis. Local auxin transport streams within the root apex redistribute auxin acropetally amongst the rapidly dividing and growing cells of the meristematic and elongation zones. On the basis of the identification of key factors of both auxin influx and efflux, the role of auxin transport in root gravitropism could be studied more closely in Arabidopsis (Chen et al. 1998, Gälweiler et al. 1998, Müller et al. 1998, Friml et al. 2002b). Specific emphasis was given to use auxin efflux and influx carriers as tools to obtain a comprehensive view on the flux of auxin from the site of gravity perception to the growth region. Important questions were which proteins besides the auxin influx facilitator AUX1 (Bennett et al. 1996) and the auxin efflux facilitator PIN2 are involved in this process and whether biosensors which might allow a direct visualisation of the auxin transport stream could be developed. Another question was directed towards the dissection of gene expression networks affected by gravity on earth and in space using microarray gene chips covering the full transcriptome of Arabidopsis.

Mutants – tools to dissect gravity-mediated signalling

Arabidopsis mutants were a major tool to dissect the molecular basis of gravitropic signalling (Wisman et al. 1998). It allowed the use of mutations within components of the Arabidopsis root gravity perception and signalling machinery for analysis. Physiological analysis of auxin transport clearly suggests a role for auxin influx and efflux carriers, which probably act in larger protein complexes (Noh et al. 2003, Vicente-Agullo et al. 2004). Genetic strategies led to the identification of mutants with defects in polar auxin transport. Of particular relevance were the *aux1* and *pin1* mutants (Gälweiler et al. 1998, Swarup et al. 2001). Both mutations typify candidate genes for the auxin influx and efflux carrier. In order to assess the role of AUX1 and members of the PIN family in gravitropic control, it was necessary to clarify in which root cells members of these families were expressed (Paponov et al. 2005). In order to assess their roles in auxin influx and efflux, their location within individual cells needed to be determined. Moreover, markers that allowed to visualise relative auxin contents at cellular resolution, and thereby the direction of auxin transport, had to be developed. Finally, gene expression profiles in the presence and absence of gravity vectors needed to be determined both on ground and in space.

Analysis of auxin influx and efflux facilitator families

Despite the fact that final biochemical proof of AUX1 function as an auxin uptake carrier is still lacking, several lines of evidence support the involvement of AUX1 in auxin influx. The strongest support came from a detailed analysis of the *aux1* mutant phenotype. Moreover, this phenotype can be mimicked by growing seedlings on a recently isolated inhibitor of auxin influx. The AUX1 proteins are related to a small subfamily of amino acid transporters (amino acid/auxin:proton symport permeases AAAP) which show homology to bacterial permeases. AUX1 was localised within Arabidopsis root tissue by an epitope-tagging approach. The AUX1 protein was detected in a remarkable pattern in a subset of stele, columella, lateral root cap, and epidermal cells exclusively in the root tips. Disruption of AUX1 caused changes in cell-specific auxin accumulation associated with tissues mediating basipetal auxin transport. AUX1 was localised with PIN1, the efflux facilitator at opposite cellular positions suggesting a role of AUX1 protein in steering the bulk flow of auxin via the protophloem to the root apical meristem and thus indicating the first molecular connection between polar and nonpolar auxin transport routes.

The PIN efflux facilitator family from Arabidopsis consists of 8 members (reviewed in Paponov et al. 2005). Homologous genes were found throughout the plant kingdom, indicating a highly conserved gene family unique to plants. Regions of identity are restricted to the amino and carboxyl termini with about ten potential transmembrane domains, five at the amino terminus and five others at the carboxyl terminus. The internal segment of the protein is less conserved in sequence than the putative membrane spanning domains. The amino acid sequence of the auxin efflux facilitator proteins indicates that they could transit the secretory pathway to the plasma membrane and suggests relationships with a major facilitator group of membrane transporters, many of which are known to be involved in removal of toxic compounds from the interior of bacterial cells.

Mutants were isolated for each member and analysed for phenotypes. Besides *pin2* also *pin3*, *pin4*, and *pin7* had altered gravitropic responses. Multiple mutants have been generated lacking different PIN genes as well as AUX1. Predictably, certain combinations of mutants revealed the role of auxin transport in different processes such as cell division, cell elongation, pattern formation, and cell differentiation (Blilou et al. 2005).

