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Plant development: new models and approaches bring progress

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In August 2006, plant biologists gathered at the FASEB 'Mechanisms in Plant Development' meeting in Vermont, which was organized by Laurie Smith and Ueli Grossniklaus. A variety of plant developmental mechanisms were presented at this meeting and, although many talks focused on *Arabidopsis thaliana* as a primary model in which to study plant development, research in maize, tomato, *Chlamydomonas* and other plants also provided insight into various topics, such as cell-type specification, small RNA biosynthesis and action, hormone perception and transport, and cell and organ size.

From the beginning: fertilization and embryonic development

Like animals, plant embryos develop from the fertilization of an egg cell by a sperm. Unlike animals, the sperm is delivered to the egg from the tip of a growing pollen tube. To reach the egg, the pollen tube must first germinate and then seek out the egg cell by growing through the tip of the female organ (the gynecium) to find the ovule that houses the egg. This seeking process involves a complex interplay between the pollen tube and the female reproductive tissues, with signals coming from both sides. The nature of these signals and the ability of a plant to distinguish its own pollen is still largely unknown. June Nasrallah (Cornell University, Ithaca, NY, USA) presented her laboratory's work on the mechanisms of self-incompatibility (SI). Although *Arabidopsis thaliana* is self-fertile, many plant species can recognize their own pollen and prevent pollen-tube development. One such system uses signaling from a receptor kinase expressed at the tip of the gynecium (the stigmatic tissue) that recognizes a cysteine-rich ligand that is localized to the pollen coat. These two proteins (which are encoded by two closely-linked genes that are referred to collectively as the *S* locus) co-evolve in such a way that the pollen ligand binds and activates only the receptor kinase encoded in the same *S*-locus haplotype, consequently triggering the arrest of self pollination. Although this ligand/receptor pair was discovered several years ago, the signal transduction events downstream of the receptor are poorly understood (Kusaba et al., 2001; Nasrallah et al., 1988). June Nasrallah described an elegant system that can be used to investigate this process further. Her group has recreated the SI system from *A. lyrata*, a species that exhibits SI, in *A. thaliana* by creating transgenic lines that express the *A. lyrata S* locus (Nasrallah et al., 2002). These plants are now amenable to genetics, and several mutants have been isolated at unique loci that show varying degrees of self-fertility.

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As the pollen tube invades the stigma, it must travel through the gynecium and seek out the ovules. Tetsuya Higashiyama (University of Tokyo, Tokyo, Japan) described an in vitro system to study this process using *Torenia fournieri,* a plant with exserted embryo sacs (Higashiyama et al., 1998). Using this system, his group discovered that the pollen tube must first pass through the maternal tissue in order to gain competence to seek out the ovules. A 70 kDa protein derived from the ovule [activation molecule for response-capability (AMOR)] is also necessary for the pollen tube to gain competence. Once competent, the pollen tube can then respond to a species-specific attractant produced from the synergid cell to find the ovule (Higashiyama, 2002; Higashiyama et al., 2006). Higashiyama's group is currently in the process of collecting over 65 million *Torenia* ovules in order to identify the AMOR protein.

After the egg is fertilized, the diploid embryo starts to develop. Polarity becomes apparent at the first cell division, which is asymmetric – giving rise to a small apical cell and a larger basal cell. The apical cell will give rise to most of the resulting embryo, whereas the basal cell will give rise to a nutritive structure (the suspensor) and a portion of the root meristem. Martin Bayer (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA) described his work on SHORT SUSPENSOR (SSP), which helps to specify the extraembryonic suspensor. *ssp* mutants have reduced, or no, suspensor growth and can be rescued by a constitutively active form of the MAPKK kinase protein YODA (YDA) (Lukowitz et al., 2004). *yda* mutants resemble *ssp* mutants, and YDA is probably downstream of SSP in suspensor specification. Bayer then showed that the *ssp* phenotype is observed only when the mutant allele is derived from the pollen, regardless of the genotype of the egg cell. This novel mode of inheritance may therefore shed light on a new mechanism of cell-type specification in the embryo. Dolf Weijers (Wageningen University, Wageningen, The Netherlands) described a mechanism that occurs later in embryogenesis: root stem cell specification. Previous work has shown that the auxin response factor MONOPTEROS (MP, also known as ARF5 and IAA24) is necessary for root apical meristem formation and is antagonized by the AUX/IAA protein BODENLOS (BDL, also known as IAA12 – *Arabidopsis thaliana* Database) (Hamann et al., 2002; Hardtke and Berleth, 1998). The mystery was that neither of these proteins are found in the cells where the first defects in cell division are observed (see Fig. 1) (Weijers et al., 2006). By analyzing microarray data sets from *mp*, *bdl* and wild-type seedlings, Weijers and colleagues have studied a set of transcription factors [referred to as targets of MONOPTEROS (TOM)] that are expressed in the same cells as MP in embryos and require MP for their expression. Weijers reported that one of these transcription factors, TOM3, may explain the non-cell autonomous action of MP, as TOM3 appears to move into the cells directly below those that express MP – the same cells that undergo aberrant cell divisions in a *mp* mutant.

