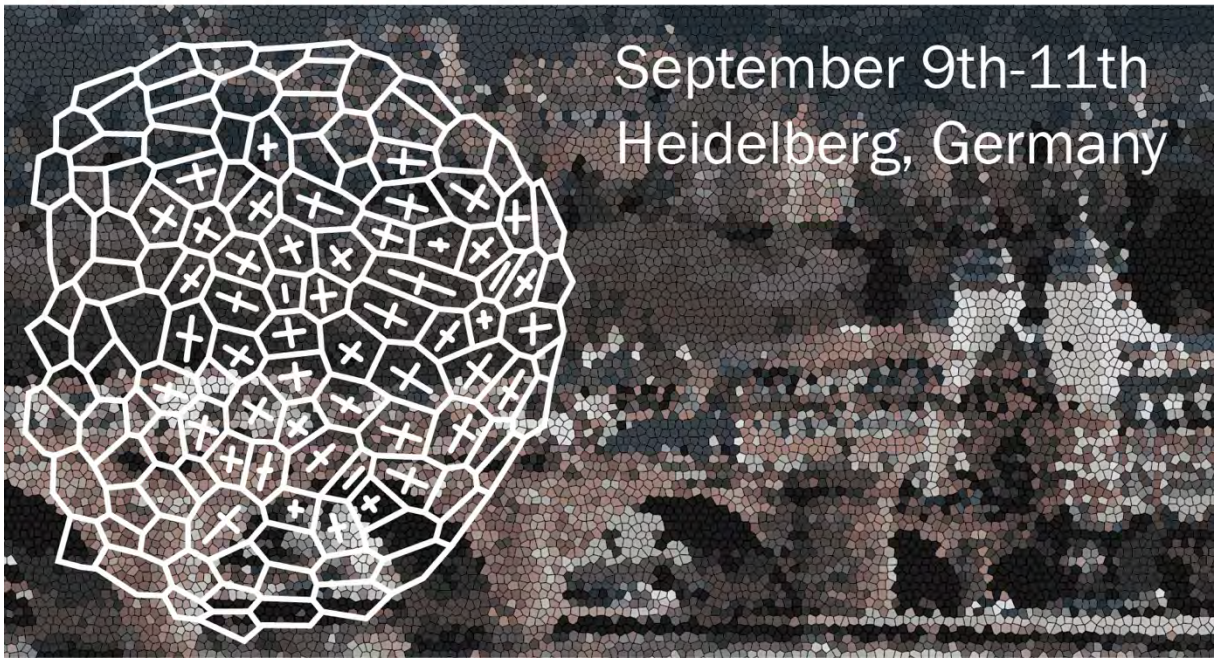
Plant Growth and Form

International Symposium on quantitative plant morphodynamics
Organised by the DFG FOR2581



UNIVERSITÄT HEIDELBERG
ZUKUNFT SEIT 1386

DFG

PLANT FOR 2581 MORPHODYNAMICS

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SCHEDULE

Monday 9.09			
		Session #1: Mechanics and patterning (tissue scale) [Chair: A. Maizel]	
14:00 - 14:15		**Opening: A. Maizel / K. Schneitz**	
14:15 - 14:45	O-1	**O. Hamant**	Suboptimal microtubule response to mechanical stress buffers growth variation
14:45 - 15:05	O-2	**K. Schneitz**	Cellular growth patterns shaping the Arabidopsis ovule
15:05 - 15:20	O-3	**C. Baroux**	Role of ovule growth dynamics on germline fate establishment in Arabidopsis
15:20 - 15:35	O-4	**R. Simon**	A peptide pair coordinates regular ovule initiation patterns with seed number and fruit size
15:35 - 16:05	O-5	**G. Ingram**	The mechanical conundrum of seed shape regulation
16:05 - 16:50		***Coffee break***	
16:50 - 17:20	O-6	**M. Heisler**	Control of plant morphogenesis by adaxial-abaxial cell type boundaries
17:20 - 17:40	O-7	**T. Greb**	Exploring the Morphodynamics of Radial Plant Growth
17:40 - 18:00	O-8	**M. Tsiantis**	A growth-based framework for leaf development and diversity.
18:00 - 18:20	O-9	**J. Lohmann**	Plant self-organisation by feedback from tissue mechanics
18:20 - 18:45		***Transfer to the Alte Aula***	
18:45 - 19:45	K-1	**E. Coen**	From Planes to Cups: The Development and Evolution of Leaf Shape
19:45 - 22:00		**Welcome reception**	

Tuesday 10.09			
		Session #2: Mechanics and patterning (cell scale) [Chair: K. Schneitz]	
09:00 - 09:30	O-10	**C. Rasmussen**	Division plane orientation in plant cells
09:30 - 09:50	O-11	**A. Maizel**	Cytoskeleton dynamics and lateral root morphogenesis
09:50 - 10:05	O-12	**P. Vaddepalli**	Molecular and cellular mechanisms regulating the cell division orientation during early plant embryogenesis
10:05 - 10:35	O-13	**M. Ueda**	Live-cell imaging of the intracellular dynamics of Arabidopsis zygote
10:35 - 11:20		***Coffee break***	
		Session #3: Quantifying and modelling cell and tissue growth [Chair: K. Schneitz]	
11:20 - 11:50	O-14	**V. Grieneissen**	Polar transport in growing plants: from maxima to minima, phytohormones and nutrients
11:50 - 12:05	O-15	**C. Kirchhelle**	Two mechanisms control growth anisotropy in Arabidopsis lateral roots
12:05 - 12:30	F-1	**J. Alonso Serra**	Mechanical control of secondary growth in trees
Flash talks	F-2	**S. Robinson**	Visualising hypocotyl elongation in response to stress-induced microtubule reorientation
	F-3	**Y. Meroz**	Towards a Framework for Collective Behavior in Plant-Inspired Growth-Driven Systems
	F-4	**J. Derr**	Fluttering of growing leaves as a way to reach flatness: Experimental evidence on Persea americana
	F-5	**R. Andrade Buono**	Tight regulation of programmed cell death in the Arabidopsis root cap regulates cellular turnover while maintaining organ integrity
	F-6	**L. Colin**	Contribution of PI(4,5)P2 in Arabidopsis Shoot Apical meristem development
	F-7	**L. Riglet**	Deciphering the Mechanisms Underlying Petal Growth & Patterning in Hibiscus trionum
	F-8	**K. Yalamanchili**	Lateral Root Priming Synergistically Arises from Root Growth and Auxin Transport Dynamics
12:30-13:30		***Lunch***	

Tuesday 10.09

13:30-16:00		**Poster Session**	
		Session #3, part 2: Quantifying and modelling cell and tissue growth [Chair: T. Greb]	
16:00 - 16:20	O-16	**S. Strauss**	Harnessing organ-centric coordinate systems for 4D biological image analysis.
16:20 - 16:35	O-17	**A. Geitmann**	From polysaccharide to polyhedron - Cell morphogenesis in the leaf epidermis
16:35 - 17:20		***Coffee break***	
17:20 - 17:50	O-18	**P. Andrey**	Deciphering cell division patterns in plant early embryogenesis by combining 3D image analysis and computer modelling
17:50 - 18:20	O-19	**L. Cerrone & A. Wolny**	Automating Cell Segmentation
18:20 - 18:35	O-20	**P. Durant-Smet**	Quantitative evaluation of feedback mechanisms between cell shape and cytoskeleton organization
20:00 - 22:00		**Speakers dinner**	

Wednesday 11.09			
		Session #3 part 3, Quantifying and modelling cell and tissue growth [Chair: J. Lohmann]	
09:00 - 09:30	O-21	**H. Jönsson**	A multi-scale approach to understand morphogenesis and differentiation
09:30 - 09:50	O-22	**K. Alim**	Plant self-organisation by feedback from tissue mechanics
09:50 - 10:05	O-23	**Y. Long**	Cellular heterogeneity in turgor pressure and growth in the shoot apex
10:05 - 10:35	O-24	**C. Godin**	Scrutinizing auxin dynamics at the shoot apical meristem … what does it mean to phyllotaxis ?
10:35 - 11:20		***Coffee break***	
		Session #4: Form and Adaptation [Chair: M. Tsiantis]	
11:20 - 11:50	O-25	**M. Fendrych**	Root gravitropism: a chain of unresolved events
11:50 - 12:10	O-26	**A. Hay**	Creating an explosion
12:10 - 12:25	O-27	**G. Grossmann**	Form follows function - shaping a cell designed to invade.
12:25-13:45		***Lunch***	
		Session #4, part 2: Form and Adaptation [Chair: M. Tsiantis]	
13:45 - 14:45	K-2	**D. Bergmann**	Multiscale investigations of leaf patterning
14:45 - 15:15	O-28	**N. Nakayama**	Informed dispersal? Environmental responses of seeds, fruits, and diaspores
15:15 - 15:45	O-29	**L. Dupuy**	Understanding limitations to root growth under mechanical stress
15:45		**Closing & departure**	

ABSTRACTS

K → Keynote

O → Oral

F → Flash talk

P → Poster

K-01 | **From Planes to Cups: The Development and Evolution of Leaf Shape**

Enrico Coen

The mechanisms by which domains of gene activity lead to the generation of tissues with intricate three dimensional shapes are poorly understood. We have been using a combination of genetic, morphological, computational and imaging approaches to address this problem in plants. Our findings suggest that spatiotemporal patterns of gene activity control formation and shaping of tissue sheets by influencing growth rates oriented by two orthogonal polarity fields. These principles will be illustrated through developmental analysis of planar and cup-shaped leaves.

K-02 | **Multiscale investigations of leaf patterning**

Dominique Bergmann, Yang Gong, Martin Bringmann, Dirk Spencer, Sophie Wallner, Juan Dong, Andrew Muroyama, On Sun Lau and Kelli Davies

Leaves can exist in a spectacular variety of forms, exhibiting size and shape variation even within an individual plant. Yet leaves also have a characteristic organization of tissue layers and cells within those tissues. In dicot leaves, multipotent, selF-0renewing populations dispersed in the epidermis (termed stomatal lineages) create the majority of cells. Among the products of this lineage, stomatal guard cells function in plant-atmosphere gas exchange. Stomatal guard cells are created through asymmetric cell divisions whose number and orientation are dictated by the interplay of specific transcription factors, local cell-cell interactions and information from the environment. In the past decade, we found that cell-type specific bHLH transcription factors (TFs) recruit entourages of more general regulators (MAPKs, chromatin remodelers, etc.) and we have used the bHLH TFs as a foothold to capture cell-type-specific gene regulation modules in Arabidopsis. Advances in time-lapse imaging, quantitative growth analysis and single cell transcriptomics have also made it possible, and necessary, to revisit classic ideas of leaf development. We are particularly interested in using the stomatal lineage as a model to link cell identity and polarity to the emergence of pattern at local, regional and organ-wide scales. We are investigating how interplay among growth, oriented divisions and cell specification could contribute to the characteristic patterning of stomata as well as to the generation of leaf shapes. We are also interested in the molecular basis of polarity generation and, starting from the polarity scaffold protein, BASL, have identified a network of activities that enable cells to produce physically asymmetric divisions and to enforce different identities after those divisions.

O-01 | **Suboptimal microtubule response to mechanical stress buffers growth variation**

Hamant O

Multicellular organisms exhibit reproducible shapes, yet at the cell level, growth can be extremely heterogeneous and variable. What are the buffering mechanisms that filter such heterogeneitity and variability? Here we take the example of plant organs where final shape only depends on cell division and cell elongation. We and others showed that shape- and growth-derived forces act as signals that orient microtubules and cellulose microfibrils in the cell walls. This response channels key biological features, such as cell shape or cell division plane orientation. We found that such mechanical feedback contributes to organ shape reproducibility. Surprisingly, the response of microtubule to stress in the wild type is not optimal, but suboptimal. Notably, we show that phenotypic variability can also emerge from a too strong response to mechanical stress. Looking for molecular regulators of developmental robustness and transcriptional noise, we identified interactions with mechanotransduction players. Altogether, this work reveals the mechanical complexity behind the robustness of organ shapes, and puts forward the question of suboptimality in biology.

O-02 | **Cellular growth patterns shaping the Arabidopsis ovule**

Tofanelli R., Vijayan A., and Schneitz K.

How the Gestalt of a plant tissue is generated remains an open question in plant developmental biology. Ovule curvature represents a unique phenomenon in plant tissue morphogenesis and invites interesting questions regarding the cellular, molecular, and mechanical mechanisms involved in the formation of this characteristic shape. The ovule of Arabidopsis thaliana represents a nice model system to address this question. At maturity, the ovule exhibits a distinctive curved shape such that the micropyle is placed next to the base of the funiculus, resulting in a 180° bending (anatropy). A qualitative description of ovule development in A. thaliana has been established, as has been a staging system based on morphological features. Ovules originate from placental tissue of the gynoecium as protrusions and three morphological pattern elements are recognized along the proximal-distal (PD) axis: funiculus, chalaza, and nucellus. A. thaliana carries bitegmic ovules as the chalaza originates two integuments, precursors of the seed coat. It has been postulated that differential growth of the outer integument contributes to curvature. Comparative evolutionary studies suggested that the presence of an outer integument is crucial for anatropy in bitegmic ovules. Moreover, genetics provided evidence on the central role of the outer integument, through the identification of mutants carrying ovules with a defect in outer integument development. We have performed a quantitative analysis of the cellular growth patterns of the integuments in wild-type and mutants at different developmental stages. Our pipeline consisted of 3D image acquisition of fixed samples at cellular resolution followed by image processing and analysis in MorphographX. The results indicate that the outer layer of the outer integument is a central regulator of ovule curvature. Furthermore, they validate our quantitative approach as a promising strategy to explore the cellular mechanisms underlying ovule curvature.

O-03 | **Role of ovule growth dynamics on germline fate establishment in Arabidopsis**

Hernandez-Lagana E., Mosca G., Mendocilla-Sato E., Pires N., Grimanelli D., Autran D., Baroux C.