Localisation of auxin influx and efflux carriers

New technologies allowed high-resolution monitoring of PIN1 and AUX proteins simultaneously. Most importantly, it was possible to monitor PIN3 localisation in root columella cells in direct response to gravity vector changes. It was shown that PIN3 accumulated predominantly at the lateral plasma membrane surface of columella cells and to vesicles that cycle in an actin-dependent manner. Upon gravistimulus, PIN3 rapidly relocalised laterally suggesting that actin-dependent relocalisation provides a mechanism for redirecting auxin flux to trigger asymmetric growth. Several other PIN proteins (i.e., PIN1, PIN4, PIN7) act in concert with PIN3. It was further demonstrated that PIN4 is important for generating a sink for auxin below the quiescent centre of the root meristem which is essential for auxin distribution (Friml et al. 2002a, b; Geldner et al. 2001; Steinmann et al. 1999).

Visualisation of auxin transport in roots

The presence of lateral auxin gradients in root tips after gravistimulation has not been verified convincingly before. Therefore, a fluorescent biosensor was developed that allowed monitoring of auxin levels at cellular resolution. The green-fluorescent protein (GFP) was fused to an endoplasmic-reticulum (ER) retention signal so that it was targeted to the ER. Expression of this fluorescent fusion protein was controlled by a synthetically constructed auxin-responsive promoter derived from elements of an auxin early-responding gene. In the absence of stimuli, GFP signals appeared almost exclusively in the quiescent centre, columella initials, and the columella cells, where gravity is perceived via amyloplasts (starch-filled plastids) containing statoliths. Upon gravistimulation the signal also appeared in the distal lateral root cap (LRC) at the lower side of the roots. Subsequently it spread to the entire LRC at the lower side, including the proximal LRC. A change in the signal could already be observed when roots were subjected to gravity stimuli for only 15 min. By examining the gravitropic response of roots in the presence of natural and synthetic auxins, it was found that roots displayed gravitropic curvature even when auxins were exogenously applied to roots at concentrations that inhibit root growth. This was one of the most vulnerable points of the Cholodny–Went hypothesis because exogenous auxins are likely to dissipate gradients of auxin, which are predicted to be formed by gravity stimuli. This theory was independently developed in the 1920s by Nicolai Cholodny and Frits Went to explain how roots and stems curved in response to light or gravity. When stimulated by light or gravity, auxin was proposed to be transported laterally in the tip, producing a lateral gradient of auxin concentration. Subsequently, the auxin gradient is brought to the elongation zone by basipetal transport of auxin, resulting in differential growth between the two sides of roots or stems. Although this model has been popular since it was first proposed, it remained a hypothesis for 70 years because almost no knowledge was available on the molecular machinery that facilitates the basipetal and lateral transport of auxin, which is crucial for this model.

Using the auxin biosensor DR5-GFP to monitor auxin concentration, the gravitropic response of roots was examined in the presence of natural and synthetic auxins, such as indole-3-acetic acid, 1-naphthaleneacetic acid, and 2,4-dichlorophenoxyacetic acid. It was observed that gravity-induced GFP signal asymmetry within the root cap was detected already 15 min after gravistimulation. Gravistimulation led to a shift of signal distribution with the LRC region. In the presence of exogenous auxins like 1-naphthaleneacetic acid, strong signal asymmetry was observed indicating that externally applied auxins do not dissipate auxin gradients within roots. From simultaneous treatments of roots with either indole-3-acetic acid or with auxin influx and efflux inhibitors it became clear that the reporter was clearly auxin responsive. It was found that efflux and influx carriers differentially contributed to the graviresponse. Lateral auxin transport in the root cap exclusively required efflux carriers, whereas basipetal auxin transport depended on both auxin influx and efflux. These findings were consistent with the localisation of the auxin efflux facilitator protein PIN3 in the columella and the influx facilitator in the LRC.

Thus, for the first time, auxin transport was shown at cellular resolution and it became possible to differentiate between gravity-induced lateral and basipetal auxin transport. Experimental pharmacological blocking of the lateral auxin transport together with PIN3-dependent relocalisation suggested regulation at the level of posttranslational protein targeting. The road map of auxin flux from the stele through the quiescent centre and columella into the lateral root cap cells raises new questions as to how the positional information provided by the statolith sedimentation affects regulation of auxin carrier location and activity. While previous analysis clearly showed that a range of physiological responses like pH control or ion transport processes are rapidly mediated by gravity, the underlying signal transduction pathways and regulatory networks remain unclear. Therefore, further systematic genomewide analysis of gravity-mediated gene regulation need to be undertaken.