Cell-type specification in the epidermis

A question common to both plant and animal development is: how does a cell become different from its neighbors in a seemingly homogenous context? The development of root hairs from the root epidermis, and of trichomes and stomates from the leaf epidermis, are excellent systems in which to study this question. John Schiefelbein (University of Michigan, Ann Arbor, MI, USA) drew on the work from his laboratory and from several others to describe how root hairs form. The epidermis of the root contains both hair cells and non-hair

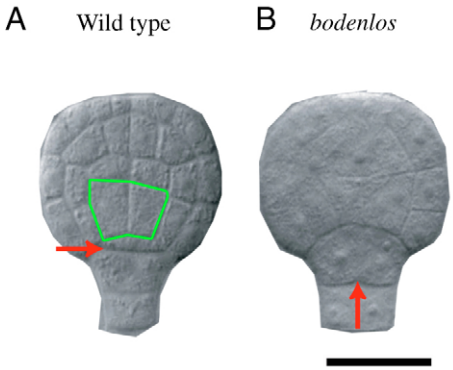

Fig. 1. A comparison of wild-type and *bodenlos* or *monopteros* seedlings and embryos. (**A**) Wild-type embryo. (**B**) Phenotype of a *bodenlos* (*bdl*) embryo. (Note that the phenotype of *monopteros* [*mp*] is identical to that of *bodenlos* [*bdl*].) Both *bdl* and *mp* mutants show a defect in the division plane of the hypophyseal cell (red arrows), and have defects in root formation, although both proteins accumulate in the cells above the hypophyseal cell (green outline in A). Scale bar: 25 μm. Fig. 1 courtesy of D. Weijers (Wageningen University, Wageningen, The Netherlands).

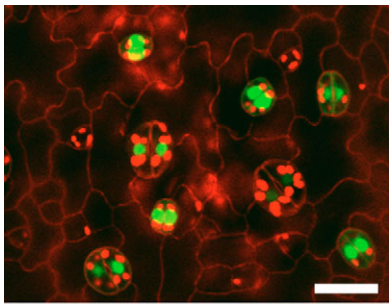

Fig. 2. *FAMAp::FAMA-GFP* expression in the guard cells of *Arabidopsis* stomates. An *Arabidopsis* leaf epidermis. FAMA protein (green) accumulates in the nucleus of the guard cells and is required for the differentiation of these cells. Chloroplasts appear red through autofluorescence. Scale bar: 25μm. Fig. 2E courtesy of D. Bergman (Stanford University, Palo Alto, CA, USA).