The differentiation of Spore Mother Cells (SMC), precursors of the plant germline, marks the somatic-to-reproductive transition. In flowering plants, female SMCs arise in the nucellus of outgrowing ovule primordia in an apical, subepidermal (L2) position. The observation that natural variation or (epi)genetic perturbations enable more than one SMC among apical L2 cells raises the question of the role of nucellus patterning on SMC specification. To answer this question, we first set to describe the growth of the ovule primordium at cellular resolution and in 3D in Arabidopsis. Combining 3D microscopy imaging and image processing, and a developmental atlas of cell divisions we provide a comprehensive analysis of cell division and cell growth events shaping ovule primordium development. Notably, our analysis reveal that SMC characteristics emerge (i) earlier than previously thought during primordium development, (ii) in a well-defined L2 niche and (iii) often for more than one cell but (iv) a developmental canalization is observed that leads to a unique SMC in well-established primordia prior meiosis. Second, we made use of a set of katanin mutants disrupted in organ shape due to lack of anisotropic growth and altered cell division patterns. We found that katanin ovules have a characteristic alteration of the primordium shape together with an altered temporal pattern of cell division in the nucellus. Remarkably, disturbance of ovule morphology is enough to break the SMC unicity rule, where more than one SMC gaining competence to express markers of reproductive fate transition. Third, and finally, 2D simulations suggest a plausible scenario for spatially differentiated cell division patterns in shaping the ovule primordium, a process which allows the emergence of one or more SMC candidate cells. In conclusion, we show that SMC establishment is tightly linked with ovule growth, connecting organ emergence with cell fate at the somatic-to-reproductive transition.

O-04 | **A peptide pair coordinates regular ovule initiation patterns with seed number and fruit size**

Kawamoto N., Pino Del Carpio D., Hofmann A., Mizuta Y., Kurihara D., Higashiyama T., Uchida N., Torii K.U., Colombo L., Groth L., and Simon R.

Ovule development in Arabidopsis thaliana involves a pattern formation process which ensures that ovules are regularly arranged in the pistils to reduce competition for nutrients and space. Mechanisms underlying pattern formation in plants, such as phyllotaxis, flower morphogenesis or lateral root initiation, have been extensively studied ,and genes controlling the initiation of ovules have been identified. However, how a regular spacing of ovules is achieved is not known. Using genome-wide association studies combined with quantitative trait locus analysis, we found that the spacing of ovules in the developing fruits is controlled by two secreted peptides, EPFL2 and EPFL9, and their receptors from the ERECTA family that act from the carpel wall and the placental tissue. We found that a signalling pathway controlled by EPFL9 from the carpel wall and acting through the LRR-receptor kinases ER, ERL1 and ERL2 promotes fruit growth. Regular spacing of ovules depends on EPFL2, which is also expressed in the carpel wall, and in the inter-ovule spaces where it acts through ERL1 and ERL2. Loss of EPFL2 signalling results in shorter fruits and irregular spacing of ovules or even ovule twinning. The EPFL2 expression pattern between ovules is negatively feedback regulated by auxin which accumulates in the arising ovule primordia. We propose here that the auxin-EPFL2 signalling module evolved to control the initiation and regular, equidistant spacing of ovule primordia, which serves to minimise competition between developing seeds. Together, EPFL2 and EPFL9 coordinate ovule patterning and thereby seed number with fruit growth through a set of shared receptors.

O-05 | **The mechanical conundrum of seed shape regulation**

Creff A., Delattre A., Ali O., Landrein B. and Ingram G.

Using the seed of Arabidopsis as a model system, we are studying how cell and tissue mechanics control the emergence of a specific shape during development. We have shown, using a combination of molecular genetics, biophysics (indentation) and modelling, that the growth of the seed is driven by the turgor of the expanding endosperm during early development (Beauzamy et al. 2016, Development). The surrounding maternal tissues are put under tension by the expanding internal tissues and a specific layer of the seed coat (the adaxial epidermis of the outer integument) is able to respond transcriptionally to this tension, and to control growth through cell wall thickening and rigidification, thus revealing the existence of mechanical signalling within the developing seed (Creff et al. 2015, Nature Communications). Ongoing work is focused on exploring in more depth the interplay between turgor pressure generation in the endosperm and mechanical responses in the testa. Even though the Arabidopsis seed has an apparently simple shape, understanding how this is achieved by the physical interplay between two genetically independent compartment is a complex undertaking. The fact that turgor within the endosperm compartment acts both to drive seed expansion, but also, by triggering tension-mediated responses in the testa, to restrict seed growth, gives what could be termed as an incoherent mechanical feed-forward loop. Thus, a mutant with an abnormally high internal pressure, can produce abnormally small seeds. We are currently exploring the outcome of changing parameters in the seed system by quantifying the evolution of seed shape during development in various mutant backgrounds. In parallel we are developing novel mathematical models that can be used to integrate processes in one compartment and their consequences in the adjoining compartment.

O-06 | **Control of plant morphogenesis by adaxial-abaxial cell type boundaries**

Yu X., Bhatia N.,, Caggiano MP., Ohno C., Heisler MG.

Fundamental feedback processes that regulate plant morphogenesis include feedback between morphology, mechanical stress and microtubule orientations as well as between auxin transport and auxin signaling. In this talk I will discuss how these feedback processes are constrained and organized by cell type boundaries in order to produce typical plant morphologies.

O-07 | **Exploring the Morphodynamics of Radial Plant Growth**

Ivan Lebovka, Dongbo Shi, Alexandra Zakieva, Theresa Schlamp, Dmitry Richter, Thomas Greb

Radial growth of plant shoots and roots is based on the activity of a group of stem cells designated as the cambium. Thereby, the cambium substantially contributes to the dominance of seed plants in terrestrial ecosystems, to wood formation and to carbon immobilization. In addition, the process represents a striking example for a constant remodeling of adult body structures without impairing their actual function. Thus, lateral growth provides excellent opportunities for addressing the fundamental question of how cellular properties are adapted during the growth of adult organisms and how postembryonic growth processes integrate constraints of existing body structures. By employing genetically encoded cell lineage-tracing, we defined functional cambium domains including an individual layer of stem cells producing both xylem and phloem tissues through periclinal cell divisions. To investigate stem cell dynamics we established a cell-based computational model visualizing cambium activity and integrating the function of central cambium regulators. Performing iterative comparisons of plant and model anatomies, we conclude that an intercellular signaling module consisting of the receptor-like kinase PXY and its ligand CLE41 builds a morphogenic gradient instructing cambium organization. Moreover, the model predicts that the orientation of cell divisions is influenced by differences in mechanical properties of cambium-derived tissues. These results highlight the importance of a multilayered communication between cells along the radial sequence of tissues within the cambium area and provide an integrated view on the dynamics of radial plant growth.

O-08 | **A growth-based framework for leaf development and diversity.**

Miltos Tsiantis, David Wilson-Sanchez, Ziliang Hu

How diversifying gene activities translate into the distinct organ morphologies of complex eukaryotes remains poorly understood. Here, we will discuss our group's work studying this problem by using two related plants with differently shaped leaves: Arabidopsis thaliana with a simple leaf shape and Cardamine hirsuta, a plant with complex leaves, that we developed into a versatile system for comparative genetics. We use live imaging, genetics, computational modelling, and analyses of growth to deconstruct these two divergent leaf forms into their cell-level constituent elements: the amount and direction of growth, and the rate of differentiation. We found that differences in leaf geometry originate from two distinct processes that act in C. hirsuta but not in A. thaliana leaves. In the first process, which requires the KNOX gene SHOOTMERISTEMLESS (STM), delayed differentiation and slower but prolonged growth throughout the leaf primordium increase the size and number of repeated outgrowths initiated along the leaf margin. These protrusions are patterned by a conserved auxin-based mechanism that also requires CUC gene activity and helps polarize growth. In the second process, local growth inhibition, mediated by the HD-ZIPI gene REDUCED COMPLEXITY (RCO), accentuates growth differences created by CUC/auxin marginal patterning. On the basis of this information, we experimentally reconstructed key aspects of the C. hirsuta leaf form in A. thaliana by concurrently expressing RCO and STM in A. thaliana leaf primordia. These findings highlight the value of conceptualizing the origin of biological shapes by linking gene activity to cellular growth parameters. Notably, leaf shape of A. thaliana transgenic lines expressing RCO and STM can still be distinguished from that of C. hirsuta, indicating that additional genes contribute to leaf shape diversity between these species. Progress towards studying those genes will also be discussed.

O-09 | **Plant self-organisation by feedback from tissue mechanics**

Christian Wenzl, Yossi Capua, Deniz T. Bak and Jan U. Lohmann

Shape and activity of the shoot apical meristem (SAM) have a profound influence on plant morphology. Since all above ground organs are directly derived from this stem cell system, regulatory programs controlling cell proliferation and organ initiation are tuned to perform within a set of morphological parameters of the shoot apex. However, neither the role of basic cellular features of the meristem, such as cell number, cell size, or cell wall stiffness, nor the influence of differential cell fate e.g. stem cell fate vs. non-stem cell fate, on shoot morphology have been fully resolved. To disentangle the influence of cellular parameters and signaling, we are using environmental variation and genetics and find that both inputs can be spatially and temporally uncoupled. While stem cell identity responds quickly and reversibly to transient environmental perturbations, cell size and number within the shoot apical meristem are fixed after a critical developmental window. Conversely, stable genetic modification of cell size and shape by a novel mutation only has a mild influence on SAM layout and the activity of signaling reporters, suggesting potent compensatory mechanisms. Using deep sequencing, we have identified the genetic lesion causal for the cell size defect, which can now be exploited experimentally.

O-10 | **Division plane orientation in plant cells**

Carolyn Rasmussen

How cells within multicellular organisms coordinately grow and divide are fundamental questions in plant biology. Pattern formation within tissues and organs relies on proper integration of cell-cell communication and developmentally regulated growth and division. We developed a cell-shape based model to predict division planes by using a soap-film minimizing approach. This model produces two equal-sized daughter cells while minimizing the surface area of the division plane similar to soap-films. In wild-type cells, the geometry of the cell is almost always sufficient to generate predictions that closely match in vivo division planes across developmental stages and in both plant and animal cells. However, when we used our model on a mutant with defects in division plane orientation, tangled1, we noticed that most divisions aligned well with predicted divisions, but some did not. Preliminary evidence showed a positive correlation between irregular cell shape and division plane offset from the prediction in the tangled1 mutant. This suggests that aberrant cell shape itself may promote improper division plane placement. When we searched for aberrantly shaped wild-type cells, they also had division planes that did not align with our predicted divisions from modeling. Together, these data suggest that the mechanism promoting soap-film minimization in plant cells is more accurate in regularly-shaped and less accurate with irregularly-shaped cells. Future studies will provide insight into how microtubule organization contributes to division plane orientation in maize and other plants.

O-11 | **Cytoskeleton dynamics and lateral root morphogenesis**

Maizel, A.

Morphogenesis of lateral roots relies on the asymmetric cell division of initially symmetric founder cells. This division is preceded by the tightly controlled asymmetric radial expansion of these cells. The cellular mechanisms that license and ensure the coordination of these events are unknown. We quantitatively analyze microtubule and F-0actin dynamics during lateral root initiation. Using mutants and pharmacological and tissue-specific genetic perturbations, we show that dynamic reorganization of both microtubule and F-0actin networks is necessary for the asymmetric expansion of the founder cells. This cytoskeleton remodeling intertwines with auxin signaling in the pericycle and endodermis in order for founder cells to acquire a basic polarity required for initiating lateral root development. Our results reveal the conservation of cell remodeling and polarization strategies between the Arabidopsis zygote and lateral root founder cells. We propose that coordinated, auxin-driven reorganization of the cytoskeleton licenses asymmetric cell growth and divisions during embryonic and post-embryonic organogenesis.

O-12 | **Molecular and cellular mechanisms regulating the cell division orientation during early plant embryogenesis**

Vaddepalli P., de Zeeuw T. and Weijers D

Plant cells are surrounded by rigid walls restricting their movement. Therefore oriented cell division is crucial in formative events during embryonic and post-embryonic development to generate cell diversity. Despite detailed knowledge of the intrinsic cell division machinery, a key unanswered question is how orientation of cell divisions is controlled by developmental regulators. Recent work has demonstrated that cell geometry constrains division plane through a simple physical rule, confirming the predictions from classical models. This geometric default rule can indeed explain many divisions in the early Arabidopsis embryo in 3D. But, all asymmetric divisions disobeyed this rule and it was shown that Auxin is required for this deviation from default rule. However, the cellular and molecular mechanisms that are responsible for the suppression of the default division are unknown. Using a combination of genetic, cell biological, molecular and computational approaches, we are investigating the contribution of nuclear positioning, cell polarity and cell shape during asymmetric cell division. Furthermore, the role of conserved signaling molecules like Rho family GTPases in regulating plane orientation is also being investigated, which are renowned for their function in cell polarity by coordinating cytoskeleton dynamics and vesicle trafficking. Thus my project aims to uncover the molecular and cellular mechanisms that regulate the cell division machinery for asymmetric cell division plane orientation.

O-13 | **Live-cell imaging of the intracellular dynamics of Arabidopsis zygote**

Kimata Y., Higaki T., Kurihara D., Kato T., Yamada T., Segami S., Morita M. M., Maeshima M., Hasezawa S., Higashiyama T., Tasaka T. and Ueda M.

In most flowering plants, the asymmetric cell division of the zygote is the initial step in establishing the apical–basal axis. In Arabidopsis thaliana, the zygote is polarized, possessing the nucleus at the apical tip in the elongated cell. In spite of its obvious asymmetry, the real-time dynamics of the zygote polarization steps was poorly understood, because the zygote develops deep in the flower. Therefore we established a live-cell imaging system by utilizing in vitro ovule cultivation system and the two-photon excitation microscopy to visualize intracellular dynamics of the zygote through the ovule. By combining this system with various specific inhibitors and mutants, we assessed the dynamics and roles of various intracellular events, such as cytoskeleton rearrangement and organelle migration in the zygote polarization. Based on these results, we would like to discuss the efficiency of this approach to understand the initiation steps of plant ontogeny from the cell biological aspect.

O-14 | **Polar transport in growing plants: from maxima to minima, phytohormones and nutrients**

Grieneisen V.

I will present combined modelling and experimental work that reveals different mechanisms underlying the creation patterns which are developmentally instructive for root development and fruit maturation. In this context, we start seeing that auxin minima should also be regarded as developmentally functional and relevant. However, to drive the actual growth of the plant, nutrient uptake from the soil is critical, and this often also comes in the form of regulated and polar transport. By quantitatively studying the uptake of the essential mineral Boron, together with collaborators from Japan and Cardiff University, we have discovered several general principles for generating stable and robust flows through plant tissue. In particular, I will highlight the hidden importance of the cell wall in these long-distance processes.

O-15 | **Two mechanisms control growth anisotropy in Arabidopsis lateral roots**

Kirchhelle C., Garcia-Gonzalez D., Jérusalem A., Moore I.