Role of lateral root cap and epidermal cells

From the report by Ottenschläger et al. (2003), it became clear that LRC and epidermal cells play important roles in the lateral and basipetal transport of auxin. These roles have been recently elegantly confirmed by Swarup and co-workers (2005). They mapped those tissues that function in auxin transport during the gravitropic response. The expression of AUX1 was visible in specific tissue domains in *aux1* roots. In addition, they examined the rescue of the mutant's agravitropic phenotype, and indeed, expressing HA-AUX1 in columella, LRC, and epidermal tissues successfully rescued *aux1* gravitropism, while expression of HA-AUX1 in stele and columella did not rescue *aux1* gravitropism. Domains of root tissues that are required to express AUX1 were thus defined. It appears that expressing AUX1 in LRC and expanding tissues rescued *aux1* gravitropism, whereas by contrast, expressing AUX1 just in the LRC did not. Taken together these results are consistent with auxin acting as intercellular signal that requires AUX1 to facilitate its transport via LRC and expanding epidermal cells during gravitropic response. Therefore, expanding epidermal cells are required to perform both auxin transport and response functions, whereas the LRC is required for auxin transport following gravitropic stimulus. For the first time, the authors also developed a mathematical model simulating the outer elongating-zone tissues and incorporating known auxin influx or efflux carrier expression and localisation patterns. They simulated the gravitropic signal by supplying an auxin asymmetry to the apical end of the virtual root model and then monitored the movement of lateral auxin through the elongation-zone tissues. The model is in agreement with previous experimental observations, highlighting its relevance for prediction of new experiments.

Gene networks underlying graviresponse

The simple question of how plants grow has challenged scientists for more than 100 years. In roots, new cells arise continually at the meristem and enlarge slowly in the meristem and then more rapidly when they move out of the meristematic region. An enormous increase in cell volume of up to 100-fold can occur, depending on internally and environmentally controlled conditions. But despite descriptive analysis, not much is known so far about the underlying mechanisms that determine growth and particularly the control of differential growth so that roots inhibit growth on the side directed towards gravity and speed up growth on the opposite side. This implicates a need for both transcriptional and translational regulation in the control of the gravity response. One way to start to obtain a blue print on which genes are active in the growing zones is using microarrays and to study the transcriptional regulation of the full complement of genes in response to gravity.

To determine which genes are up-regulated and which ones are down-regulated during the time course of minutes and hours after gravity stimulation, gene expression profiles were obtained from the early stage of the Arabidopsis root gravitropic response with high-density oligonucleotide probe microarrays representing about 23,000 Arabidopsis genes. This corresponds to almost the full Arabidopsis genome. Several attempts were undertaken to obtain first views on the transcriptional networks of gravity stimulation in the Arabidopsis root apex after reorientation, monitoring transcript levels of 22,744 genes in a time course during the first hour after the stimulus (Moseyko et al. 2002, Kimbrough et al. 2004). The majority of the gravity-regulated genes belonged to well known categories of genes important in oxidative stress and plant defence, heat shock, metabolism, transcription, cell wall/plasma membrane, signal transduction, and hormonal control. 7.6% of the genes were altered after gravity stimulation and a similar number was measured in response to mechanical stimulation. Both responses share many genes, but 65 were found to be exclusively up-regulated under the conditions used by Kimbrough et al. (2004). Interestingly, several of these genes encode transcription factors like MYB and KNAT and proteins involved in coping with environmental stresses like cold, dehydration, and pathogen responses. Under conditions of gravitropic and mechanic stimulation, cell cycle activities, cell wall biosynthesis, and production of new cytoskeletal elements were all down-regulated, whereas energy metabolism, electron transfer, defence mechanisms, and transcriptional processes were up-regulated. However, in most instances genes were not simply switched on or off, but rather their relative expression levels seemed to be modulated.

The most surprising response was the up-regulated expression of a large group of genes belonging to the functional category of oxidative burst and plant defence. A functional significance of this, however, remains to be determined. Similar, but more detailed studies are in progress on many of the stimuli-specific regulated transcripts in an effort to elucidate their function in the mechanism of response to gravity and mechanical stimulation (P. Wolff and K. Palme unpubl.). Follow-up localisation studies and mutant analysis will allow us to determine whether the differentially regulated genes are really essential for gravity sensing and/or response. Further identification of the effectors regulating fast and transient gravity-induced gene expression will aid in determining the initial signal transduction events responsible for gravity sensing in plant root tips.