cells. Positional cues are thought to arise from the underlying cortex, as hair cells sit above two cortex cells (the H position) and non-hair cells sit above only one (the N position) (Galway et al., 1994). Although several proteins are required for N and H cell specification, many of them can move between cells, resulting in nearly identical complexes in all epidermal cells. Schiefelbein presented on how the negative regulation of these complexes ultimately defines the cell type. A major regulator of this process is a receptor-like kinase called SCRAMBLED (SCM) that probably receives a signal from the cortex cells and negatively regulates the transcription factor WEREWOLF (WER) in the H position (Kwak et al., 2005; Lee and Schiefelbein, 1999). This tips the balance of the transcriptional complexes and allows the root hair to form. Many of these same regulators are involved in trichome initiation and spatial patterning in the leaf epidermis. Martin Hülskamp (University of Cologne, Cologne, Germany) described how the TRANSPARENT TESTA GLABRA 1 (TTG1) protein is initially found in all cells, but then moves and is concentrated in the trichome cells (Schnittger et al., 1999). Using a TTG1/yellow florescent protein fusion, his group was able to visualize this movement, and he showed that TTG1 expressed in the subepdermis could rescue the *ttg* mutant. Finally, Dominique Bergman (Stanford University, Palo Alto, CA, USA) discussed the role of two related basic helix-loop-helix proteins [SPEECHLESS (SPCH) and FAMA] in stomate formation in the leaf epidermis (Fig. 2). Although related, these two proteins act at distinct steps in stomatal patterning, and FAMA cannot rescue *spch* when under the control of the *SPCH* promoter. Ongoing research should reveal which other proteins regulate the specific actions of these two genes.

Hormone production, perception and transport

The hormone auxin has been implicated in almost all aspects of plant growth and development. In the past several years, new tools and technologies have allowed plant biologists to investigate how auxin may be involved in the particular developmental process that they study. The discovery of auxin biosynthetic genes, the cloning of the PINFORMED (PIN) family of auxin efflux carriers and the creation of an auxin-responsive reporter (DR5) has advanced the field considerably, and these new reagents have been used extensively to answer specific developmental questions (Galweiler et al., 1998; Muller et al., 1998; Palme and Galweiler, 1999; Ulmasov et al., 1997; Zhao et al., 2001). Three groups presented work on computer models, based on the flow and localized concentration of auxin, that can simulate a growing shoot apical meristem (SAM) and the pattern (or phyllotaxy) of emerging primordia. Elliot Meyerowitz (California Institute of Technology, Pasadena, CA, USA) presented a model from his laboratory based on live imaging of PIN1-GFP and other cell type-specific reporters (Heisler et al., 2005). Drawing from data on PIN1 localization, PIN1 concentration, and PIN1 trafficking between the membrane and internal cellular compartments, Meyerowitz and his collaborators generated a model of the SAM that produced primordia in a phyllotactic pattern (Jonsson et al., 2006). The model also accurately reproduced the changes in PIN1 subcellular localization and concentration that occur as primordia are established. Cris Kuhlemeier (University of Bern, Bern, Switzerland) and his collaborator Przemyslaw Prusinkiewicz (University of Calgary, Calgary, Canada), presented their own computer model, which took into account cell division patterns observed in live meristems, the localization of PIN1 and the pattern of a *DR5-GFP* reporter (Smith et al., 2006). This model could reproduce the switch from distichous to spiral phyllotaxy and mimic defects observed in live meristems, such as the loss of PIN1. These 'computable' plant meristems should allow researchers to predict how live meristems will react to perturbations introduced into the computer model. Michael Sauer (University of Tubingen, Tubingen, Germany) showed data indicating that the flow of auxin either towards or away from an auxin source may be tissue dependent. When roots were exposed to exogenous auxin, the PIN2 protein (located in the outer cell layers of the root) relocalized to the plasma membrane nearest the auxin source, whereas the PIN1 protein (expressed in the center of the root) relocalized from the bottom of cells to the top, pointing away from the auxin source. Angela Hay (University of Oxford, Oxford, UK) discussed how auxin gradients control leaf initiation and shape. By combining mutations in genes known to affect leaf shape. such as *asymmetric leaves 1* (*as1*), and those involved in auxin transport and perception, such as *pin1* and *auxin resistant 1* (*axr1*), she showed that these proteins all function to keep the meristem-specific transcription factor *BREVIPEDICELLUS* (*BP*, also known as KNAT1 – *Arabidopsis thaliana* Database) out of leaves (Hay et al., 2006). John Bowman (Monash University, Melbourne, Australia) presented data that implicated auxin flow in the outgrowth of ectopic organs as well.