Multicellular plants robustly and reproducibly generate organs of astonishing morphological diversity. As plant cells are encased by a rigid cell wall and cannot migrate from their position in the tissue, this diversity in shape depends on the plant's ability to establish and control directional cell growth. Cellular growth rate and direction depend on the precisely targeted deposition and modification of cell wall macromolecules (principally cellulose and pectins) at different faces of the cell. Intracellular transport in eukaryotes is regulated by a complex molecular machinery, of which the Rab family of small GTPases have emerged as key regulators. We have recently reported that a plant-specific member of the Rab GTPase family, RAB-A5c, identifies a selective exocytic trafficking pathway to geometric edges of meristematic cells (where two faces meet), thus identifying edges as a novel domain of 3D cell patterning. In lateral roots, inhibition of Rab-A5c-mediated edge-directed trafficking caused loss of directional growth at the cell and organ scale, severely perturbing cell and organ morphology. I will present our latest modelling, pharmacological and genetic data investigating the role of RAB-A5c-mediated trafficking during growth control. Our data demonstrates that growth direction control through RAB-A5c is independent of oriented cellulose microfibril deposition at faces, the leading paradigm for growth direction control. However, cellulose microfibril reorganisation can partially compensate for loss of RAB-A5c function. Why have plants evolved at least two independent mechanisms to control directional growth in lateral roots – structural anisotropy at cell faces through oriented deposition of cellulose microfibrils, and RAB-A5-mediated activity at cell edges? I will present our latest hypotheses for why these different mechanisms have evolved and how they interact with each other.

O-16 | **MorphoGraphX 2.0 Harnessing organ-centric coordinate systems for 4D biological image analysis**

Soeren Strauss, Adam Runions, Brendan Lane, Richard Smith Group, Miltos Tsiantis Department (MPIPZ Cologne)

The study of plant morphogenesis requires knowledge about the geometry and the development of whole organs as well as individual cells. Modern imaging techniques such as confocal microscopy provide high quality 3D images of biological tissues and also allow to track a region of interest over time through time-lapse. The open source software MorphoGraphX (De Reuille & Routier-Kierzkowska et al., 2015) enables researchers to analyze biological images on a cellular level and has been used in numerous studies in the last years (e.g. Kierzkowski et al., 2012; Scheuring et al., 2016; Hong et al., 2016; Hofhuis et al., 2016; Kierzkowski et al., 2019). The core feature of the first release version of MorphoGraphX was a computational pipeline to create segmented curved meshes of the organ surface from 3D confocal microscope images. Alternatively volumetric segmentation can be created if the image quality allows it. Next, cell geometry and marker expression as well as developmental features such as cell growth and growth directions can be quantified. Due to its modular structure, MorphoGraphX can easily be extended by additional plug-ins. Since the first release of the software, a large number of plug-ins have been implemented to enable new kinds of data analysis and visualization which we will publish together with an extended documentation as MorphoGraphX version 2.0. For instance, it is now possible to create custom organ centric coordinate systems or cell atlases to objectively compare different samples, time points or genotypes (Montenegro-Johnson et al., 2015 & 2019). Moreover, cellular geometry and growth can be quantified in greater detail (Sapala et al., 2018; Kierzkowski et al., 2019). Further new processes allow to semi-automatically classify cell types and to create morphing animations of time-lapse data.

O-17 | **From polysaccharide to polyhedron - Cell morphogenesis in the leaf epidermis**

The generation of simple plant cell geometries such as cylindrical shoot epidermal cells is known to be regulated by the extensibility pattern of the primary cell wall, thought to be largely determined by cellulose microfibrils. However, the mechanism leading to more complex shapes such as the interdigitated, jigsaw puzzle-like patterns in the epidermis of eudicotyledon leaves is poorly understood. We investigated how the cell wall regulates the morphogenetic process in these cells and which initial steps lead to the characteristic undulations in the cell circumference. Brillouin microscopy and polarized fluorescence imaging allowed us to untangle the respective roles of cellulose and homogalacturonan pectin during lobe formation in the epidermal pavement cells of the cotyledons of Arabidopsis thaliana. We show that non-uniform distribution of cellulose microfibrils and demethylated pectin correlate with spatial differences in cell wall stiffness but intervene at different developmental stages. Challenging the widely accepted paradigm of cellulose as a crucial morphogenetic agent, we discovered that lobe initiation involves a modulation of cell wall stiffness through the local enrichment in demethylated pectin, whereas only the subsequent increase in lobe amplitude is mediated by the stress-induced deposition of aligned cellulose microfibrils. Finite element simulations lead us to propose that both steps are preceded by a turgor-driven mechanical buckling event that serves as the initial trigger for the multi-step morphogenetic process.

O-18 | **Deciphering cell division patterns in plant early embryogenesis by combining 3D image analysis and computer modelling**

Laruelle E, Moukhtar J, Trubuil A, Belcram K, Legland D, Khadir Z, Palauqui JC, Andrey P

In plants, cell division and oriented growth are the major cellular determinants of tissular organizations and developmental shape transitions. A major challenge in plant developmental biology is thus to understand how the position and the orientation of division planes are selected. Since the XIXth century, several phenomenological rules have been proposed to relate division plane selection and cell geometry. It is generally considered that symmetric cell divisions, which contribute to cell proliferation, correspond to a default mechanism driven by physical constraints. In particular, the minimum plane area principle embedded in Errera's rule has been shown to explain a number of experimental observations. However, most research efforts to date have concentrated on symmetric divisions and specific systems where cell shapes can be assimilated to 2D geometries. Therefore, to what extent 3D cellular geometry may influence symmetric and asymmetric divisions and consequently cell division patterns remains poorly explored. Here, we show how we combined 3D confocal imaging, automated image analysis, cell lineage reconstruction, and stochastic computational modelling to analyze cell division patterns during early embryogenesis in the model plant Arabidopsis thaliana. Our results highlight invariant principles relating mother cell shape and division plane positioning. Introducing a new cell division model, we demonstrate the existence of a new geometrical rule allowing to predict the selection of the division plane in both symmetric and asymmetric divisions. In addition, we show that the same rule can explain both stereotyped and variable cell division patterns depending on cell generation. An important consequence of our findings is that the apparently complex cell organization of the Arabidopsis early embryo could be interpreted as a selF-0organized structure emerging from a geometrical feedback loop between cell geometry and division plane positioning.

O-19 | **Volumetric plant cell instance segmentation with neural networks**

Wolny A., Cerrone L., Hamprecht F. A., Kreshuk A.

Instance segmentation is the task of partitioning an image into meaningful regions relevant for the problem at hand. In particular, plant cell instance segmentation of 3D volumetric images is a fundamental step in quantitative biological analysis, but it is also an inherently difficult and time-consuming task. In recent years Deep Learning has revolutionized the field of machine learning, bringing automated algorithms closer to human performance. In particular, for instance segmentation, coupling a deep convolutional network for boundary detection with a clustering algorithm proved to be extremely successful. Despite its great performance, so far deep learning did not become a practical tool. Indeed training such networks requires a large amount of human curated data sets and generalization to even a slightly different image domain is generally not guaranteed. We collected a large number of plants organs datasets coming from different modalities such as confocal and light sheet microscopy, and performed extensive experiments across a large variety of segmentation techniques. Based on our findings we built an instance segmentation pipeline which gives the best performance across different datasets. The proposed pipeline is capable of accurate automatic segmentation and generalizes to different microscope modalities. We aim to provide our pipeline as a user friendly tool for 3D cell instance segmentation.

O-20 | **Quantitative evaluation of feedback mechanisms between cell shape and cytoskeleton organization**

Durand-Smet P., Spelman T., Meyerowitz E., Jonsson H.

Specific cell and tissue form is essential to support many biological functions of living organisms. During development, the creation of different shapes fundamentally requires the integration of genetic, biochemical and physical inputs. In plants, it is well established that the cytoskeletal microtubule network plays a key role in the morphogenesis of the plant cell wall by guiding the organisation of new cell wall material. Moreover, it has been suggested that mechanical stresses orient the microtubules along their principal direction, thereby controlling wall architecture and plant cell shape. The cell cytoskeleton is thus a major determinant of cell shape. What is less clear is how cell geometry in turn influences cytoskeletal organization. Here we present an experimental approach to explore the relative contribution of geometry to the final organization of actin and microtubule cytoskeletons in single plant cells. In addition, the study of actin and microtubules at the same time and in the same system has allowed us to compare the organization of the two networks and how they interact. A model of self organizing microtubules in 3D predicts that severing of microtubules is an important parameter controlling the anisotropy of the microtubules network. We experimentally confirmed the model predictions by analysis of the response to shape change in plant cells with altered microtubule severing dynamics. This work is a first step towards assessing quantitatively how cell geometry controls cytoskeletal organization.

O-21 | **A multi-scale approach to understand morphogenesis and differentiation**

Jönsson, H.

The plant shoot meristem produces new cells to feed the above ground plant. Coordinated cell expansion and differentiation leads to the initiation of complex tissue shape and organ initiation. We use a Computational Morphodynamics approach, combining live imaging with computational modelling for a quantitative and predictive understanding of mechanisms regulating the development. In this talk I will focus on how we combine models at different scales to gain an understanding of feedback between shape, mechanics and molecular regulation driving these processes.

O-22 | **Plant selF-0organisation by feedback from tissue mechanics**

Alim K.

Plant organ outgrowth superficially appears like the continuous mechanical deformation of a sheet of cells. Yet, how precisely cells as individual mechanical entities can act to morph a tissue reliably and efficiently into three dimensions during outgrowth is still puzzling especially when cells are tightly connected as in plant tissue. In plants, the mechanics of cells within a tissue is particularly well defined as individual cell growth is essentially the mechanical yielding of cell-wall in response to internal turgor pressure. Cell wall stiffness is controlled by biological signalling and, hence, cell growth is observed to respond to mechanical stresses building up within a tissue. What is the role of the mechanical feedback during morphing of tissue in three dimensions? Here, we develop a three dimensional vertex model to investigate tissue mechanics at the onset of organ outgrowth at the tip of a plant shoot. We find that organ height is primarily governed by the ratio of growth rates of faster growing cells initiating the organ to slower growing cells surrounding them. Remarkably, the outgrowth rate is higher when cells growth responds to the tissue-wide mechanical stresses. Our quantitative analysis of simulation data shows that tissue mechanical feedback on cell growth can act via twofold mechanism. First, the feedback guides patterns of cellular growth. Second, the feedback modifies the stress patterns on the cells, consequently amplifying and propagating growth anisotropies. This mechanism may allow plants to grow organs efficiently out of the meristem by reorganizing the cellular growth rather than inflating growth rates.

O-23 | **Cellular heterogeneity in turgor pressure and growth in the shoot apex**

Yuchen Long, Ibrahim Cheddadi, Vincent Mirabet, Gabriella Mosca, Mathilde Dumond, Jan Traas, Christophe Godin, Arezki Boudaoud

Cell-to-cell fluctuations are observed in many biological phenomena like gene expression, signaling, cell size regulation and growth. Notably, heterogeneity in cell size and growth rate often prevails and impacts tissue patterning and macroscopic growth robustness (1). Plant cell expansion is driven by turgor pressure, and is restrained by cell wall. Despite numerous studies (2, 3), the spatial variation of turgor pressure and its relation with cell-to-cell growth variability in multicellular tissues remain elusive. Here, using micro-indentation by atomic force microscope, we demonstrate that turgor pressure is heterogeneous between adjacent cells in the epidermis of Arabidopsis shoot apical meristem. Combining experiments and physical modelling of cell wall mechanics and water movement, we show that cell size and tissue topology pattern heterogeneities in pressure and growth, reminiscent of a liquid foam. Pressure correlates either positively or negatively with cellular growth rate depending on conditions, suggesting that the meristem is at a tipping point between limitation of growth by cell wall extensibility and by water conductivity. Together, our results shed light on spatial variations of pressure and growth rate, with potential roles in tissue homeostasis. References 1. L. Hong et1 1a1l1.1,1 1A1n1n1u1a1l1 1R1e1v1i1e1w1 1o1f1 1P1l1a1n1t1 1B1i1o1l1o1g1y1.1 16191,1 1416191419151 1(121011181)1.1 121.1 1J1.1 1K1.1 1O1r1t1e1g1a1,1 1P1l1a1n1t1 1P1h1y1s1i1o1l1.1

O-24 | **Scrutinizing auxin dynamics at the shoot apical meristem … what does it mean to phyllotaxis ?**

Godin C.

While auxin is one of the main hormonal regulators of phyllotaxis patterning at the shoot apical meristem, its distribution at the surface of the SAM is still largely not understood. In the past decade, a number of computational models have been used to test different hypotheses about auxin dynamics and transport. However, whether these models predict consistent auxin distributions within the SAM is largely debated. To proceed further, we used quantitative imaging to demonstrate that auxin provides graded information at the SAM in space and time. We showed that this information is integrated in time to differentiate temporally potential organ initiation sites. I will discuss how these dynamic fluctuations of auxin provide new insights on how phyllotaxis is regulated at the SAM and the impact of these new data on the development of phyllotaxis models.

O-25 | **Root gravitropism: a chain of unresolved events**

Fendrych, M

Plant roots grow in soil and use gravity as a reference to navigate in this environment. Root gravitropism is a complex chain of events: gravity vector change materialized by statolith sedimentation is perceived in the columella cells (by an unknown mechanisms). Auxin flux is redirected by PIN auxin efflux proteins to the lower side of the root (how PINs are repolarized or activated is not clear). Then auxin flows to the lower side of the root by a coordinate action of PIN2 and AUX1. Finally, cells on the lower side respond to the rise of auxin concentration by inhibiting their elongation and the root bends downwards. The pathway connecting auxin and the inhibition of cell elongation is not understood either. The reaction of cells to auxin is in the center of our research and I will discuss the recent progress in understanding auxin perception and the physiological response of root cells to this hormone. Studying gravitropic behavior of roots requires specific approaches and hardware equipment; I will present our vertical imaging platform and our pursuit for establishing microfluidic imaging pipeline with well growing Arabidopsis roots. Finally, studying gravitropism and reaction to auxin calls for the ability to precisely measure physiological parameters dynamics in an unbiased way, and I will present some of the image analysis tools we use for this purpose.

O-26 | **Creating an explosion**

Adibi M., Weber G., Smith R. and Hay A

How mechanical and biological processes are coordinated across cells, tissues, and organs to produce complex traits is a key question in biology. Cardamine hirsuta, a relative of Arabidopsis thaliana, uses an explosive mechanism to disperse its seeds. We have shown that this trait evolved through morphomechanical innovations at different spatial scales. At the organ scale, tension within the fruit wall generates the elastic energy required for explosion. This tension is produced by differential contraction of fruit wall tissues through an active mechanism involving turgor pressure, cell geometry and wall properties of the epidermis. Here, we investigate how growth and tension interact to produce explosive fruit.

O-27 | **Form follows function - shaping a cell designed to invade.**

Grossmann G.