New approaches: chemical genetics and RNA interference

Apart from the classical genetic and advanced molecular studies, new strategies have been recently applied to study gravitropic growth. Chemical genomics is a powerful way to circumvent problems associated with lethality and redundancy when dissecting dynamic and complex pathways (Darvas et al. 2004, Shogren-Knaak et al. 2001, Torrance et al. 2001). Complex gene networks like those identified by transcriptional profiling of gravitropic responses call for such approaches. As many mutations in the endomembrane system can result in agravitropic phenotypes similar to those found for auxin, a 10,000-member small compound library was screened for substances causing aberrant gravitropic responses (Surpin et al. 2005). By microscopic inspection of vacuole morphology, 219 chemicals were identified as primary hits, from which 199 seemed to inhibit and 20 seemed to promote the gravitropic response. One of the chemicals was found to be structurally related but not identical to 2,4-dichlorophenoxyacetate, a synthetic auxin. This chemical is probably hydrolysed to active auxin, thereby inhibiting root and shoot gravitropic responses. The other chemicals were unrelated to auxin but still affected gravitropism and vacuole morphology in a tissue-specific manner, suggesting a close link between the endomembrane system and the gravitropic response. Interestingly, one of the chemicals was antagonistic to the response of roots to auxin and another chemical corresponded to phyocyanin, a bacterial metabolite important for endomembrane targeting in yeast cells.

Altogether these and other attempts clearly demonstrate the power of chemical genomics to investigate the function, modification, and interaction of proteins and modulators of complex signalling pathways in plants (Zouhar et al. 2004, Armstrong et al. 2004). Such investigations will be particularly useful for the study of proteins which differ with respect to their dynamic cellular localisation. RNA interference, a posttranscriptional method of gene silencing, discovered in *Caenorhabditis elegans* (Fire et al. 1998), also proved an important tool for deeper understanding of gene networks and signalling pathways. In the animal research field, RNA interference technology is often used for disruption of specific targets or cellular pathways followed up by further analysis of specific changes in gene expression in cells (Boutros et al. 2004). In concert with chemical compound screens where small molecules mimic specific RNA interference disruption profiles, small interfering RNA molecules could be extremely useful to define mechanisms, networks, and rules involved in gravitropic response at cellular as well as at whole-organ levels (MacBeath et al. 2001). These approaches will complement established genetic methods such as mutation genetics or knockout organisms as the small molecules will react rapidly within spatially restricted areas, because reversible biological effects thereby enable transient studies of protein function with tuneable and conditional degrees of phenotype expression. As gene knockouts in important pathways are frequently lethal and not easily accessible, chemical genomics provides unique opportunities to probe and investigate gravitropism in future.

Perspective

Important advances have been made over the past few years to unravel the mysteries of gravitropic signal perception and transduction that enable the root cells to distinguish their position within the organ and with respect to the gravity vector. A novel fluorescent biosensor makes possible the visualisation of in vivo changes in auxin levels and links them to differential growth responses with elongation rates on the upper side being faster than those on the lower side of the tissue. In addition, transcriptional response mechanisms were delineated from whole-genome microarray analyses of Arabidopsis root apices after gravity stimulation. Functional gene categories were determined for up- and down-regulated transcripts and rapid transient changes in the relative abundance of specific transcripts detected and clustered in coordinating regulated genes related to specific events.

Molecular systems biology in gravitational research

However, identifying all the genes and proteins, i.e., the system elements that are important for gravitropism, is like listing all the parts of a technical product like an automobile. No matter how complete such a list may be, by itself it will never allow an understanding of the complex machinery underlying the graviresponse. What we really need to know before we can understand the Arabidopsis gravitropism is how all the system elements integrate this response. Systems biology is a new branch of biology that attempts to discover and understand biological properties that emerge from the interactions of many system elements. The major reason why systems biology is gaining so much interest today is that progress in molecular biology, particularly in high-throughput genomics and proteomics, has allowed us to collect comprehensive data sets on a wide variety of organisms and responses which now need to be put into a functional context. The power of joining biology with mathematics and modelling and the synergies resulting from these interdisciplinary activities are enormous. We therefore need methods which allow to generate quantitative data on the graviresponse suitable for systems analysis and to integrate this knowledge at the molecular, cellular, and organismal level; that is, to gain a systems level understanding. As the Arabidopsis root has a unique architecture which lends itself very well to systems biology approaches, we will profit from high-throughput experimental tools to gather and configure highly specific sets of data ready to be integrated into interactive models. We need methods to study interacting proteins, metabolites, and cellular processes dynamically and quantitatively. We need to organise and store massive data sets in standard and easily accessible format and we must be able to create model simulations of key root processes. First steps into this direction have been successfully taken but we now need to convince funding agencies to support activities in the area of integrative and systems biology.