By combining mutations in a related family of transcription factors that play a role in leaf polarity [*KANADI1* (*KAN1*, also known as KAN – *Arabidopsis thaliana* Database), *KAN2* and *KAN4* (also known as *ABBERANT TESTA SHAPEI*; ATS)], the resulting plants produced ectopic leaves from the seedling stem (Emery et al., 2003; McAbee et al., 2006). PIN1-GFP was ectopically expressed in these outgrowths and appeared to be transporting auxin to the tips, just as in normal leaf development. Although auxin flow has been well researched, the timing and location of auxin production has remained largely unknown. Yunde Zhao (University of California San Diego, San Diego, CA, USA) presented his work on a family of flavin monooxygenases (the YUCCA proteins) that are the possible rate-limiting step in tryptophan-dependent auxin biosynthesis (Zhao et al., 2001). Zhao showed that many of these genes, initially identified by their overexpression phenotypes, are expressed in unique patterns, and their loss-of-function phenotypes indicate that the site of auxin production is as important as where it is transported to (Cheng et al., 2006).

Sizing things up: determinants of cell and organ size

How does a cell know what size it should be, and how is the overall size and shape of an organ or organism determined? Plants provide an excellent system to study these questions, as tissue-specific genetic screens can be performed. Domestication and recent selections have also resulted in closely related cultivars of crop plants that differ in organ size and shape. Esther van der Knaap (Ohio State University, Wooster, OH, USA) presented work that identified the tomato *sun* locus, which controls whether fruit shape is elongated rather than being typically round (van der Knaap et al., 2004). *sun*, however, does not control fruit size, indicating that the gene underlying this locus controls organ shape via a redistribution of fruit mass (Fig. 3). High-resolution fine mapping and sequencing of the *sun* locus by van der Knaap and colleagues has revealed that a segmental duplication causes the altered fruit shape. Two genes in this duplicated region are differentially expressed, and are therefore probably responsible for the fruit-shape phenotypes. These findings provide a starting point for identifying other fruit-shape genes in plants. Michael Lenhard (University of Freiburg, Freiburg, Germany) reported a mutant called *kluh* (*klu*), which formed smaller floral organs because of a decreased number of cell divisions. *KLU* encodes a cytochrome P450, which, when overexpressed in petals, increases their size. Although the substrate of KLU is currently unknown, it may generate a non-cell autonomous signal for growth, as organs that do not express *KLU*, but are near those that do, can also grow larger. James Umen (Salk Institute for Biological Studies, La Jolla, CA, USA) introduced the audience to the utility of *Chlamydomonas reinhardtii* as a system in which to study cell-size specification. *Chlamydomonas* mother-cell sizes can vary widely, but they divide to produce daughter cells with a similar size distribution over a population. Therefore the number of cell divisions a mother cell undergoes is controlled by a sizing mechanism, and several mutants defective in this process were presented. One mutant, *mat3*, produces abnormally small daughter cells, and the *MAT3* locus was shown to encode a retinoblastoma (RB)-related protein (Umen and Goodenough, 2001). Screens for suppressors of *mat3* identified alleles of DP1- and E2F-related proteins – conserved targets of RB-related proteins. On their own, these two mutants result in larger daughter cells than normal. With these and other mutants in hand, a genetic and biochemical framework is emerging for how *Chlamydomonas* and, probably, other organisms regulate cell size.

Fig. 3. Nearly isogenic tomato lines differing at the *sun* locus. Fruit on the left carry the elongated allele of *sun*, whereas fruit on the right carry the round allele of *sun* in *S. lycopersicum* cv Sun1642. Scale bar: 5cm. Image courtesy of V. D. Knaap (Ohio State University, Wooster, OH, USA).