Tip growth enables cells to penetrate their surrounding and thereby tap into nutrient resources, infect host organisms or find mating partners. Root hairs and pollen tubes are tip growing, single cell structures in plants that are specifically designed to invade soil and maternal tissue, respectively. We use root hairs of Arabidopsis as model to understand the architectural principles and regulatory mechanisms that drive morphogenesis of a functional cell shape. The root hair is a remarkable example of robustness and plasticity going hand in hand to ensure functional cell morphology. While root hair positioning, diameter and growth angle are robust features with little observed variation, the time window and trigger of outgrowth, growth rate and final length can be highly variable. In this presentation, I will discuss theoretical considerations regarding the emergence of specialized cell shapes and report our recent findings on root hair formation and growth regulation. We followed the targeted recruitment of the growth machinery to the emerging site of outgrowth over time and found that polarization and growth initiation are temporally separate processes that each involve interaction of ROP GTPases with phase-specific RopGEFs. A novel super-resolution imaging approach revealed the formation of stable nanodomains in the polar root hair initiation domain of the plasma membrane that recruit and locally accumulate ROPs. After this robust polarization, growth regulation is influenced by environmental conditions. By allowing a single root to grow in asymmetric microfluidic environments, we could demonstrate that hair growth regulation occurs largely in a cell-autonomous fashion, likely through direct modulation of the apical growth machinery.

O-28 | **Informed dispersal? Environmental responses of seeds, fruits, and diaspores**

Nakayama N

Finding a new habitat is sometimes a necessary but difficult challenge. Animals are known to select the timing and direction of migration to increase the chance of reaching a favourable new niche, in a process known as 'informed dispersal'. Plant migration largely occurs in the form of seed dispersal, which relies on anatomical and morphological adaptations for the use of physical or biological dispersal vectors. Recently, studies of interactions between the dispersal unit and physical environment have uncovered fluid dynamic mechanisms of seed flight, protective measures against fire, and release mechanisms of explosive dispersers. Although environmental conditions generally dictate dispersal distances, plants are not purely passive players in these processes; some plants may also enact informed dispersal, where dispersal-related traits are modified according to the environment. This can occur via developmental regulation, but also on shorter timescales via structural remodelling in relation to water availability and temperature. Linking interactions between dispersal mechanisms and environmental conditions will be essential to fully understand population dynamics and distributions.

O-29 | **Understanding limitations to root growth under mechanical stress**

Matthias Mimault, Daniel Patko, Yangminghao Liu, Ilonka Engelhardt, Vincent Ladmiral, Bruno Ameduri, Mike MacDonald, Mariya Ptashnyk, Lionel Dupuy

Land use is facing extraordinary pressures from a growing world population. Knowledge of where and how crop yield can be enhanced has become crucial, and this requires fundamental understanding of limitations to growth under biotic and abiotic stresses from the soil. We have developed a suite of techniques for detailed quantification of root development in realistic but controlled growth environment. Techniques include fluidics-based approaches for control of soil conditions, quantification of particle forces, transparent soil substrate for live measurements in situ, and large field of view light sheet microscopy for quantitative imaging. We are also developing particle-based (SPH) approaches to model apical meristem development in response to mechanical stress. The framework identifies SPH particles with individual cells in a tissue, with anisotropic poro-mechanics, turgor pressure, cell division and cell walls biosynthesis controlling the maintenance of the organ in response to external pressure. Models show it is possible to simulate the growth of entire meristems at cellular resolution and we reveal how variations in soil particle forces can predict growth patterns in soil.

[1] Dupuy et al “Micromechanics of root development in soil”, Current Opinion in Genetics and Development (2018).

F-01 & P-02 | **Mechanical control of secondary growth in trees**

Alonso-Serra J., Shi X., Peaucelle A., Rastas P., Bourdon M., Immanen J., Takahashi J., Koivula H., Eswaran G., Muranen S., Help-Rinta-Rahko H., Smolander O-P., Su C., Safronov O., Gerber L., Salojärvi J., Hagqvist R., Mähonen A-P., Nieminen K. and Helariutta Y.

The weight of trees encompasses a vertical physical challenge to cambium-derived tissues, which in turn, undergo radial growth thereby supporting the above-ground biomass. This feedback between sensing and responding to weight orchestrates plant allometric growth and posture, a phenomenon we define here as vertical proprioception. To integrate multidimensional physical stimuli, critical mechanical conditions at the cambium may be important, yet they remain unknown. Here we show that after manipulating stem weight, the proprioceptive response modulates radial growth in birch. By taking advantage of the recessive mutation at the ELIMÄKI locus (EKI) in Betula pubescens we were able to dissect this response from the gravitropic responses associated with reaction wood formation. The eki trees collapse after three months of vertical growth because of a defective vertical proprioception response at the stem base. Based on atomic force microscopy the cambial zone in eki is mechanically more elastic than WT, which correlates with cell expansion defects of pectin-enriched cell walls during wood morphogenesis. At the genetic level, the mechanosensing pathway associated to touch is transcriptionally misregulated in eki, indicating that the ELIMÄKI locus is required for the integration of the weight-growth feedback during vertical proprioception. Taken together, we provide evidence for the local and systemic role of mechanics during cambial growth in plants. Furthermore, our genetic analysis performed under accelerated flowering conditions, within four years, introduces a fast track to Mendelian genetics in trees.

F-02 & P-45 | **Visualising hypocotyl elongation in response to stress-induced microtubule reorientation**

Sarah Robinson and Cris Kuhlemeier

Morphogenesis in plants is controlled by the spatial pattern of the mechanical properties across the tissue. During growth, internal mechanical stresses can develop and also serve as an important determinant of plant development. To investigate the mechanical properties and responses to mechanical stress in the developing tissues of the model plant Arabidopsis we developed an automated confocal micro-extensometer (ACME). ACME enables forces to be applied to tissues, while they are imaged with a confocal microscope. These images were analysed to extract 3D cellular strain measurements; revealing spatial gradients in mechanical properties that correlate with the pattern of growth. We also used ACME to investigate responses to mechanical stress. We imaged the cytoskeleton in the different layers of the hypocotyl while mechanical stress was applied. The results were analysed by building a finite element model. We saw that when a relative compressive force was applied the pattern of tissue stress changed, leading to a reorientation of the microtubules in the epidermis but not the inner layers. As the epidermis usually restricts growth, this reorientation led to growth increasing in these samples. This may mimic the response of a seedling whose growth is restricted as it pushes through the soil and responds with an increase in growth.

F-03 & P-36 | **Towards a Framework for Collective Behavior in Plant-Inspired Growth-Driven Systems**

Bastien R., Porat A. and Meroz Y.

A variety of biological systems are not motile, but sessile in nature, relying on growth as the main driver of their movement. Groups of such growing organisms can form complex structures, such as the functional architecture of growing axons, or the adaptive structure of plant root systems. These processes are not yet understood, however the decentralized growth dynamics bear similarities to the collective behavior observed in groups of motile organisms, such as flocks of birds or schools of fish. Equivalent growth mechanisms make these systems amenable to a theoretical framework inspired by tropic responses of plants, where growth is considered implicitly as the driver of the observed bending towards a stimulus. We introduce two new concepts related to plant tropisms: point tropism, the response of a plant to a nearby point signal source, and allotropism, the growth-driven response of plant organs to neighboring plants. We first analytically and numerically investigate the 2D dynamics of single organs responding to point signals fixed in space. Building on this we study pairs of organs interacting via allotropism, i.e. each organ senses signals emitted at the tip of their neighbor and responds accordingly. In the case of local sensing we find a rich state space. This work sets the stage towards a theoretical framework for the investigation and understanding of systems of interacting growth-driven individuals.

F-04 & P-14 | **Fluttering of growing leaves as a way to reach flatness: Experimental evidence on Persea americana**

Derr J., Bastien R., Couturier E. and Douady S.

Simple leaves show unexpected growth motions: the midrib of the leaf swings periodically in association with buckling events of the leaf blade, giving the impression that the leaf is fluttering. The quantitative kinematic analysis of this motion provides information about the respective growth between the main vein and the lamina. Our three-dimensional reconstruction of an avocado tree leaf shows that the conductor of the motion is the midrib, presenting continuous oscillations and inducing buckling events on the blade. The variations in the folding angle of the leaf show that the lamina is not passive: it responds to the deformation induced by the connection to the midrib to reach a globally flat state. We model this movement as an asymmetric growth of the midrib, which directs an inhomogeneous growth of the lamina, and we suggest how the transition from the folded state to the flat state is mechanically organized.
[Derr et al., (https://doi.org/10.1098/rsif.2017.0595)]

F-05 & P-03 | **Tight regulation of programmed cell death in the Arabidopsis root cap regulates cellular turnover while maintaining organ integrity**

Rafael Andrade Buono, Robert P. Kumpf, Sören Strauss, Matyas Fendrych, Jiri Friml, Richard S. Smith, and Moritz K. Nowack

The root cap surrounds the growing root tip of most land plants. In Arabidopsis and other plants, root cap cells are short-lived and turned over constantly. As a last step of cellular differentiation a programmed cell death (PCD) process allows for the removal of root cap cells in a tightly regulated fashion, thus ensuring the maintenance of root cap organ size. We use a combination of imaging techniques to capture the generation, differentiation, and degeneration of root cap cells on live growing roots. The 4D datasets are processed using machine learning, tracking, and cell shape extraction to generate detailed morphometric analysis of root cap development and progression towards PCD. We aim to unravel the tightly controlled temporal and spatial pattern of cell removal by PCD and its dependency on the cell's position in the organ and on its underlying neighbour cell. We explore potential mechanisms of PCD in root cap cells entering the root elongation zone in contrast with outermost root cap cells that undergo PCD while not in contact with epidermal cells.

F-06 & P-10 | **Contribution of PI(4,5)P2 in Arabidopsis Shoot Apical meristem development**

Plants can sense mechanical perturbations from the environment (Braam 2005), as well as mechanical stress derived from their own growth. For instance, cortical microtubules (Green King et al., 1966, Hamant et al., 2008) and gene expression (Coutand 2009, Landrein et al., 2015) are affected by growth-derived stress in plants. However, how all these signals are integrated and transduced through the plant, and what kind of roles they could play in development remain unknown. Here we address this question in the Arabidopsis shoot apical meristem (SAM), which controls the development of the aerial part of the plant. Many signals have been characterized in the SAM (Eilon Shani et al., 2006, Weits et al., 2019, Nature). Although this has not been thoroughly studied, the plasma membrane composition could play a key role in the transduction of these signals. There is accumulating evidence that PI(4,5)P2, a minor phosphatidylinositol phosphate lipid with a big polar head and highly anionic moiety is present at the plasma membrane and contributes to plant development, notably in roots (Rodriguez-Vilalon et al., 2014, Rodriguez-Vilalon et al., 2015, Ischebeck et al., 2013, Tejos et al., 2014). More recently, the use of PI(4,5)P2 biosensors lines have highlighted a stereotypical PI(4,5)P2 pattern at the SAM. Indeed, the PI(4,5)P2 biosensor is accumulated in organ-meristem boundary zones, which is crucial for organ separation, axillary meristem emergence and meristem selF-0maintenance. The central zone of the SAM, where stem cells are located, does not display an accumulation of PI(4,5)P2 biosensors (Stanislas et al., 2018). Interestingly, this pattern could correlate, in part, with the predicted mechanical stress pattern at the SAM, but also partially with predicted auxin peaks. These data suggest that the establishment of such a PI(4,5)P2 pattern could contribute to SAM functions. This project aims to investigate the contribution of PI(4,5)P2 in SAM function and development.

F-07 & P-44 | **Deciphering the Mechanisms Underlying Petal Growth & Patterning in Hibiscus trionum**

Lucie Riglet, May Yeo, Stefano Gatti, Alice Fairnie, Valentina Travaglia, Joseph Walker, Edwige Moyroud

In flowers, epidermal cells play a major role in reproduction processes such as catching pollinators' attention through petal patterning. In Hibiscus trionum, petals are white with a purple spot at the base, producing a pattern known as bullseye. This pigmented proximal region is characterized by flat elongated and striated cells whereas the white, distal region displays conical cells with a smooth surface. The mechanisms that control the specification of different cell types in a reproducible pattern across the petal epidermis are not yet understood. The bullseye geometry depends on the relative growth experienced by each petal region. Using confocal imaging of H. trionum petal, we found that cells already start to behave differently along the proximo-distal axis of the petal at a very early stage of petal development, long before any of the characteristic features of the pattern appears. We divided this stage (stage 0) into 3 substages and discovered that at substage 0a, cell behaviour is homogeneous along the petal axis. To monitor the cell proliferation rate along the petal proximo-distal axis, we imaged young petals at t0 and t18h and tracked cells across those time points using MorphographX. Combining these data will allow us to simulate petal growth with cellular resolution. We will use this virtual petal as a mesh to test the ability of various models to recapitulate the patterning process. Additionally, we used a transcriptomic approach to identify genes differentially expressed in the proximal vs distal compartments at the very beginning of petal development. Interestingly, some of the genes differentially expressed along the petal axis are homologs of Arabidopsis genes involved in abaxial/adaxial polarity and/or in boundary formation between organs. We are currently producing transgenic H. trionum lines overexpressing those genes to decipher if they could contribute to proximo/distal polarity and boundary formation within the petal epidermis.

F-08 & P-62 | **Lateral Root Priming Synergistically Arises from Root Growth and Auxin Transport Dynamics**

Yalamanchili K., Van den Berg T., De Gernier Hugues., Santos Teixeira J.A., Beeckman T., Vermeer J., Scheres B., Willemsen V. and Ten Tusscher K.H.W.J.

The root system is a major determinant of plant fitness. Its capacity to supply the plant with sufficient water and nutrients strongly depends on its architecture, which arises from the repeated branching off of lateral roots. The first critical step in lateral root formation is priming, which pre-patterns sites competent of forming a lateral root. Priming refers to temporal oscillations in auxin levels, auxin signalling and gene expression in the root meristem, which leads to the specification of cells competent for future lateral root formation. So far, the mechanism underlying oscillations has remained unclear. In this study, we developed a novel multi-scale computational root model incorporating a realistic root tip architecture and reflux loop properties as well as root growth dynamics to investigate the impact of auxin reflux loop properties combined with root growth dynamics on priming. The model shows that root tip architecture and reflux loop properties result in an auxin loading zone at the start of the elongation zone, with preferential auxin loading in narrow vasculature cells. Furthermore, we demonstrate how meristematic root growth dynamics causes regular alternations in the sizes of cells arriving at the elongation zone, which subsequently become amplified during cell expansion. These cell size differences translate into differences in cellular auxin loading potential. Combined, these properties result in temporal and spatial fluctuations in auxin levels in vasculature and pericycle cells. Our model predicts that temporal priming frequency predominantly depends on cell cycle duration, while cell cycle duration together with meristem size control lateral root spacing. At the moment, to validate our model experimentally and possibly refute other models, we apply perturbations resulting in changes in division frequency or meristem size and study its effect on oscillation.