Root growth constitutes a complex biological system in which the four different phases of gravity response, namely, perception, transduction, transmission, and response need to be described in quantitative terms. Future activities need to define a gravity process pipeline starting with standardised quantitative and qualitative data collection and integration of data for reconstruction and mathematical modelling. Due to the precisely defined architecture of Arabidopsis roots, analysis of root growth will be the key model for quantitative understanding of plant growth in which part lists generated by several "omics" technologies will be integrated, opening up a new area for a systems-based analysis of these important pathways.

Acknowledgement

We are grateful to Arthur Molendijk for critical reading of the manuscript. We also gratefully acknowledge support by the DLR, ESA, the Deutsche Forschungsgemeinschaft (SFB 388, SFB 592), and the FCI.

References

Armstrong JI, Yuan S, Dale JM, Tanner VN, Theologis A (2004) Identification of inhibitors of auxin transcriptional activation by means of chemical genetics in Arabidopsis. Proc Natl Acad Sci USA 101: 14978–14983

Bennett MJ, Marchant A, Green HG, May ST, Ward SP, Millner PA, Walker AR, Schulz B, Feldmann KA (1996) Arabidopsis AUX1 gene: a permease-like regulator of root gravitropism. Science 273: 948–950

Blancaflor EB, Masson PH (2003) Plant gravitropism. Unraveling the ups and downs of a complex process. Plant Physiol 133: 1677–1690

Blilou I, Wildwater M, Willemsen V, Paponov I, Friml J, Heidstra R, Aida M, Palme K, Scheres B (2005) The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. Nature 433: 39–44

Boonsirichai K, Guan C, Chen R, Masson PH (2002) Root gravitropism: an experimental tool to investigate basic cellular and molecular processes underlying mechanosensing and signal transmission in plants. Annu Rev Plant Biol 53: 421–447

Boutros M, Kiger AA, Armknecht S, Kerr K, Hild M, Koch B, Haas SA, Heidelberg Fly Array Consortium, Paro R, Perrimon N (2004) Genome-wide RNAi analysis of growth and viability in *Drosophila* cells. Science 303: 832–835

Chen R, Hilson P, Sedbrook J, Rosen E, Caspar T, Masson PH (1998) The *Arabidopsis thaliana AGRAVITROPIC 1* (*AGR1*) gene encodes a component of the polar auxin-transport efflux carrier. Proc Natl Acad Sci USA 95: 15112–15117

Darvas F, Guttman A, Dorman G (eds) (2004) Chemical genomics. Marcel Dekker, New York

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature 391: 806–811

Friml J, Benkova E, Blilou I, Wisniewska J, Hamann T, Ljung K, Woody S, Sandberg G, Scheres B, Jürgens G, Palme K (2002a) AtPIN4 mediates sink-driven auxin gradients and root patterning in Arabidopsis. Cell 108: 661–673

Friml J, Wisniewska J, Benková E, Mendgen K, Palme K (2002b) Lateral relocalisation of auxin efflux regulator PIN3 mediates tropic growth responses in Arabidopsis. Nature 415: 806–809

Gälweiler L, Guan C, Müller A, Wisman E, Mendgen K, Palme K (1998) Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. Science 282: 2226–2230

Geldner N, Friml J, Stierhof YD, Jürgens G, Palme K (2001) Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. Nature 413: 425–428

Kimbrough JM, Salinas-Mondragon R, Boss WF, Brown CS, Sederoff HW (2004) The fast and transient transcriptional network of gravity and mechanical stimulation in the Arabidopsis root apex. Plant Physiol 136: 2790–2805

Kitano H (2005) International alliances for quantitative modelling in systems biology. Mol Systems Biol 1: DOI 10.1038/msb4100011

MacBeath G (2001) Chemical genomics: what will it take and who gets to play? http://genomebiology.com/2001/2/6/comment