The roles of small RNAs in development

Plants continue to be a rich source of information for how small RNAs are generated and regulate the function of other genes. Small interfering RNAs (siRNAs), micro RNAs (miRNAs) and trans-acting siRNAs (ta-siRNAs) play diverse roles in plants, including in viral defense and cell-type specification, and are produced through the action of four dicer-like (DCL) proteins with specialized functions (Bouche et al., 2006; Gasciolli et al., 2005; Voinnet, 2002). Olivier Voinnet (CNRS, Strasbourg, France) presented the results of large forward and reverse genetic screens designed to identify new factors that play a role in small RNA activities and biogenesis. In one screen, a miRNA sensor was constructed that contained a ubiquitously expressed form of GFP that had a miRNA binding site at the 3′ end. In wild-type plants, the reporter was only active in cells that did not express the miRNA. Mutants were then isolated that showed increased accumulation of the sensor and fell into two classes. In class-1 mutants, the sensor RNA and protein were increased, whereas in class-2 mutants, the sensor RNA levels did not increase significantly, but protein levels were increased. This indicates that there may be separable pathways present for transcript cleavage and translational inhibition. Scott Poethig (University of Pennsylvania, Philadelphia, PA, USA) discussed the role of miRNAs and ta-siRNAs in juvenile-to-adult transition in plants. In *Arabidopsis*, this phase change is manifested by increased numbers of abaxial (or ventral) trichomes, as more adult leaves are produced. Mutations in *ZIPPY* [*ZIP*, also known as *ARGONAUTE7* (*AGO7*) – *Arabidopsis thaliana* Database] had previously been shown to produce precocious trichomes, and a suppressor screen of *zip* yielded mutations in two genes, *ETTIN* (*ETT*) and *AUXIN RESPONSE FACTOR 4* (*ARF4*) (Hunter et al., 2003). *ETT* and *ARF4* mRNA accumulation is controlled by a ta-siRNA (*tasiR-ARF*) derived from the *TAS3* locus, and Poethig showed that *zip* mutants had an increased level of *ETT* and *ARF4*, and a reduced level of *tasiR-ARF* (Hunter et al., 2006). Marja Timmermans (Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, USA) discussed the role of miRNAs and ta-siRNAs on the correct adaxial/abaxial (dorsal/ventral) patterning of the leaf. *miR166* controls the accumulation of the class III homeodomain leucine-zipper gene *rolled leaf1* (*rld1*), and Timmermans showed that *miR166* is abaxially expressed in a pattern complementary to *rld1* and that it appears to accumulate in a gradient (Juarez et al., 2004). A second gene, *leafbladeless1* (*lbl1*), is necessary for adaxial fate and for the production of *ta-siRNA2142* (Timmermans et al., 1998). *lbl1* mutants in maize misexpress *miR166* throughout the leaf primordial, indicating that leaf polarity in maize is under the control of two opposing small regulatory RNAs. Timmermans also

showed that the ta-siRNA pathway is active in the SAM, and postulated that *ta-siRNA2142*, like other DCL4-dependent siRNAs, may act as a mobile signal to set up leaf polarity by restricting *miR166* expression. Finally, Yuval Eshed (Weizmann Institute of Science, Rehovot, Israel) showed the power of using synthetic miRNAs to overcome genetic redundancy in plants (Alvarez et al., 2006; Schwab et al., 2006). By using synthetic miRNAs, Eshed reduced the expression of nine genes from two different families involved in growth and uncovered phenotypes not observed in any single-mutant line. These sorts of experiments should rapidly advance research on closely related gene families and uncover roles that are difficult to discern through classical genetics.

Conclusions

In the 2 years since the last FASEB conference, a remarkable amount of progress has been made in our understanding of plant development. The roles of hormones, small RNAs and moving transcription factors have been carefully dissected, and problems with genetic redundancy have been tackled. It has become clear that, work in many plant model organisms strengthens the field and provides insight into not only plant developmental mechanisms, but also into questions that arise in the development of all organisms.

I apologize to the many speakers whose results I could not include due to space constraints. I would like to thank H. Szemenyei and M. Hannon for sharing their notes on the meeting.

References

Alvarez, J. P., Pekker, I., Goldshmidt, A., Blum, E., Amsellem, Z. and Eshed, Y. (2006). Endogenous and synthetic microRNAs stimulate simultaneous, efficient, and localized regulation of multiple targets in diverse species. *Plant Cell* **18**, 1134-1151.

Bouche, N., Lauressergues, D., Gasciolli, V. and Vaucheret, H. (2006). An antagonistic function for Arabidopsis DCL2 in development and a new function for DCL4 in generating viral siRNAs. *EMBO J.* **25**, 3347-3356.

Cheng, Y., Dai, X. and Zhao, Y. (2006). Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in Arabidopsis. *Genes Dev.* **20**, 1790-1799.

Emery, J. F., Floyd, S. K., Alvarez, J., Eshed, Y., Hawker, N. P., Izhaki, A., Baum, S. F. and Bowman, J. L. (2003). Radial patterning of Arabidopsis shoots by class III HD-ZIP and KANADI genes. *Curr. Biol.* **13**, 1768-1774.