P-01 | **The contribution of phosphatidic acid in transducing mechanical stress during shoot apical meristem development**

Ackermann F., Stanislas T.

Plants are able to sense their mechanical environment. This mechanical signal is used by the plant to determine its architecture with plant stems becoming stiffer and shorter when exposed to wind. This is true also at a smaller scale. Morphogenesis, both at the cell and tissue level, involves mechanical signals that influence specific patterns of gene expression and trigger cytoskeletal reorganisations. How a mechanical stress is perceived and how this signal is transduced into the cell to exert these effects is still poorly understood, and remains a challenging question in both the plant and animal community. Among the structural components of plant cells, the plasma membrane (PM) has received very little attention. Yet its position at the interface between the cell wall and the cytoplasmic microtubule arrays makes it a key factor at the nexus between biochemical and mechanical cues. Phospholipids have emerged as an important class of cellular messenger molecules in various cellular and physiological processes. Among them, phosphatidic acid (PA) is a simple phospholipid observed in most organisms that is an important regulator of the cytoskeleton. We are currently developing several strategies to interfere specifically (in time and space) with PA at the plasma membrane. This will give us the tool to understand how this signaling lipid play a role during mechano-perception and -transduction needed during morphogenesis both at the tissue and cell level.

P-04 | **Imaging in 4D ovule primordium development in Arabidopsis**

Mendocilla-Sato E., Hernandez-Lagana E., Mosca G., Cerrutti G., Godin C., Wightman R., Tarr P., Grimanelli G., Baroux C.* and Autran D.*

The specification of the precursor of female germ cells (spore mother cell) in plants is contemporary to ovule primordium development. To understand how germ cell specification and morphogenesis are coordinated in the ovule, we combine quantitative imaging, modeling and functional approaches. Indeed, the ovule primordium is an apparently simple biological object, consisting of around 10 contacting cells at initial stages, and amenable to monitor morphogenetic events at the cellular level and to modeling. However, imaging early ovule development is not trivial because, in angiosperms, ovules emerge from the placental tissue, deeply enclosed in the carpel at the centre of the flower. We will present our current approaches using Multiphoton microscopy to achieve time-lapse imaging of the emergence and growth of the ovule primordium in 3D.

P-05 | **Hypocotyl endodermis in the dark: keeping the pace with fast growth and differentiation**

Barbosa I. and Geldner N.

The hypocotyl is an embryonic organ responsible for seedling emergence from soil to the surface during seed germination, that later becomes indistinguishable from roots after secondary growth. In the dark, hypocotyls elongate at extreme pace fueled by energy stored in the cotyledons. The cellular basis of hypocotyl elongation in dark has mostly been studied in epidermis, whose cells undergo exclusively elongation, from base to top; no division and are kept undifferentiated. Upon light, elongation ceases and some epidermal cells differentiate into stomata. Little is known how inner layers, such as the endodermis, accommodate this fast growth and whether or not they differentiate. Old botanical studies describe the hypocotyl endodermis contain a Casparian Strip (CS), a differentiation landmark for root endodermis. As a stiff deposition of lignin surrounding the whole cell, the CS is expected to limit cell elongation, however this has not been formerly tested. We investigate how CS is deposited in fast growing hypocotyls in the dark, using histological and genetic markers. The analysis revealed similar basal-to-apical gradient of cell elongation but, in contrast to the epidermis, hypocotyl endodermis divides and differentiates. The molecular machinery for CS deposition is similar to the one in the root but with different requirements. We discuss the temporal and spatial events of hypocotyl endodermis differentiation and its possible function in this temporary organ in plant development, but fundamental in seedling establishment.

P-06 | **De novo establishment of a closed meristem during lateral root formation in Arabidosis thaliana**

De novo formation of roots is crutial for Arabidopsis thaliana ability to absorbe water and nutrients from its environment. Lateral root, a model for de novo organogenesis, develop post-embryonically to reproduce the structure and function of the primary root, hense a new closed meristem with indeterminate growth is formed. This type of meristem organization is associated with a gradiant of proliferation ranging from quiescence in the stem cell niche organizer to highly proliferative in the transit amplifying cells. While tissue patterning has been studied in early stages of lateral root development, it remains unclear when a primary-root like stem cell niche is established. Transcriptional reporters of key stem cell niche regulators, combined with cell proliferation assays based on EdU incorporation and cell cycle progression reporter were used to assess the onset of tissue identity and behavior of the stem cell niche components. Laser ablation experiments and genotoxic stress sensitivity assay were used to test the functionality of the stem cell niche. While stem cell niche identity patterning start from the very first stages of lateral root development, a quiescent stem cell niche organizer is estbalished much later, post emergence in lateral root up from 200 µm. Cell ablation experiments suggest that some stem cells are present and contribute to tissue growth prior emergence, in absence of a functional stem cell niche organizer.
All together this suggests that tissues identity patterning and functionalisation of those tissues are tow distinct developmental events,which can but do not necessarily have to be concomittant during de novo stem cell niche formation in lateral root.

P-07 | **A small, affordable and modular system to automatically follow root system architecture evolution**

Daric V., Lamber E., Legend S., Gaggion N., Jeuffrard M., Roule T., Crespi M., Bares J.P., Di Vozzo R., Ariel F., Ferrante E. and Blein T.

The plant root system is the major organ for the absorption of water and mineral elements necessary for plant growth and development. Root developmental plasticity allows plants to cope with environmental changes in the soil. The natural variation of root systems allows exploring the genetic link between environmental adaptation and root system architecture (RSA). This requires the precise phenotyping of a high number of samples to obtain reliable measurements. Nowadays, numerous root phenotyping devices and platforms are available, although they are generally expensive to setup, require large space and are fixed. Here, we propose a small modular system to automatically follow RSA development on agar plates at a high time resolution. The time resolution is even short enough to be able to follow the impact of plate rotation on primary root gravitropism. The small size of each unit allows it to be placed in any conventional growth chamber or cabinet without any significant modification or the need to dedicate permanently a specific space to this end. Its modular design allows unlimited scaling-up according to the need of each experiment. In addition, it has the advantage of being built using 3D printed parts and ordinary electronic components. Therefore, this device is affordable by small structures other than large phenotyping facilities. Our system leverages the last advances in artificial intelligence and computer vision to perform automatic analysis of growing plant video sequences. We implemented a deep learning segmentation model based on convolutional neural networks to automatically delineate the root. We then perform feature extraction about both, root structure (such as main root length and number of lateral roots) and temporal attributes (growing speed and dynamic along time) without human intervention, enabling fully automatic high-throughput phenotyping of plant roots. This system allows us to explore the natural variation of Arabidopsis ecotypes.

P-08 | **Contribution of auxin to initiating, maintaining and terminating stem-cell fate at the embryonic SAM**

Capua Y and Lohmann J

The plant shoot apical meristem (SAM) is the source of all its aerial organs. It comprises a pool of stem cells (SC), which are tightly and robustly regulated by genetic and hormonal interactions. The phytohormone auxin is classically associated with cellular differentiation at the SAM periphery, where it specifies lateral organs. However new findings from our lab suggest that auxin may be also needed for SC maintenance, and that SCs are at the same time resistant to its differentiating effects. How can the same hormone exert two such opposite effects only a few cells apart is poorly understood. Here, we propose to address this question using the embryonic SAM as a system, and by employing fluorescent marker imaging and advanced genetic and molecular toolset. We will first record SC and hormone signaling dynamics in the Arabidopsis embryo to reveal their spatio-temporal interactions during SAM formation. We will then use spatially and temporally resolved genetic perturbations to decode the functional contributions of hormone signaling to stem cell initiation and maintenance. Finally, we will use our quantitative imaging data as a rich resource to model the interactions. By bringing together these three aims, we hope to elucidate how auxin signaling effects SC fate initiation, maintenance and termination.

P-09 | **The receptor kinase STRUBBELIG promotes the response to cellulose deficiency in Arabidopsis thaliana**

Chaudhary A., Chen X., Gao J., Leśniewska B., Wolf S., Dawid C., and Schneitz K.

Cell wall remodeling is essential for the orchestration of cell division and growth patterns during plant development. In addition, alterations in the cell wall play a pivotal role in cellular and tissue responses to abiotic and biotic stresses. The necessary cell wall monitoring mechanisms remain poorly understood. In Arabidopsis previous data suggested that the receptor kinase STRUBBELIG (SUB) and the C2 domain protein QUIRKY (QKY) form a complex at plasmodesmata involved in the control of tissue morphogenesis. We show that SUB and QKY also promote stress gene induction, jasmonic acid production and ectopic lignin accumulation either upon exogenous application of the cellulose biosynthesis inhibitor isoxaben or in plants with a genetic defect in the cellulose synthase complex. Moreover, our data reveal that SUB signaling is required for the recovery of root growth after transient exposure to isoxaben. Genetic data further indicate that SUB promotes the isoxaben-induced cell wall stress response independently from other known receptor kinase genes mediating this response, such as THESEUS1 or MIK2. We also show that cell wall stress eventually attenuates SUB activity by a post-transcriptional mechanism. The observed downregulation of SUB correlates with the finding that treating wild-type plants with sub-lethal doses of isoxaben results in roots and floral organs that exhibit a sub-like phenocopy. Our combined data reveal a novel role for SUB signaling in the response to cell wall damage induced by a reduction in cellulose biosynthesis. Thus, we propose SUB to function in a least two distinct biological processes: the control of tissue morphogenesis and the cell wall stress response. Moreover, SUB appears to be subject to the control mechanism coordinating growth and stress responses. Finally, our data establish plasmodesmata as important organelles involved in the cell wall stress response.

P-11 | **Function of IQDs as cellular scaffold in spatial control of cell division plane orientation in Arabidopsis**

Dahiya P., Kumari P. and Bürstenbinder K.

Precise control of division plane orientation is important for growth and morphogenesis of multicellular organisms. In plants, unlike in animals, the plane of cell division directly determines the direction of growth due to the presence of a rigid cell wall that physically connects neighboring cells and prevents cell migration. The bundling and spatial organization of microtubules (MTs) plays an important role in this process. Networks of MT associated proteins (MAPs) control MT dynamics, stability and organization. One class of plant-specific MAPs are IQD proteins which comprise 33 members in Arabidopsis thaliana. Functional insights into molecular mechanisms of IQDs however, are sparse. Studies conducted in our group showed that a subclade of Arabidopsis IQDs are expressed in unique and overlapping domains of the root apical meristem. Loss of function lines display defects in division plane selection. Translational GFP fusions of the proteins localize to mitotic MT arrays, including the preprophase band (PPB) and phragmoplast, and to the MT-depleted cortical division zone (CDZ) that serves as PPB memory. We further revealed physical interaction of IQDs with key players in plant cytokinesis and provide first evidence for a requirement of IQDs as determinants for CDZ recruitment of interacting proteins. A hallmark of IQD proteins is the presence of the central IQ67 domain, which mediates binding to calmodulins (CaMs). CaMs are archetypal Ca2+ sensors that transduce signals via the second messenger Ca2+ into cellular responses. Our work on IQDs thus provides a platform and framework to study roles of Ca2+ signaling in MT organization and control of cell division. Intriguingly, IQDs contain large regions of predicted intrinsic disorder, which is a hallmark of scaffold proteins embarked in assembly of macromolecular complexes. Collectively, we propose that IQDs serve as cellular scaffolds during cytokinesis that link Ca2+ signaling to division plane control.

P-12 | **Characterization of the EPF1 and EPF2 genes of Phaseolus vulgaris in the development of stomata and their response to water deficit**

de la Sancha-Pérez R. B., Covarrubias-Robles A., Chater C.

Research on the genetic basis of stoma development in Arabidopdis thaliana has provided information on the mechanisms that control the stomatal pattern and its differentiation. These analyzes have shown that there are peptide signals secreted by certain epidermal cells that exert a regulatory action to control where and when stomata are formed, optimizing the gas exchange, this factors are known as EPIDERMAL PATTERNING FACTORS (EPF). Among the EPFs, the EPF1 and EPF2 stand out. The lack of EPF1, which is normally expressed in stem cells and young stomata, results in an increase in stomatal density and its distribution in the epidermis of the leaf. The lack of EPF2 produces a greater density of precursor and stomatal cells; distribution of stomata is aberrant. On the other hand, the constitutive overexpression of EPF1 or EPF2 results in the reduction of the number of stomata. The natural variation of stomatal patterns has been evaluated in several species, showing that it is often associated with a greater water use efficiency (WUE). This correlation between stomatal variation and WUE has been studied in several crops resulting in an increased WUE by reducing the amount of stomata in the leaves when they are under water deficit. From this, we can consider the reduction of density and stomatal index as a target to be genetically manipulated in order to increase WUE. In the case of common bean, it has been reported that when plants are exposed to water deficit the yield reduces specially during the flowering period and pods filling. With this project we will characterize the EPF1 and EPF2 genes of Phaseolus vulgaris using mutant lines of A.thaliana. For the evaluation of WUE we will overexpress the EPF1 and EPF2 genes, using soybean as a model of study, to evaluate the response of the plants during water deficit using as a parameter the yield of the pods.

P-13 | **Patterning the cell wall for complex tasks**

Deinum E.E., Scheider R., Klooster K. van 't, Jacobs B., Persson S. and Ketelaar T.

Cell walls are highly complex structures, enabling complex cell shapes and/or highly demanding functions. As a model system, we look at secondary cell wall patterning in protoxylem elements. In these cells, a banded or spiralled pattern of secondary cell wall reinforcements is formed. In metaxylem, different, more densely connected patterns are formed, which corresponds to the later maturation of metaxylem. Protoxylem elements can still stretch considerably after maturation/cell death, whereas metaxylem matures after elongation is complete. The increased reinforcements of metaxylem, on the other hand, allow for more efficient water transport because the resulting vessels are stronger. Although the patterns look different, it is likely that the underlying mechanism is the same but tuned differently as a consequence of different functional requirements. The location of these reinforcements is determined by plant cortical microtubules, which organize into the corresponding patterns prior to the patterned deposition of wall materials. Whereas cortical microtubules can selF-0organize themselves into an aligned array through frequent collisions, more complex patterns require the interaction with an additional patterning system, likely involving ROPs and other proteins. The full problem can be approached from two directions: 1) How are cortical microtubules able to adjust to an external spatial pattern? Are observed spatial variations in microtubule dynamics sufficient to reproduce their observed collective dynamics, and under which conditions? 2) How can ROP proteins, which are typically associated with cell polarity, produce stable many-peaked patterns and how could these patterns, in turn, be affected by the cortical microtubules? Through a combination of quantitative microscopy and detailed computer simulations we aim to unravel the requirements of local pattern formation in plant cell walls, an important driver of plant growth and form.

