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MEETING REPORT

Auxin Research Flourishes in the Desert: Auxin 2008

John Chandler

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Introduction

The "Auxin 2008" meeting was held at the Eldorador Club Palmeraie Hotel, Marrakech, Morocco, 4–9 October, and was the latest in a series of meetings initiated to focus on all aspects of the single hormone auxin. This meeting followed successful meetings in Crete in 2004 and Corsica in 2000. The meeting was organized by Ottoline Leyser, Catherine Bellini, and Mark Estelle, with local help from Mohamed Benichou. Participants represented at least 17 countries and there were 56 oral contributions and 49 poster abstracts in an exotic resort hotel setting.

The advantages of a small intensive meeting on a focused topic was not only the possibility of a high ratio of talks to participants, which gave students and postdoctorates an opportunity to present their work, but it provided plenty of opportunity for informal discussion, networking, and collaboration-building throughout the week. The meeting hotel was comfortable and also geographically isolated enough to allow few distractions, enabling full participation from all delegates. In addition, a social program, including an afternoon with an organized tour, and ample free time in the evenings made the nonscientific aspects of this meeting particularly memorable.

The meeting reported on many significant research advances in the field of auxin biology from leading researchers in the field. Auxin affects almost all aspects of plant growth and development and since the last auxin meeting much progress has been made in the study of perception via the discovery and isolation of the crystal structure of the TIR1 auxin receptor, and in the study of auxin biosynthesis, with new pathways being confirmed by *Arabidopsis* mutant characterization. Also, advances in understanding the mechanisms of auxin transport and auxin signalling have led to an increase in the understanding of many signal transduction cascades involving the auxin regulation of transcription factors in specific developmental processes. The hugely diverse roles of auxin were reflected in the breadth of the session topics, which covered all aspects of development and were entitled homeostasis and biosynthesis; signalling; cell division, elongation, and polarity; cell polarity and specification; transport; embryogenesis; modelling; vascular development; meristem function; and architecture.

J. Chandler (✉)
Department of Developmental Biology, University of Cologne, Gyrhofstrasse 17, Cologne 50931, Germany
e-mail: John.chandler@uni-koeln.de

Keynote Talks

The meeting opened with a keynote session from Ning Zheng (University of Washington, Seattle, WA, USA). Ning Zheng's group was involved in the discovery of the crystal structure of the TIR1 auxin receptor protein. He described how auxin acts as "molecular glue" to increase substrate binding of the TIR1 complex to its substrate AUX/IAA proteins and how inositol hexakisphosphate is a potential cofactor for TIR1. He outlined how the mechanism by which TIR1 binds auxin and is an agonist of ubiquitin ligase function represents a novel receptor mechanism which can be applied to mammalian drug discovery, where compromised SCF complex binding capacities are linked to many diseases including cancers. The search for mammalian ubiquitin ligase agonists represents a new basis for drug discovery. The important point was therefore made that plant science research can often

unexpectedly have major consequences for other research fields.

The second keynote lecture from Ben Scheres (Utrecht University, Utrecht, The Netherlands) described the function of the *Arabidopsis PLETHORA* (*PLT*) gene family, which has been implicated in root and root stem cell specification. The *PLT* genes are induced by auxin and also control auxin biosynthesis and response and function redundantly in a broader range of developmental patterning processes than previously thought, such as phyllotaxis and embryogenesis, leading to a model in which they might function within feedback circuits to fine-tune auxin-controlled gene responses.

The final keynote session was presented by Thomas Laux (Freiburg University, Freiburg, Germany) who outlined the role of the *WUSCHEL* homeobox (*WOX*) gene family in *Arabidopsis* in the formation of the embryonic plant axis and in embryonic patterning, with some of the *WOX* genes being expressed in the zygote.

Biosynthesis and Homeostasis

In many plants, auxin can be synthesized by various alternative pathways acting in parallel, because no auxin auxotroph to date has been characterized. The highly redundant and complex nature of auxin biosynthesis makes this area a challenging and active one within auxin research.