Moseyko N, Zhu T, Chang HS, Wang X, Feldman LJ (2002) Transcription profiling of the early gravitropic response in Arabidopsis using high-density oligonucleotide probe microarrays. Plant Physiol 130: 720–728

Müller A, Guan C, Gälweiler L, Tänzler P, Huijser P, Marchant A, Parry G, Bennett M, Wisman E, Palme K (1998) *AtPIN2* defines a locus of Arabidopsis for root gravitropism. EMBO J 17: 101–109

Noh B, Bandyopadhyay A, Peer W, Spalding E, Murphy A (2003) Enhanced gravi- and phototropism in plant *mdr* mutants mislocalising the auxin efflux protein PIN1. Nature 423: 999–1002

Ottenschläger I, Wolff P, Wolverton C, Bhalerao R, Sandberg G, Ishikawa H, Evans M, Palme K (2003) Gravity-regulated differential auxin transport from columella to lateral root cap cells. Proc Natl Acad Sci USA 100: 2987–2991

Paponov I, Teale WD, Trebar M, Blilou I, Palme K (2005) The PIN auxin efflux facilitators: evolutionary and functional perspectives. Trends Plant Sci 10: 170–177

Perrin RM, Young LS, Murthy UMN, Harrison BR, Wang Y, Will JL, Masson PH (2005) Gravity signal transduction in primary roots. Ann Bot 96: 737–743

Shogren-Knaak MA, Alaimo PJ, Shokat KM (2001) Recent advances in chemical approaches to the study of biological systems. Annu Rev Cell Dev Biol 17: 405–433

Somerville CR, Meyerowitz EM (eds) (2001) The Arabidopsis book. American Society of Plant Biologists, Rockville, Md, http://www.aspb.org/publications/arabidopsis/

Steinmann T, Geldner N, Grebe M, Mangold S, Jackson C, Paris S, Gälweiler L, Palme K, Jürgens G (1999) Coordinated polar localization of auxin efflux carrier PIN1 by GNOM ARF GEF. Science 286: 316–318

Surpin M, Rojas-Pierce M, Carter C, Hicks GR, Vasquez J, Raikhel NV (2005) The power of chemical genomics to study the link between endomembrane system components and the gravitropic response. Proc Natl Acad Sci USA 102: 4902–4907

Swarup R, Friml J, Marchant A, Palme K, Bennett M (2001) AUX1 localisation has uncovered a novel transport pathway for IAA in the Arabidopsis root apex. Genes Dev 15: 2648–2653

Swarup R, Kramer E, Perry P, Knox K, Leyser O, Haseloff J, Beemster G, Bhalerao R, Bennett M (2005) Root gravitropism requires lateral root cap and epidermal cells for transport and response to a mobile auxin signal. Nat Cell Biol 7: 1057–1065

Teale W, Paponov I, Ditengou F, Palme K (2005) Auxin and the developing root of *Arabidopsis thaliana*. Physiol Plant 123: 130–138

Torrance CJ, Agrawal V, Vogelstein B, Kinzler KW (2001) Use of isogenic human cancer cells for high-throughput screening and drug discovery. Nat Biotechnol 19: 940–945

Utsuno K, Shikanai T, Yamada Y, Hashimoto T (1998) AGR, an agravitropic locus of *Arabidopsis thaliana*, encodes a novel membrane-protein family member. Plant Cell Physiol 39: 1111–1118

Vicente-Agullo F, Rigas S, Desbrosses G, Dolan L, Hatzopoulos P, Grabov A (2004) Potassium carrier TRH1 is required for auxin transport in Arabidopsis roots. Plant J 40: 523–535

Wisman E, Hartmann U, Sagasser M, Baumann E, Palme K, Hahlbrock K, Saedler H, Weisshaar B (1998) Knock-out mutants from an *En-1* mutagenized *Arabidopsis thaliana* population generate phenylpropanoid biosynthesis phenotypes. Proc Natl Acad Sci USA 95: 12432–12437

Wolverton C, Ishikawa H, Evans M (2002) The kinetics of root gravitropism: dual motor and sensors. J Plant Growth Regul 21: 102–112

Zouhar J, Hicks GR, Raikhel NV (2004) Sorting inhibitors (Sortins): chemical compounds to study vacuolar sorting in Arabidopsis. Proc Natl Acad Sci USA 101: 9497–9501