P-15 | **A group 6 LEA protein participates in lateral root emergence and primary root growth in Arabidopsis thaliana under well-irrigated and water deficit growth conditions.**

Diaz-Ardila, H.N., Arroyo, I.A. & Covarrubias A.A.

Late Embryogenesis abundant proteins (LEA) are abundant proteins in dry seeds and in vegetative tissues of plants grown under water deficit; also, most of them are predicted as intrinsically disordered proteins (IDPs). In vitro evidence indicates that these proteins protect other macro-molecules and cell structures under stress. This research project has focused on group 6 LEA (LEA6) proteins, first reported in Phaseolus vulgaris (PvLEA6). PvLEA6 accumulates in response to water deficit and ABA treatments in most cell types from different organs. In Arabidopsis thaliana, there are three genes encoding LEA6 proteins. Even though, LEA proteins were discovered long time ago, we have little information regarding its role in plants. Our research group has characterized a mutant in the AtLEA6-1 gene (atlea6-1) and found that the absence of this protein in Arabidopsis leads to sensitivity to hyperosmotic or salt stress. The results obtained show that atlea6-1 presents changes in root architecture compared to wild-type. When mutant plants are grown under non stress conditions, their roots display reduction in lateral root density and length, as well as longer primary roots; these phenotypes are more evident under salinity and osmotic stress. Further analysis of atlea6-1 root apical meristems showed longer cortical cells, more cells in the proliferation domain, and shorter cell cycle. Furthermore, atlea6-1 presents altered lateral root emergency but not lateral root initiation. Considering the function described for LEA proteins in vitro, it is possible that AtLEA6-1 not only prevents the negative impact of these stress conditions on key elements participating in cell proliferation, but also, its protecting role is essential for root development under non-stress conditions. Consistent with these observations, we found a high AtLEA6-1 promoter activity in vascular cylinder, and cortical and epidermal cells surrounding lateral root primordium.

P-16 | **The Edge Factor? Receptor-Like Proteins and edge-directed trafficking in the maintenance of directional growth in Arabidopsis**

Elliott L., Kirchhelle C. and Moore I.

Cell polarity is a crucial requirement for plant growth, influencing both the growth of individual cells and of entire organs. The plant-specific GTPase RAB-A5c defines a set of membrane compartments that localise to the geometric edges of cells in growing tissues of Arabidopsis (Kirchhelle et al., 2016). Interestingly, inhibition of this edge-directed trafficking pathway using a Rab mutant variant results in severe cellular growth defects. This work has identified edge-directed transport as a new type of polarity within plant cells, with important functions in plant growth control. Despite this, the mechanism by which edge-localised RAB-A5c acts on cell growth remains unknown and no pathway cargoes have been identified. We sought to understand the molecular mechanisms by which edge-directed transport acts on anisotropic growth, using a proteomics-based approach. We have identified that the Receptor-Like Protein RLP4 and its previously unrecognised relative RLP4-L are the first known cargoes of RAB-A5c cell-edge compartments. RLP4 and its relatives are well conserved across plants, and whilst other Receptor-Like Proteins are well known to have roles in cell wall maintenance and control of plant growth, our work constitutes the first investigation of the function of the RLP4 family. We provide evidence that RLP4 and RLP4-L are components of edge-directed trafficking through investigating their dependency for correct localization on functional RAB-A5c. Moreover, we also use a series of protein truncations coupled with 3D quantitative image analyses to give greater insights into RLP4 and RLP4-L dynamics, and function, in anistropically-growing tissues. We are now able to present hypotheses for the functions of the RLP4 family in edge-directed trafficking and the maintenance of anisotropic growth. Through this, we provide the first insights linking the molecular composition of the newly-discovered edge-directed trafficking to control of growth anisotropy in Arabidopsis.

P-17 | **Microtubule behaviour influences pavement cell shape complexity**

Eng R.C., Schneider R., and Sampathkumar A.

Cotyledon surfaces are covered by pavement cells (PCs) with alternating lobe outgrowths and indenting neck regions. It is postulated that cellulose microfibril alignment mediated by microtubules (MTs) determines the final shape of PCs by either restraining or promoting PC growth. While most studies to date focus on understanding the role of MTs in the initiation of the symmetric breaking process in PCs, very little effort on how MT behaviour influences overall shape post-initiation is lacking. Here, we demonstrate how MT behaviour and its association to different domains of the PCs influence overall cell shape and growth. Kinematic imaging of developing PCs revealed differential behaviour of MTs at a subcellular scale during morphogenesis. A tight gradient of MT association to regions of negative curvatures (i.e. lobe domains) at certain time-points significantly contributed to cell shape changes and growth rate differences. We further utilized mutants lacking the MT-regulators, KATANIN and CLASP, that contain different degrees of MT organization to test how local MT array organization modulates cell shape. Evaluation of time-lapse data sets revealed that while lobe initiation occurred in all mutants, highly stable arrays at negative curvatures contributed to the enhancement of the lobe and neck domains whereas disordered MTs resulted in less pronounced lobing of cells. Assessment of physical stress using an in silico tool showed that MT behaviour via its control over lobe and neck dimensions influences the level of stress experienced by the cells.

P-18 | **ROP-Ric signaling during Physcomitrella patens organs development and maturation**

Freire-Rios A., Tang H., Ketelaar T., Scheres B., Vermeer J. and Willemsen V.

Plant cell polarity is not only essential for the formation of new organs (asymmetrical cell divisions), their proper growth (e.g. tip growth of root hairs or pollen tubes) and shape (e.g. leaf pavement cells). Cell polarity is also important to trigger developmental changes by keeping balance between hormones (e.g. auxin polar transport) and signaling changes in environmental conditions. In eukaryotes, small Rho-GTPases have important roles as polarity cues. These proteins are evolutionarily conserved and in plants are known as ROPs (Rho of plants). Interactions of locally activated ROPs with specific effectors trigger intracellular responses. The ROP family in the moss Physcomitrella patens consists of 4 members (11 in A.thaliana) and only 1 canonical effector RIC (11 in A.thaliana). Due to the high degree of homology within ROP proteins (and therefore, functional redundancy) moss becomes a more suitable organism to study conserved mechanisms of this protein family in plants. We have focused our study of ROP function during the gametophore bud formation and development. Our preliminary localization and functional studies indicate that ROP-RIC signalling may indeed play a role during formation, development and maturation of this organ. Unravelling the mechanisms of such role could be later extrapolated to higher plant systems.

P-19 | **A discordance of seasonally covarying cues unmasks a hidden reaction norm in the heterophyllous pitcher plant Cephalotus follicularis**

Fukushima K., Narukawa H. and Hasebe M.

Organisms can cope with environmental challenges by changing their development. This is called phenotypic plasticity and played a role in the survival of sessile organisms including land plants. Although multiple variables consist environmental conditions, how their combinations influence plastic traits is not well characterized. To understand the outcome of the multiple cue integration, we studied the leaf shape plasticity of the carnivorous pitcher plant Cephalotus follicularis under controlled environments. We show that the plastic response is regulated by at least two cues covarying in natural habitats, temperature and photoperiod. Notably, maladaptive leaves were frequently produced under a non-natural combination of benign temperature and photoperiod, highlighting the role of multiple cue integration in exposing so-called “hidden reaction norm”. Our results suggest that, even if individual cues are within the range of natural fluctuations, a phenotypic plasticity can fail under their discordant combinations that have not been well tested by natural selection.

P-20 | **Dehiscence zone as a developmental timer for explosive seed dispersal in Cardamine hirsuta**

Anahit Galstyan , Penny Sarchet, Hugo Hofhuis and Angela Hay

Adaptations for dispersal are ubiquitous in nature and fruits play an important role in the seed dispersal of flowering plants. Seed dispersal occurs via a process called pod shatter in both explosive fruit of Cardamine hirsute and the non-explosive fruit of Arabidopsis thaliana, and relies on the precise patterning of fruit tissues during development. The dehiscence takes place by physical separation of specialized tissues at the valve margin in both species. In A. thaliana, this occurs as the fruit dries out, separating the valve from the replum and exposing seeds for dispersal. In contrast to this, seed dispersal by explosive pod shatter occurs before the fruit dries out in C. hirsute where established pre-tension and accumulation of elastic energy drive the explosive coiling of the vales. This raises the hypothesis that the developmental control of dehiscence zone formation may trigger the process of explosive seed dispersal in C. hirsute. To test this hypothesis, we have performed comprehensive genetic analysis of fruit patterning genetic network in C. hirsute. Mutation in valve margin regulator INDEHISCENT (IND) abolishes dehiscence zone formation. In chind mutants, the energy required for explosion is trapped due to inability of valve tissue physically separate from the replum. We also found that in C.hirsuta the function of FRUITFULL (FUL) is conserved. FUL specified valve tissue by repression of valve margin genetic network and ful mutants exhibit homeotic conversion of valve into valve margin. However, unlike A. thaliana ful, where the ectopic valve margin identity consists mostly of lignified cells, the ectopic valve margin identity in C. hirsuta ful consists mostly of separation layer cells. Collectively, these results suggest that the genetic network controlling valve margin identity in C. hirsuta shows conservation with A. thaliana but has been re-wired to set separation layer as predominant valve margin fate.

P-21 | **Subsidiary Cells: the right-hand of guard cells for improved stomatal movements in grasses**

Nunes T. D. G. , Zhang D., Lindner H., Raissig M. T.

Stomata are cellular gateways on leaves that allow gas-exchange and balance carbon dioxide uptake and water loss. Grasses, such as the important food crops maize and wheat, present physiological improved stomata with four cells contrasting the typical two guard-cell morphology in most of plants. Grasses recruit two lateral subsidiary cells (SCs), which is triggered by the mobile bHLH transcription factor BdMUTE in the model grass Brachypodium distachyon. The Arabidopsis orthologue AtMUTE is non-mobile and specifies guard mother cell identity instead. It is unknown if BdMUTE mobility is required for SCs recruitment nor which motifs within MUTE are responsible for BdMUTE mobility and functionality. Generally, MUTE orthologues are well conserved but some differences between the grass and non-grass peptides were identified particularly within MUTE-specific and SMF domains. To identify required domains or motifs for grass-specific function and mobility, domain swaps between BdMUTE and AtMUTE will be performed and reporter chimeric proteins expressed. To study the contribution of SCs to stomatal gas exchange efficiency in grasses, a transcriptome analysis between wt and bdmute mutant mature leaves (with and without subsidiary cells respectively) identified differentially expressed candidate genes potentially associated with SCs function. Reverse genetic approaches (CRISPR-based gene editing and use of available mutant collections), reporters and/or overexpression constructs combined with infrared gas-exchange analysis are used to determine the candidate genes' role in SC function and contribution to stomatal physiology. We hope to better understand how SCs form and contribute to improved gas exchange efficiency and plant performance observed in the grass family.

P-22 | **Novel roles for CRC in adaxial-abaxial regulation during carpel development**

Gross T. and Becker A.

The development of carpels is one of the most crucial steps in the life cycle of angiosperms, as they are the most complex plant organs. They harbor seeds and later develop into fruits. Hence, they are of great ecological and economic importance. CRABS CLAW (CRC), a YABBY protein, is an important part of the carpel developmental network as it is involved in multiple steps during carpel development such as carpel fusion, floral meristem termination, determination of the abaxial-adaxial axis, and nectary formation. Previous work revealed its genetic interactions with other carpel developmental regulators and elucidated the functions of its protein domains. During carpel development, the temporal and spatial expression pattern of CRC is rather complex with multiple expression domains throughout the young gynoecium. However, only few regulators of CRC expression are described. Here we present the identification of transcriptional regulators, responsible for the proper temporal and spatial expression of CRC. The regulation of CRC expression has been analyzed via a large scale Yeast-1-Hybrid screen and we identified over 100 potential regulators of CRC expression, integrating CRC tightly into the carpel developmental regulatory protein network. Further analysis of CRC function through expression analysis led to the identification of target genes like mir165/166, members of the KANADI gene family, and the HD ZIP III gene family. Both gene families are major players in the adaxial-abaxial regulatory network, involved in the development of all lateral plant organs such as leaves and floral organs. CRC supports KANADI action and activates the expression of other involved factors. In addition, CRC directly targets members of the HD ZIP III family. Thus, we propose a new model of CRC action during carpel development, structuring it into an early leaf like phase and a late carpel specific phase, to establish adaxial-abaxial and apical-basal polarity.

P-23 | **Gurzadyan A., Formosa-Jordan P., Jönsson H.**

Dynamical analysis of single cell PIN polarity

We reconnoitre biological networks and physical laws responsible for plant development. During multicellular development the hormonal signalling machinery is indispensable for regulation of the morphodynamics. The elucidation of mechanisms of auxin transport and PIN efflux protein polarity plays pivotal role in revealing the fundamentals of stem cell differentiation and organ formation in Arabidopsis thaliana. To this end, we study the pattern formation in isolated cells, i.e. at the lowest systematic level, to understand single cell regulatory networks manifesting cellular polarity. We are elaborating integral framework of protoplast extraction, time-lapse imaging at different conditions with and without microfluidic chips, quantification and theoretical modelling to elucidate the PIN dynamics at the single cell level. By undertaking comprehensive assay of constructed reaction-diffusion systems, estimating the key similarities and differences of their capabilities, we propose a generalised model that embraces dynamics of several others if enzymatic mass action rates are restricted within their particular subsets. The global analysis of the multi-dimensional parameter space and in silico integration provides intricate picture of bifurcations. That yields distinct characteristics of possible PIN dynamics: the spontaneous or transient polarity, the coarsening tendency, scale-invariant feature or polarity emergence as response to localised inhomogeneity in imposed initial concentrations, etc. Finally, we model tissue-wide auxin transport by extrapolating the single cell polarity model to the tissue-scale via intracellular partitioning in accordance to segmented images, e.g of shoot apical meristem, leaf or primary root. Given these we aim to deploy mechano-chemical multi-scale simulations of intracellular processes, cell-to-cell signalling and mechanical interaction to establish a comprehensive approach of modelling the auxin transport and, hence, the morphogenesis.

P-24 | **Learning to segment an image**

Wolf S., Pape C. , Cerrone L., Li Y., Wolny A., Kreshuk A. and Hamprecht F.A.

Learning to segment an image I will give a tutorial-style overview of the state of the art in semantic segmentation (which pixel belongs to which class?) and instance segmentation (e.g., which pixel belongs to which cell?).