Stephan Pollmann (Bochum University, Bochum, Germany) reported how different plants can synthesize IAA from indole-3-acetamide (IAM) via IAM hydrolases and how inducing AMIDASE1 activity in *Arabidopsis* results in IAA overproduction. Alternatively, IAA can be synthesized via indole-3-acetaldoxime (IAOx) and Hiroyuki Kasahari (RIKEN, Yokohama, Japan) showed that IAOx levels were nondetectable in *cyp79b2 cyp79b3* double mutants, confirming that the cytochrome P450 enzymes encoded by these two genes provide the major way in which IAOx is produced. The recent characterization of TAA1/WEI8, an aminotransferase encoded by a member of a small gene family within the indole-3-pyruvic acid pathway (IPA) of auxin biosynthesis, was described by Jose Alonso (NCSU, Raleigh, NC, USA). TAA1/WEI8 is responsive to ethylene and represents a focus of hormone crosstalk for developmental signals and environmental responses. Local auxin production via TAA1/WEI8 is thought to be required for root meristem maintenance and the creation of auxin gradients. Higher-order mutants between members of the TAA1/WEI8 family phenocopy *monopteros* mutants, showing that they also contribute to embryo development. Understanding the contribution of local auxin biosynthesis in various organs is an increasingly important topic in auxin biology. Yunda Zhao (UCSD, San Diego, CA, USA) outlined the importance of the role of flavin monooxygenases encoded by the *Arabidopsis YUCCA* genes in local auxin biosynthesis as well as the progress in understanding the upstream genetic regulation of this redundant gene family.

Karin Ljung (Umeå University, Umeå, Sweden) reported on a method to construct a map of IAA metabolism and distribution in the root using fluorescence-activated cell sorting of protoplasts from many different GFP marker lines, coupled with mass spectrometry. Results confirm that clear IAA gradients exist, with both the highest synthesis and turnover rates in the apical region of dividing cells. Downregulation of IAA pools is mediated prinicipally by oxidation in *Arabidopsis* roots, not by conjugation, and data show that root development is dependent on IAA biosynthesis and catabolism as well as auxin transport. This technology can now be used to address the contributions of auxin biosynthesis, catabolism, and conjugation at cellular levels.

Conjugation of IAA with alanine or leucine, catalyzed by members of the GH3 protein family, is an important means by which IAA is removed from the available active auxin pool. Jutta Ludwig-Müller (Dresden University, Dresden, Germany) reported data from *Physcomitrella patens* where targeted knockout of either single or double endogenous GH3 genes resulted in higher levels of free IAA and lower conjugated IAA levels, demonstrating that IAA conjugation is an important contributor to auxin homeostasis in mosses. In *Arabidopsis*, the *SHI/STY* family of transcription factors regulate *YUCCA4*. Two homologs of this gene family have also been characterized in *Physcomitrella patens*, shown in a poster by Eva Sundberg (Uppsala University, Uppsala, Sweden), and expression patterns and knockout phenotypes suggest that they serve a conserved ancestral function to their *Arabidopsis* counterparts in auxin biosynthesis.

Embryogenesis

The importance of auxin in embryonic patterning was recognized at the meeting by devoting a whole session to it. The auxin response factor *MONOPTEROS* (*MP*) is a key specifier of the basal embryo domain, especially the hypophysis where it exerts a non-cell-autonomous function. MP physically interacts with and is repressed by its Aux/IAA partner BODENLOS (BDL). Dolf Weijers (Wageningen University, Wageningen, The Netherlands) and a poster from Barbara Müller in his group presented the characterization of targets of MP (TOM), one of which moves as a protein into the vasculature and hypophysis, thus partly explaining the non-cell-autonomy of MP. The

Arabidopsis genome encodes 23 different auxin response factors and dissecting which specific developmental functions some have during embryogenesis is one goal of Weijers's research. In contrast, the apical domain of the *Arabidopsis* embryo is partly specified by the transcriptional corepressor TOPLESS (TPL), which acts physically with BODENLOS (BDL) and MP in auxin-mediated gene regulation as presented by Jeff Long (Salk Institute, La Jolla, CA, USA). Mutants at the *TPL* locus confer a basal domain phenotype to the apical domain, showing that they repress basal domain transcription responses here in wild-type embryos. Two other targets of MP were also presented: *DORNRÖSCHEN* (John Chandler, Cologne University, Cologne, Germany) in the tips of the developing cotyledons, and *BREVIX RADIX* (*BRX*; Christian Hardtke, Lausanne University, Lausanne, Switzerland). Posters by Ive De Smet and Steffen Lau (Tübingen University, Tübingen, Germany), from the Gerd Jürgens group, presented work aimed at isolating regulators of BDL (ROB): a yeast one-hybrid screen using the *BDL* promoter resulted in several ROB proteins, which appear to be negative regulators of BDL.