Galway, M. E., Masucci, J. D., Lloyd, A. M., Walbot, V., Davis, R. W. and Schiefelbein, J. W. (1994). The TTG gene is required to specify epidermal cell fate and cell patterning in the Arabidopsis root. *Dev. Biol.* **166**, 740-754.

Galweiler, L., Guan, C., Muller, A., Wisman, E., Mendgen, K., Yephremov, A. and Palme, K. (1998). Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. *Science* **282**, 2226-2230.

Gasciolli, V., Mallory, A. C., Bartel, D. P. and Vaucheret, H. (2005). Partially redundant functions of Arabidopsis DICER-like enzymes and a role for DCL4 in producing trans-acting siRNAs. *Curr. Biol.* **15**, 1494-1500.

Hamann, T., Benkova, E., Baurle, I., Kientz, M. and Jurgens, G. (2002). The Arabidopsis BODENLOS gene encodes an auxin response protein inhibiting MONOPTEROS-mediated embryo patterning. *Genes Dev.* **16**, 1610-1615.

Hardtke, C. S. and Berleth, T. (1998). The Arabidopsis gene MONOPTEROS encodes a transcription factor mediating embryo axis formation and vascular development. *EMBO J.* **17**, 1405-1411.

Hay, A., Barkoulas, M. and Tsiantis, M. (2006). ASYMMETRIC LEAVES1 and auxin activities converge to repress BREVIPEDICELLUS expression and promote leaf development in Arabidopsis. *Development* **133**, 3955-3961.

Heisler, M. G., Ohno, C., Das, P., Sieber, P., Reddy, G. V., Long, J. A. and Meyerowitz, E. M. (2005). Patterns of auxin transport and gene expression during primordium development revealed by live imaging of the Arabidopsis inflorescence meristem. *Curr. Biol.* **15**, 1899-1911.

Higashiyama, T. (2002). The synergid cell: attractor and acceptor of the pollen tube for double fertilization. *J. Plant Res.* **115**, 149-160.

Higashiyama, T., Kuroiwa, H., Kawano, S. and Kuroiwa, T. (1998). Guidance in vitro of the pollen tube to the naked embryo sac of torenia fournieri. *Plant Cell* **10**, 2019-2032.

Higashiyama, T., Inatsugi, R., Sakamoto, S., Sasaki, N., Mori, T., Kuroiwa, H., Nakada, T., Nozaki, H., Kuroiwa, T. and Nakano, A. (2006). Species preferentiality of the pollen tube attractant derived from the synergid cell of Torenia fournieri. *Plant Physiol.* doi: 10.1104/pp.106.083832.

Hunter, C., Sun, H. and Poethig, R. S. (2003). The Arabidopsis heterochronic gene ZIPPY is an ARGONAUTE family member. *Curr. Biol.* **13**, 1734-1739.

Hunter, C., Willmann, M. R., Wu, G., Yoshikawa, M., de la Luz Gutierrez-Nava, M. and Poethig, S. R. (2006). Trans-acting siRNA-mediated repression of ETTIN and ARF4 regulates heteroblasty in Arabidopsis. *Development* **133**, 2973-2981.

Jonsson, H., Heisler, M. G., Shapiro, B. E., Meyerowitz, E. M. and Mjolsness, E. (2006). An auxin-driven polarized transport model for phyllotaxis. *Proc. Natl. Acad. Sci. USA* **103**, 1633-1638.

Juarez, M. T., Kui, J. S., Thomas, J., Heller, B. A. and Timmermans, M. C. (2004). microRNA-mediated repression of rolled leaf1 specifies maize leaf polarity. *Nature* **428**, 84-88.

Kusaba, M., Dwyer, K., Hendershot, J., Vrebalov, J., Nasrallah, J. B. and Nasrallah, M. E. (2001). Self-incompatibility in the genus Arabidopsis: characterization of the S locus in the outcrossing A. lyrata and its autogamous relative A. thaliana. *Plant Cell* **13**, 627-643.

Kwak, S. H., Shen, R. and Schiefelbein, J. (2005). Positional signaling mediated by a receptor-like kinase in Arabidopsis. *Science* **307**, 1111-1113.