P-25 | **A 3D model of phyllotaxis**

Hartmann F., Barbier de Reuille P. and Kuhlemeier C.

Auxin-driven patterning operates in two main modes: convergence and canalization. The shoot apical meristem is a potentially unique system in which these two modes co-occur in a coordinated manner and in a fully three-dimensional geometry. In the epidermal layer, convergence points form, from which auxin is canalized towards inner tissue. Each of these two patterning processes has been extensively investigated separately, but the integration of both in the shoot apical meristem remains poorly understood. We present a first attempt of a three-dimensional model of auxin-driven patterning during phyllotaxis. Our simulations are based on a biochemically plausible mechanism of auxin transport proposed by Cieslak et al. (2015) which generates both convergence and canalization patterns. We are able to reproduce most of the dynamics of PIN1 polarization in the meristem, and we explore how the epidermal and inner cell layers act in concert during phyllotaxis. In addition, we discuss the mechanism by which initiating veins connect to the already existing vascular system.
References:
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- Hartmann FP, Barbier de Reuille P, Kuhlemeier C (2019) Toward a 3D model of phyllotaxis based on a biochemically plausible auxin-transport mechanism. PLoS Comput Biol 15(4): e1006896

P-26 | **Comparative Angiosperm Transcriptome (CAT) database - a website to digitally explore angiosperm carpel development and evolution**

Herbert DB., Finke H., Kivivirta K., Lange M., Beuerlein K., Altmüller J. and Becker A.

Until today relatively little is known about the origin of angiosperms, a group of plants that dominates most ecosystems and provides the majority of our food. Carpels are the female reproductive structure of flowers, the unifying organ of all angiosperms. The general aim of this project is to identify a minimal set of genes required for carpel development in angiosperms to provide a better understanding of flower development of present and ancient angiosperm species. Furthermore the molecular functions and genetic interactions of this set of genes will be identified to predict the carpel developmental network and to learn which genes carry out which functions in angiosperm flowers. This project is the first to analyze carpel developmental regulators in a wide range of phylogenetically important taxa by a combination of LMD and transcriptome analysis. Therefore transcriptomes of four carpel developmental stages within four species (A. thaliana, S. lycopersicum, E. californica and O. sativa) have been sequenced and analyzed. The comparisons within/between species and between tissues enable to identify the major carpel developmental genes. To overcome the problem of big datasets, we show here an innovative solution to make large datasets accessible for researchers, to facilitate downstream analyses of transcriptome analysis. Here we present CAT database ("Comparative Angiosperm Transcriptome database"), an e-solution for evolutionary and developmental questions within the group of angiosperms. CAT is based on the four species and four stages of carpel development; in addition several transcriptome data of other plant tissues of the four species are available. The database CAT provides the user with all relevant information about annotation, combined with basic bioinformatics analyzes (like digital gene expression, tau value and individualized correlation analysis.), furthermore CAT presents all results in a user-friendly graphical way.

P-27 | **Dynamics of cell division patterns and their role on female Spore Mother Cell fate acquisition during ovule primordium development in Arabidopsis**

Hernández-Lagana E., Mendocilla-Sato E., Ingouff, M., Michaud C.,Grimanelli, D., Baroux, C., and Autran, D.

The ovule primordium of Arabidopsis is the site where the onset of the female germline precursor or female Spore Mother Cell (SMC) takes place. The SMC is developed from a single somatic subepidermal cell at the tip of the ovule. How the ovule primordium grows and achieves its shape while integrating the differentiation of the SMC remains poorly understood. Some of the genetic and epigenetic factors controlling the number of SMCs formed within the ovule primordium have been identified. Yet, we still lack of a better understanding of how these factors act in synergy with architectural cues to coordinate this process. To elucidate how cell division dynamics drives ovule primordia patterning and to investigate its influence in SMC fate and plasticity, we generated 2D and 3D microscopy dataset with distinct fluorescent markers of cell cycle phases. Our recent findings show that the regulation of cell proliferation activity is temporally and spatially controlled within the ovule primordium and is a key parameter contributing to the organ growth. Furthermore, disturbances of specific cell divisions patterns, are associated with the formation of ectopic MMCs .Our perspectives include testing the role of specific ovule domains on MMC establishment by generating domain-targeted constructs to affect cell division parameters.

P-28 | **Genome-wide identification of CUC1 regulated genes by RNA-seq and ChIP-seq**

Ziliang Hu, David Wilson-Sanchez, Madlen I. Rast-Somssich, Lachezar A Nikolov, Francesco Vuolo, Chidi Afamefule, Miltos Tsiantis

The genetic basis underlying leaf morphological diversity is largely unknown. Current evidence suggests that diversification of key developmental regulators contributes to trait diversity. NAM/CUC3 (NO APICAL MERISTEM/CUP-SHAPED COTYLEDON3) genes encode NAC transcription factors and have been proved to be conserved regulators in leaf margin dissection across eudicots. Phenotypic analysis into C. hirsuta indicates that ChCUC1 (CUP-SHAPED COTYLEDON1) plays an important role in compound leaflet formation. Specifically, introduction of ChCUC1 allele is sufficient to transform A. thaliana leaves into more complex ones. On this basis, we suggest that species-specific expression of ChCUC1 in developing leaves contributes to leaf shape diversification between C. hirsuta and A. thaliana. However, the mechanism through which CUC1 regulates leaf shape diversity remains elusive. To resolve this issue, we performed transcriptomic and chromatin immunoprecipitation analysis to identify downstream target genes of ChCUC1. By RNA-seq and ChIP-seq approaches, we identified organ boundary genes represented by LIGHT SENSITIVE HYPOCOTYLS 6 (LSH6) and LATERAL SUPPRESSOR (LAS). These genes may mediate the cell-autonomous function of ChCUC1 to repress cell growth. It will also be intriguing to investigate the interactions between CUC genes with Reduced Complexity (RCO).

P-29 | **Multiple Auxin-Response Regulators Enable Stability and Variability in Leaf Development**

Israeli A., Capua Y., Efroni I and Ori N.

Auxin-signal transduction is mediated by the antagonistic activity of transcriptional activators and repressors. Both activators and repressors belong to gene families, but the biological importance of this complexity is not clear. Here, we addressed this question using tomato leaf development as a model by generating and analyzing mutants in multiple auxin-response components. In developing compound tomato leaves, auxin promotes leaflet formation and blade growth, and in the intercalary regions between leaflets, auxin response is inhibited by the Aux/IAA protein ENTIRE (E). e mutants form simple leaves due to ectopic blade growth in the intercalary domain. Using this unique loss-oF-0function phenotype and genome editing of auxin-response factor (ARF) genes, encoding auxin-response activators, we identified the contribution of specific ARFs to the e phenotype. Mutations in the related ARFs SlMP, SlARF19A, and SlARF19B, but not SlARF7, reduced the leaf blade and suppressed the e phenotype in a dosage-dependent manner that correlated with their relative expression, leading to a continuum of shapes. While single e and slmp mutants affected blade growth in an opposite manner, leaves of e slmp double mutants were similar to those of the wild type. However, the leaf shape of e slmp was more variable than that of the wild type, and it showed increased sensitivity to auxin. Our findings demonstrate that the existence of multiple auxin-response repressors and activators stabilizes the developmental output of auxin and that tuning their activity enables shape variability. The increased complexity of the auxin response therefore balances stability and flexibility in leaf patterning.

P-30 | **Role of a GDSL esterase, CGM4, in stomatal dynamics**

Kar R., Asseck L., Livanos P., Sampathkumar A., Schwarzländer M., Stahl M., Kilian J., Harter K., Bayer M. and Grefen C

Stomata dynamically regulate gas exchange, a mechanism critical to the survival of plants in terrestrial ecosystems. The development, mechanism and biochemical composition of stomata are well studied; however, many stomata-related genes remain uncharacterized. Here, we focus on the gene CGM4, belonging to the GDSL like lipase/esterase family, expressed in early developmental stages of stomata. We use mutants of CGM4, and its homolog CGM3, to show that cgm3cgm4 plants have significantly smaller width to length ratio of the stomatal pore. Further cgm3cgm4 plants show significantly reduced transpiration rate compare to wild type (Col-0) and a significantly increased drought tolerance. Analyses of cell wall composition of mutant plants show a reduction in the abundance of polyphenol derivatives compare to Col-0. These results indicate a mechanical defect in stomatal dynamics of cgm3cgm4 plants. We thus suggest, that CGM4, and its homolog CGM3, function during the stomata cell wall biogenesis, which influences the dynamics of the mature stomata.

P-31 | **High-resolution spatial and temporal transcript atlas of barley spike meristems offers insights into the developmental dynamics of spike type inflorescences**

Koppolu R., Thiel J., Erbe S., Trautewig C., Himmelbach A., Mascher M., Kale S., Bräutigam A., Schnurbusch T.

The gross morphology of an organism can be traced to its early developmental events, particularly to the changes in genes controlling development. In plants specification, of various organ primordia such as roots, leaves, and flowers is majorly driven by the local transcriptional regulation at the site of their specification. Hence understanding the precise control of organ specification, necessitates the need to dissect the transcriptional regulation at the site of organ initiation. Barley inflorescence called spike has a unique structure called triple spikelet (TS) [(one central (CS) and two lateral spikelets (LS)] along the inflorescence axis. The CSs are always fertile. The fertility of LSs at the TS distinguishes barley spike into, two- (sterile LSs) and six-rowed (LSs fertile). To understand the transcriptional regulation specifying LS development, we have precisely isolated immature LS and CS organs in a two-rowed barley cv. Bowman by laser capture microdissection across seven spike primordia developmental stages and subjected to RNA-seq analysis. We also analyzed inflorescence apical meristem, spike pro-vascular tissue, root apical meristem, and leaf meristems to understand the differences in transcriptional programs of various meristematic tissues. Our analysis of differential genes between CS and LS tissues revealed all known regulators of LS development along with several unknown genes. By mutational studies and phenotypic evaluation of four of the novel LS development genes identified, we validated the observed differential transcriptional regulation. In summary, we have developed a high-resolution tissue-specific transcriptome atlas of developing barley spike, illuminating the precise regulation of spike development.

P-32 | **Arabidopsis thaliana ROP-GAPs involved in pavement cell morphogenesis**

Lauster T., Stöckle D., Zimmermann S. and Müller S.

We identified two closely related GTPase activating proteins (PH-GAP1 and PH-GAP2) involved in cell shape morphogenesis. Simultaneous mutation in two independent allele combinations of ph-gap1 ph-gap2 double mutants resulted in altered leaf shape, displaying more lancet-formed leaves. Furthermore, the characteristic puzzle shape of pavement cells in cotyledons was compromised, indicating a role of PH-GAP1 and PH-GAP2 in cell polarity establishment. PH-GAPs contain an N-terminal pleckstrin-homology (PH) domain, a conserved GTPase activating protein (GAP) domain and a C-terminal coiled-coil region. Phenotypic analysis of PH-GAP2 deletion mutants showed that the C-terminal domain is required for their function during cell shape morphogenesis. GAPs function in the inactivation of small GTPases called Rho of plants (ROPs). The antagonistically acting ROP2 and ROP6 are well characterized in mediating the reorganization of the cytoskeleton in leaf epidermis cells, to establish their characteristic puzzle shape with pronounced lobes and indentations. We analyzed the microtubule organization in ph-gap1 ph-gap2 mutants. In wild type, developing pavement cells show highly bundled microtubules in neck regions and fewer microtubules in adjacent lobes. In contrast, the difference in microtubule abundance between lobe and neck regions was less pronounced in ph-gap1 ph-gap2 mutants. Nevertheless, microtubules anisotropy was not affected in ph-gap1 ph-gap2. To elucidate whether PH-GAP1/2 contribute to the regulation of ROP2 and/or ROP6 activity during pavement cell development, we performed interaction studies between PH-GAP1/2 and ROP2/6 revealing that PH-GAP1/2 preferentially interact with ROP2. Taken together our results suggest that PH-GAP1/2 might regulate ROP2 activity and consequently lobe formation during pavement cell shape development.

P-33 | **The cap size and shape of Arabidopsis thaliana primary roots impact the root responses to an increase in medium strength**

J. Roué, H. Chauvet, N. Brunel-Michac, F. Bizet, B. Moulia, E. Badel and V. Legué

Roots grow in a complex soil environment, which exhibits spatial and temporal heterogeneities. The ability of roots to penetrate the soil and to overcome physical obstacles depends either on their ability to find a path of least resistance, or on their ability to generate sufficient force to go through the obstacle. During root progression in soil, root cap cells are the first to encounter obstacles, and are known to sense environmental cues, making it a relevant candidate for a mechanosensing site. The objective of our study is to investigate the role of the root cap shape in the establishment of root responses to medium strength. We hypothesized that pointed caps facilitated root penetration and domed caps made this root penetration harder. This hypothesis was investigated by analysing the root responses to medium strength of Arabidopsis thaliana mutant lines with altered caps, such as fez-2 and smb-3 mutant which exhibited more acute and more rectangular cap shapes, respectively. Changes in soil strength have been mimicked with a two-layer Phytagel-based growth medium, containing two layers of distinct Phytagel concentrations, which modulated growth medium strength and submitted an axial force to roots. Using a spatiotemporal analysis, we characterized the ability of the root to penetrate the harder medium, the root growth rate, and the root curvature of wild-type and mutant roots. When Arabidopsis primary roots encounter medium layers with increasing strength , they adopt three main types of responses including root penetration, root buckling without apex deviation leading to a curly shape, or root buckling followed by apex deviation leading to a step-like shape. The alteration in penetration ability, reduced in fez-2 roots and enhanced in smb-3 roots, seems to result from altered resistances to root buckling. These results suggest that the root cap shape could impact root responses to medium strength by influencing root resistance to buckling.

P-34 | **Concerted expression of a cell-cycle regulator and a metabolic enzyme from a polycistronic transcript in plants**

Lorenzo-Orts, L., Witthoeft J., Deforges, J., Martinez, J., Loubéry, S., Placzek, A., Poirier, Y., Hothorn, L. A., Jaillais, Y., Hothorn, M.