Modelling

Modelling auxin fluxes in developmental responses was a new feature of the meeting, reflecting the increase in both the numbers of models and the recognition that models have been extremely successful in mimicking auxin fluxes in vivo. Models can be tested directly by experimental data and are useful tools for probing the properties and parameters of the systems involved. Models were described concerning the function of auxin gradients in wood grain pattern formation in *Populus* trees (Eric Kramer, Bard College at Simon's Rock, Great Barrington, MA, USA), auxin fluxes involved in phyllotaxis, vascular patterning, and branching architecture (Przemyslaw Prusinkiewicz, Calgary University, Calgary, AB, Canada), root curvature (Marta Laskowski, Oberlin College, Oberlin, OH, USA), and auxin transport in tobacco BY-2 cells (Eva Zazímalová, Czech Academy of Sciences, Prague, Czech Republic). The last speaker demonstrated how the parameters of a model can be tested and honed by comparing modelling data with experimental data. Auxin controls gene transcription via the derepression of auxin response factors by degradation of members of the AUX/IAA repressor family, but it is not clear how this can lead to qualitatively varying transcriptional responses in specific developmental contexts. Stephan Kepinski (Leeds University, Leeds, UK) outlined his research approach to generate quantitative and biochemical data describing dynamic aspects of the conceptual auxin signalling scheme, to define parameters and model auxin signalling in developmental contexts. Chris Kuhlemeyer (Berne University, Berne, Switzerland) outlined the differences between "up-the-gradient" PIN1 polarization types of feedback loops between PIN1 and its own induction by auxin, which generate auxin gradients by directing auxin toward cells with a higher concentration as occurs during leaf initiation, and "with-the-gradient" PIN1 polarization that reinforces the direction of auxin flux, as in the canalization hypothesis of leaf vascularization. His modelling of PIN1 localization coupled with experimental observation during midvein initiation shows that both types of feedback loops operate in dynamic interplay.

Signalling

Auxin Binding Protein 1 (ABP1) has been considered to be an auxin receptor due to its auxin-binding properties and role in auxin-regulated cell elongation. However, studies with *abp1* have been hampered by its embryo lethality. Now, Catherine Perrot-Rechenmann (CNRS, Gif-sur-Yvette, France) and coworkers have established *abp1* conditional knockout plants by antisense and expression of an anti-ABP1 monoclonal antibody Sc-Fv fragment. This approach led to defects in leaf growth and in shoot and root meristems, which relate to cell division and expansion and implicate a developmental role for ABP1 in many developmental processes.

Three talks from the vasculature and architecture sessions highlighted the importance of long-range signalling in auxin-regulated growth responses controlling shoot branching and embryogenesis and vascular development. The function of the *BYPASS* (*BPS*) gene family was introduced by Leslie Sieburth (Utah University, Salt Lake City, UT, USA), who showed that *bps1* mutants can produce only wild-type leaves after removal of the roots and, via grafting experiments, that mutant roots can induce growth arrest in wild-type shoots. This suggests that an as yet unknown root-derived mobile signal affects shoot growth. The signal is not auxin but affects auxin signalling as shown by DR5 responsiveness. The talk by Ottoline Leyser (York University, York, UK) reported on how the *MORE AXILLARY GROWTH* (*MAX*) genes modulate *Arabidopsis* branching: the *MAX1*, *MAX3*, and *MAX4* loci encode genes acting in carotenoid cleavage pathways and *MAX2* encodes an F-box protein. The talk by Philip Brewer (University of Queensland, Brisbane, QLD, Australia) linked into the MAX story by reporting on the identification of strigolactones as the signal that represses branch outgrowth in diverse species such as *Arabidopsis*, pea, petunia, and rice and that strigolactones are presumed downstream products of *MAX* gene carotenoid cleavage. Strigolactones, a class of terpenoid

lactones have long been known to be signalling molecules in mycorrhizal associations and the germination of parasitic plant seeds. The challenge is now to elucidate the pathway in more detail. Plant architecture was also addressed by Paula McSteen (University of Penn State, Pennsylvania, PA, USA), who presented her work on the role of auxin in maize in controlling inflorescence architecture following screens for mutants that do not initiate axillary meristems, including *barren inflorescence2* (*bif2*), a mutant in the ortholog of *Arabidopsis* PID which phosphorylates ZmPIN1 and interacts with BARREN STALK1, a bHLH transcription factor, and *sparse inflorsecence1* (*spi1*), a monocot *YUCCA*-like monooxygenase involved in local auxin biosynthesis. A comparison of monocot maize orthologs involved in auxin biosynthesis and transport with those *Arabidopsis* dicot genes reveals both conservation and differences. Miltos Tsiantis (Oxford University, Oxford, UK) is using *Cardamine hirsuta* as a model system in comparison with *Arabidopsis* for studying architecture in terms of the generation of leaf form. *Cardamine* has a compound leaf, and it is the generation of local auxin maxima that initiates growth foci and gives rise to leaflet initiation, in combination with the activity of Class I KNOTTED-like homeobox (KNOX) proteins. Therefore, polar auxin accumulation is responsible for leaf form diversification during evolution.