Lee, M. M. and Schiefelbein, J. (1999). WEREWOLF, a MYB-related protein in Arabidopsis, is a position-dependent regulator of epidermal cell patterning. *Cell* **99**, 473-483.

Lukowitz, W., Roeder, A., Parmenter, D. and Somerville, C. (2004). A MAPKK kinase gene regulates extra-embryonic cell fate in Arabidopsis. *Cell* **116**, 109-119.

McAbee, J. M., Hill, T. A., Skinner, D. J., Izhaki, A., Hauser, B. A., Meister, R. J., Venugopala Reddy, G., Meyerowitz, E. M., Bowman, J. L. and Gasser, C. S. (2006). ABERRANT TESTA SHAPE encodes a KANADI family member, linking polarity determination to separation and growth of Arabidopsis ovule integuments. *Plant J.* **46**, 522-531.

Muller, A., Guan, C., Galweiler, L., Tanzler, P., Huijser, P., Marchant, A., Parry, G., Bennett, M., Wisman, E. and Palme, K. (1998). AtPIN2 defines a locus of Arabidopsis for root gravitropism control. *EMBO J.* **17**, 6903-6911.

Nasrallah, J. B., Yu, S. M. and Nasrallah, M. E. (1988). Self-incompatibility genes of Brassica oleracea: expression, isolation, and structure. *Proc. Natl. Acad. Sci. USA* **85**, 5551-5555.

Nasrallah, M. E., Liu, P. and Nasrallah, J. B. (2002). Generation of self-incompatible Arabidopsis thaliana by transfer of two S locus genes from A. lyrata. *Science* **297**, 247-249.

Palme, K. and Galweiler, L. (1999). PIN-pointing the molecular basis of auxin transport. *Curr. Opin. Plant Biol.* **2**, 375-381.

Schnittger, A., Folkers, U., Schwab, B., Jurgens, G. and Hulskamp, M. (1999). Generation of a spacing pattern: the role of triptychon in trichome patterning in Arabidopsis. *Plant Cell* **11**, 1105-1116.

Schwab, R., Ossowski, S., Riester, M., Warthmann, N. and Weigel, D. (2006). Highly specific gene silencing by artificial microRNAs in Arabidopsis. *Plant Cell* **18**, 1121-1133.

Smith, R. S., Guyomarc'h, S., Mandel, T., Reinhardt, D., Kuhlemeier, C. and Prusinkiewicz, P. (2006). A plausible model of phyllotaxis. *Proc. Natl. Acad. Sci. USA* **103**, 1301-1306.

Timmermans, M. C., Schultes, N. P., Jankovsky, J. P. and Nelson, T. (1998). Leafbladeless1 is required for dorsoventrality of lateral organs in maize. *Development* **125**, 2813-2823.

Ulmasov, T., Murfett, J., Hagen, G. and Guilfoyle, T. J. (1997). Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. *Plant Cell* **9**, 1963-1971.

Umen, J. G. and Goodenough, U. W. (2001). Control of cell division by a retinoblastoma protein homolog in Chlamydomonas. *Genes Dev.* **15**, 1652-1661.

van der Knaap, E., Sanyal, A., Jackson, S. A. and Tanksley, S. D. (2004). High-resolution fine mapping and fluorescence in situ hybridization analysis of sun, a locus controlling tomato fruit shape, reveals a region of the tomato genome prone to DNA rearrangements. *Genetics* **168**, 2127-2140.

Voinnet, O. (2002). RNA silencing: small RNAs as ubiquitous regulators of gene expression. *Curr. Opin. Plant Biol.* **5**, 444-451.

Weijers, D., Schlereth, A., Ehrismann, J. S., Schwank, G., Kientz, M. and Jurgens, G. (2006). Auxin triggers transient local signaling for cell specification in Arabidopsis embryogenesis. *Dev. Cell* **10**, 265-270.

Zhao, Y., Christensen, S. K., Fankhauser, C., Cashman, J. R., Cohen, J. D., Weigel, D. and Chory, J. (2001). A role for flavin monooxygenase-like enzymes in auxin biosynthesis. *Science* **291**, 306-309.