Evidence is accumulating that auxin is involved in rapid responses to the environment, such as the shade avoidance response to light quality. Ida Ruberti (Rome University, Rome, Italy) explained how responses to low R/FR light, such as hypocotyl elongation and leaf primordium arrest, are related to auxin-induced cytokinin breakdown, thereby implicating a previously unrecognized regulatory circuit to underlie plant response to canopy shade. Low blue light can also induce shade avoidance responses and lead to hypocotyl elongation and the poster of Diederik Keuskamp (Utrecht University, Utrecht, The Netherlands) outlined how the dependency of this process on auxin is being addressed by monitoring PIN expression and using *pin* mutants.

Crosstalk is an increasingly important area within hormone biology. Ethylene is the hormone most usually considered in developmental crosstalk with auxin, although little is known about the basis of this dialog at the molecular level. Mondher Bouzayen (Ecole Nationale Superieure Agronomique, Toulouse, France) reported that Aux/IAA genes in tomato are also regulated by ethylene and, in particular, SI-IAA, an Aux/IAA gene, is transcriptionally regulated by both auxin and ethylene pathways and mutants show auxin and ethylene-related phenotypes. Thus, Aux/IAA genes may represent an important point of intersection between auxin and ethylene developmental regulation pathways.

Transport

Auxin transport is mainly polar and dependent on the asymmetric localization of PIN efflux proteins within individual cells. Some of the regulatory pathways controlling PIN localization have been elucidated. One of these is via the serine-threonine protein kinase PINOID (PID). Remko Offringa (Leiden University, Leiden, The Netherlands) showed that the phosphorylation of three serine residues within the PIN1 protein is necessary for normal PIN1 localization. PID belongs to a family of AGC3 kinases in *Arabidopsis*, whose other members—WAG1, WAG2, and AGC3-4—also regulate PIN polarity and act redundantly with PID. A poster presentation by Hélène Robert from Remko Offringa's group showed that PID interacts in a yeast two-hybrid screen with four of five members of the *Arabidopsis* BTB and TAZ domain (BT) protein family via their BTB domain. Evidence suggests that the function of these interaction partners is to repress PID kinase activity, possibly by recruiting PID to the nucleus. Nicola Krieghoff (Freiburg University, Freiburg, Germany) presented a poster outlining how members of the *Arabidopsis* AGC family also have a role in early gametophyte development and ovule maturation.

Christian Luschnig (BOKU, Vienna, Austria) elaborated the role of the *MODULATOR OF PIN* (*MOP*) loci in *Arabidopsis* in controlling PIN1 expression: these loci influence PIN protein levels post-transcriptionally, without affecting *PIN* transcription or distribution, but it is not yet known what these loci encode. The characterization of *PIN1* genes in maize, reported by Serena Varotto (Padova University, Padova, Italy), suggests that several different PIN proteins are involved in auxin transport in the embryo and endosperm, with PIN1-mediated auxin fluxes being responsible for generating the shoot and root apical meristem. However, in contrast to *Arabidopsis*, some PIN genes in maize have splice variants.

Jiri Friml (VIB, Ghent, Belgium) summarized how polar PIN localization mediates local auxin gradients necessary for organogenesis and that PIN5 and PIN8 localize to the endoplasmic reticulum, having an as yet unknown function. Auxin is also transported by PGP1 and PGP19, homologs of human multiple drug resistance/P glycoproteins, so-called ABC ATP-binding transporters. Interestingly, although PGP1 and PGP19 are required for directional auxin flux, for example, through the stem and root and from the shoot apical meristem into the cotyledons, as reported by Gabriele Monshausen (University of Wisconsin-Madison, Madison, WI, USA), they are uniformly distributed within cells. Sakai Tatsuya (RIKEN, Yokohama, Japan) presented evidence that the ABC auxin transporter PGP19, encoded by the *FLABBY* gene in *Arabidopsis*, is transcriptionally downregulated by

phytochromes and cryptochromes, leading to reduced auxin distribution in hypocotyls and to enhanced hypocotyl bending. Jürgen Klein-Vehn from the Friml group talked about how rapid changes in PIN polarity could be plausibly effected by an ARF GEF-dependent transcytosis, causing endocytic recycling of PIN proteins between opposite cell faces, which can be observed in vivo. Pankal Dhonukshe from the same group furthermore showed with real-time imagery that PIN proteins are initially delivered to plasma membranes via endocytosis in a nonpolar manner and subsequent polarity is established by endocytic recycling.

Root Development

Root development remains the best studied auxin developmental process and there were over 15 oral presentations/posters on all aspects of this topic drawn from many different sessions. Lateral root initiation is a good model system for studying its promotion by auxin. Tom Beeckman (VIB, Ghent, Belgium) explained how auxin is required but not sufficient for lateral root formation by stimulating the cell cycle at the G1-to-S phase transition. He outlined his work on isolating which additional auxin-induced factors are required for lateral root formation. Steffan Vanneste from the Beeckman group presented a poster on a chemical genetics approach to identify activators of lateral root formation.

Hormone crosstalk in lateral root development was addressed by several presentations: A poster by Joseph Dubrovsky (VIB, Ghent, Belgium) from the Benková group showed that IAA is the instructive signal for lateral root founder cells and as a mechanism is probably evolutionarily conserved in plants. Eva Benková (VIB, Ghent, Belgium) described her research to address the antagonistic action between auxins and cytokinins in lateral root primordium development in *Arabidopsis*. Sangeeta Negi (Wake Forest University, Winston-Salem, NC, USA) instead is interested in auxin-ethylene cross-talk in lateral root formation: tomato and *Arabidopsis* mutants with a block in ethylene responses have enhanced lateral root formation, and, conversely, mutations which enhance ethylene synthesis or signalling inhibit lateral root formation, perhaps by enhancing AUX1 expression and redirecting auxin away from lateral roots and into the primary root polar transport stream.

The effect of auxin on adventitious root growth was addressed by Catherine Bellini (Umeå University, Umeå, Sweden) who reported that ARF6 and ARF8 are positive regulators of adventitious root initiation and of GH3-3, GH3-5, and GH3-6, which are negatively regulated by ARF17.

The control of root hair formation was outlined by Yoshihisa Ikeda (Umeå University, Umeå, Sweden) who found that root hair initiation is dependent on a concentration gradient of auxin toward the root tip, with hairs being formed at the basal end of hair-forming cells. The *BEATNIK*/*CTR1* gene, when mutated, leads to more root hairs, suggesting that it serves as a repressor of an auxin gradient that controls planar polarity and leaf hair initiation.

Cell division and differentiation in the root meristem were addressed by Sabrina Sabatini (Rome University, Rome, Italy). She showed how the balance between cell differentiation and division necessary for root meristem function is controlled by a simple relationship between cytokinin and auxin activity: Cytokinins antagonize auxin action by transcriptional repression of SHY2/IAA3, leading to an alteration in PIN expression and subsequent auxin redistribution. Conversely, auxin causes SHY2/IAA3 degradation, maintaining PIN expression and cell division.

Conclusions

The meeting highlighted progress in fundamental auxin biology from leaders in the auxin field. One major development has been the positive power of computer modelling to model fluxes and gradients in developmental processes, which enables new parameters to be established and tested against in vivo data to help understand the dynamic and complicated nature of auxin transport. Advances in understanding auxin biosynthesis and particularly how local auxin biosynthesis contributes to specific developmental programs and in response to rapid changes in the environment is a further noticeable development. Among the many remaining challenges is the establishment of other possible auxin receptors and the generation of more data on auxin biosynthesis and signalling from other species apart from the *Arabidopsis* paradigm that will enable comparative evolutionary questions to be asked about the conservation of key pathways and the study of aspects of auxin function not able to be addressed in *Arabidopsis*. I am sure the auxin community is eagerly looking forward to the next meeting and the only disappointment is that the meeting is held so infrequently for such a dynamic and rapidly evolving research field. However, the Third International Symposium on Auxin and Cytokinins in Plant Development (ACPD) serves to partly fill this gap and will be held in Prague on 10–14 July 2009. Please visit the website for further details (http://acpd.cas.cz).

I apologize to any participants who feel that their work was excluded from this summary. Space limitations and thus the need to focus on a selection of themes meant that not every individual contribution could be covered in detail.