Eukaryotic mRNAs frequently contain upstream open reading frames (uORFs) encoding small peptides which may regulate translation of the main ORF (mORF) by various mechanisms. We have discovered a larger uORF encoding an ortholog of the CELL DIVISION CYCLE PROTEIN 26 (CDC26) in Arabidopsis. CDC26 is a member of the Anaphase Promoting Complex/Cyclosome (APC/C), an E3-ubiquitin ligase involved in cell cycle progression in all eukaryotes. CDC26 regulates accumulation of APC/C target proteins and controls cell division, plant growth and embryo development. CDC26 is translated together with the inorganic polyphosphatase TTM3 from a bicistronic mRNAwhich has been conserved in the plant lineage for over 700 million years. While TTM3 translation coordinates CDC26 expression by targeting the transcript into polysomes, CDC26 translation promotes mRNA degradation via nonsense-mediated decay (NMD). Hence, the bicistronic configuration of the CDC26-TTM3 transcript allows fine-tune regulation of CDC26 expression and thereby the cell cycle. Our work highlights the role of polycistronic transcripts in regulating gene expression.

P-35 | **Lockhart with a twist: The effect of variations in mechanical properties through the plant cell wall thickness on growth and twisting.**

Luo J, Chakraborty J and Dyson R.J.

Plant cell expansion is governed via manipulation and rearrangement of polymers within the cell wall via either passive reorientation as the wall expands or active biological control. In particular, rapid cell expansion must be accompanied by new material deposition to prevent rupture of the cell wall. These newly-incorporated components, combined with passive motion of material through the thickness of the cell wall, can lead to local variations in the effective mechanical properties of the composite wall material. We derive, analyse and interpret a mathematical model, treating the cell wall material as a composite fibre-reinforced fluid, which takes these variations into account, in particular considering the mechanical anisotropy given by oriented cellulose microfibres (and their angle at deposition) and the effect of time-dependent modification in the pectin matrix properties. We present our findings, and discuss their consequences with a particular focus on the resultant growth and twist of the cell.

P-37 | **MorphomechanX, a novel tool to model plant morphogenesis and its application on A. thaliana ovule development**

Mosca G., Lane B., Hernandez-Lagana E., Mendocilla-Sato E., Autran D., Smith R., Baroux C.

Mechanically based modeling of plant morphogenesis is becoming an essential tool to integrate experimental data and explore new scenarios. Here we introduce the novel software MorphomechanX, which allows to create models at cellular and continuous tissue resolution with cutting edge features (like mixed elements) in a user friendly and interactive environment. Some test cases illustrating the main features will be presented. As a specific application we will discuss the case of A.thaliana ovule development and patterning, where multiple modeling approaches, combined with 3D image analysis, allow to provide novel insights in how the ovule coordinates its growth and the spore mother cell emerges within it.

P-38 | **Structural basis for recognition of RALF peptides by LRX proteins during pollen tube growth**

Moussu S., Broyart C., Santos-Fernandez G., Augustin S., Wehrle S., Grossniklaus U. and Santiago J.

Plant reproduction relies on the highly regulated growth of the pollen tube for proper sperm delivery. This process is controlled by secreted RALF signaling peptides, which have been previously shown to be perceived by CrRLK1Ls membrane receptor-kinases and leucine-rich (LRR) extensin proteins (LRXs). Here we demonstrate that RALF peptides are active as folded, disulfide bond-stabilized proteins, which can bind to the LRR domain of LRX proteins with nanomolar affinity. Crystal structures of the LRX-RALF signaling complexes reveal LRX proteins as constitutive dimers. The N-terminal LRR domain containing the RALF binding site is tightly linked to the extensin domain via a cysteine-rich tail. Our biochemical and structural work reveals a complex signaling network by which RALF ligands may instruct different signaling proteins – here CrRLK1Ls and LRXs – through structurally different binding modes to orchestrate pollen tube growth.

P-39 | **Mechanism of localized lignin deposition in explosive fruit**

Pérez-Antón M., Hofhuis H., Kroll P., Metzger S. and Hay A.

A major goal in biology is to identify the molecular basis of complex trait innovations, such as explosive seed dispersal, found in Cardamine hirsuta. The asymmetric deposition and lignification of secondary cell walls (SCWs) in endocarp b cells of the fruit valve was found to be a key morphomechanical innovation underpinning explosive dispersal. To gain insights into the mechanisms controlling this lignin patterning in C. hirsuta, we identified less lignin (lig) mutants that showed reduced lignification of the fruit valve. The lig1 mutant is deficient in lignin deposition in endocarp b cells, resulting in a reduced seed dispersal range. By positional cloning, complementation, and the isolation of additional alleles, we demonstrate that an ortholog of the transcription factor SPL7, a central regulator of copper homeostasis, is the causal locus for the phenotype. Using ICP-MS to measure the concentration of copper, we show that SPL7 is both necessary and sufficient to regulate the concentration of copper in fruit. We find that SPL7 is expressed in endocarp b cells and lignified cells of the dehiscence zone, suggesting a local role in lignification. Three members of the lignin-polymerizing laccases, which are Cu-requiring enzymes, are expressed in endocarp b cells, where we show they precisely co-localize with lignin in the asymmetric SCWs. We propose that copper deficiency in the lig1 fruit, due to loss of SPL7 function, reduces laccase activity in endocarp b cells, resulting in reduced lignification. This provides a testable hypothesis for the control of localized lignin deposition in explosive fruit.

P-40 | **Developmental innovations of stomatal form and function in grasses**

Dan Zhang, Tiago DG Nunes, Heike Lindner, Dominique C Bergmann, Michael T Raissig

Plants optimize carbon assimilation while limiting water loss by adjusting stomatal aperture. In grasses, a developmental innovation—the addition of subsidiary cells (SCs) flanking two dumbbell-shaped guard cells (GCs)—is supposedly linked to the grass family's improved stomatal gas exchange efficiency. A mutant screen in the wheat relative and model grass Brachypodium distachyon identified a transcription factor necessary and sufficient for SC formation. Unexpectedly, the transcription factor is an ortholog of the stomatal regulator AtMUTE, which defines GC precursor fate in Arabidopsis. The novel role of BdMUTE in specifying lateral SCs appears linked to its acquisition of cell-to-cell mobility in Brachypodium. Physiological analyses on bdmute mutant plants lacking SCs experimentally support classic hypotheses that SCs permit greater stomatal responsiveness to enhance water use efficiency and larger range of pore apertures to increase photosynthetic capacity. Recently, we profiled the transcriptome of developing and mature leaf regions of both wild-type and SC-less bdmute plants to identify novel factors regulating SC development and SC function, respectively. Discovering genes required for SC function will help understand how SCs actually improve stomatal gas exchange dynamics in grasses. Understanding how SCs are formed and enable grasses to breathe more efficiently might allow engineering of stomatal properties in many different crops to improve water use efficiency and plant performance.

P-41 | **Petal number robustness and its evolutionary loss in Cardamine hirsuta**

Rambaud-Lavigne L., Monniaux M., and Hay A.

Petal number is a robust phenotype in many flowering plants, including Arabidopsis thaliana, where each flower produces four petals. However, in the closely related species Cardamine hirsuta, petal number is sensitive to natural genetic, environmental and stochastic perturbations, showing that phenotypic robustness was lost in C. hirsuta. The genetic basis for this difference between species is being investigated in our lab. We showed previously that divergence in APETALA1 (AP1) gene function can account for the difference in petal number robustness between species. The A. thaliana version of AP1 masks the effects of petal number QTL in C. hirsuta. This epistatic effect is lost in C. hirsuta, causing petal number to vary in response to natural genetic variation. Therefore, rewiring of the gene regulatory network, in which AP1 acts to regulate petal formation, led to evolutionary loss of robustness in C. hirsuta. Here, we use a morphodynamics approach to investigate the robustness and rewiring of the petal number gene regulatory network in A. thaliana and C. hirsuta, respectively. This approach calls for dynamic gene expression data from time-lapse confocal imaging of genes that we selected based on their petal-loss phenotype upon mutation. These genes include the petal identity gene APETALA3, the boundary gene CUP-SHAPED COTYLEDON 2, and the auxin transporters PIN-FORMED1 and AUXIN RESISTANT1, along with the auxin sensor DR5 in both species. We aim to use quantitative data from time-lapse imaging and genetics to inform the development of computational models of auxin-induced patterning of petal number. In this way, we expect to address mechanisms underlying robustness and the genetic changes contributing to morphological evolution.

P-42 | **Tissue-wide integration of mechanical cues promotes auxin patterning efficiency**

Ramos J. R. D., Maizel A. and Alim K.

Transport of the growth hormone auxin drives morphogenesis of new plant organs. Mechanical stresses are observed to regulate the localization of auxin's key carrier proteins PIN. In turn auxin accumulation changes the mechanical properties of cell walls unfolding a feedback that drives the emergence of auxin patterns. Here, we explore the collective mechanical response of the tissue to auxin patterns and how it feeds back onto auxin transport. In order to study the tissue's collective dynamics, we use a hybrid model composed of a vertex model for plant tissue mechanics, and a compartment model for auxin transport. As a reference to this full mechanical description we analyze its nearest neighbour approximation in a static tissue. By comparing the full mechanical description and the reference model, we find that large auxin maxima require less coupling of mechanical stress to PIN in the full mechanical description due to tissue-wide mechanical behaviour. This more efficient auxin patterning magnitude goes hand in hand with a dramatic increase in net auxin flow. These results show that auxin patterning feeds back onto auxin transport through tissue stress selF-0organization reinforcing cell-cell communication.

P-43 | **Interplay between auxin and cytoskeleton in the endodermis during the lateral root primordium**

Blanca Jazmín Reyes-Hernández, Amaya Vilches Barro and Alexis Maizel

Cortical microtubules (CMT) are cytoskeletal structures and one of its functions is to guide the deposition of cellulose at the cell wall (CW) and thereby participate in the consolidation of cell shape. Early stages of lateral root primordium (LRP) development need to be accompanied by the deformation of the overlying tissues, being the first the endodermis, in order to allow LRP emergence. Although CW remodeling processes in the endodermis during the emergence of LRP have been described, little is known about CMT dynamics during this morphological process. We have found that the CMT have heterogeneous organization in the cell face of the endodermis, at the face in contact with the cortex side they are more disorganized (isotropic) than in the one in contact with the vascular side where they are anisotropic. We have identified that during early stages of the LRP growth, in the endodermal face in contact with the vascular side, CMT changed their anisotropic organization to isotropic. Monitoring the DR5rev reporter and the auxin/indole-3-acetic acid (Aux/IAA) 3 /SHY2 expression we found that this CMT arraignment comes after the auxin signaling onset. This data together with the analyses of CMT organization in auxin signaling related-mutants suggest that CMT-organization might be dependent of auxin, via SHY2, and not by the mechanical pressure of the LRP morphology. This data and our genetic approach to disrupt the polymerization of the CMT specifically in the endodermis sheds light on how this cytoskeletal components are necessary for the early spatial accommodation in the endodermis and allow the endodermis thickness during LRP development.

P-46 | **On the significance of mechanosensing during seed development**

Rolletschek H., Muszynska A., Borisjuk L.

Mechanosensing describes the process whereby physical forces act as a trigger for a developmental event. Own previous work demonstrated that the availability of space influences the embryo's growth pattern and metabolism (Borisjuk et al., Plant Cell 25/2013). The growing embryo within a seed comes in contact with maternal tissues, and starts to bend and fold. This process is accompanied by the initiation of storage product accumulation in the embryo and seed maturation. In our DFG-funded project, we investigate how mechanical constraints induce the switch from growth into storage mode of embryo metabolism. We developed a unique experimental setup to study the regulation of the structural, metabolic, and molecular responses to physical restraint in the developing embryo of oilseed rape/canola (Brassica napus). Evidences for the mechanistic link between a physical constraint and growth, metabolic alterations, and seed/embryo maturation events are presented. Mechanosensing by the embryo appears to be a key component of seed development, which to date has not been recognized.

P-47 | **Spatio-temporal control of cell wall properties and signalling networks in the shoot apical meristem of A. thaliana**

Paola Ruiz Duarte, Anne-Kathrin Schürholz, Sebastian Wolf

Imposed by their sessile lifestyle, plant development and post-embryonic growth are tailored to environmental conditions. Developmental plasticity is conferred by pluripotent stem cells embedded in meristems, which continuously generate new plant organs. The balance between stem cell replenishment and cell proliferation is among others, controlled through CLE-peptide signalling pathways with the participation of cell wall remodelling. In the shoot apical meristem (SAM), new cells acquire different tissue identities along their trajectory from the centre to the periphery and adopt positional/domain properties. The interplay of cell wall elasticity, achieved through cell wall modifying enzymes like pectin methyl esterases (PMEs) and phytohormone cues, can drastically affect the SAM's morphology. The spatio-temporal regulation of the cross-talk between phytohormones, CLAVATA3/ENDOSPERM SURROUNDING REGION (CLE) signalling peptides, and cell wall remodelling genes is largely unknown. Previous studies have revealed reduced PME expression in the SAM but also that they might be targets of WUSCHEL (WUS), an important transcription factor that regulates CLV3 expression and SAM homeostasis. Here we demonstrate that expression of the PME VANGUARD 1 (VGD1ox) in the SAM, results in an extreme dwarf phenotype and larger inflorescences, with a reduced chess board-like cell shape. We observe that the CLVATA3 domain expands laterally when pectin methylesterification is decreased in the stem cells. We generated PME promoter reporter lines and observed that pPME5, is only expressed at the boundaries of the SAM and young primordial. Our results demonstrate that PME has a yet undescribed, but essential functions in plant developmental plasticity and stem cell homeostasis. We predict our study could reveal part of the complex network that regulates SAM, for example, the individual and combined action of PMEs, as well as to characterize PME functions that are elusive until now.

P-48 | **Spatio-temporal organization of microtubules and cellulose synthases in developing cotelydons**

René Schneider, Ryan Christopher Eng, and Arun Sampathkumar

All plant cells are surrounded by cell walls that act as a key player in controlling where a cell expands and1 1w1h1e1r1e1 1i1t1s1 1c1o1n1t1o1u1r1 1r1e1m1a1i1n1s1 1s1t1a1t1i1c1.1 1l1n1 1m1a1n1y1 1p1l1a1n1t1s1,1 1t1h1e1 1e1p1i1d1e1r1m1a1l1 1c1e1l1l1s1 1o1f1 1c1o1t1e1l1y1d1o1n1s1 1s1h1o1w1 1a1 1r1i1c1h1 1v1a1r1i1e1t1y1 1i1n1 1s1h1a1p1e1s1,1 1e1.1g1.1 1i1n1 1A1r1a1b1i1d1o1p1s1i1s1 1w1h1e1r1e1 1c1e1l1l1s1 1f1o1r1m1 1a1 1w1a1v1y1,1 1i1n1t1e1r1l1o1c1k1e1d1 1p1a1t1t1e1r1n1 1r1e1m1i1n1i1s1c1e1n1t1 1o1f1 1j1i1g1-1s1a1w1 1p1u1z1z1l1e1 1p1i1e1c1e1s1.1 1D1e1s1p1i1t1e1 1a1t1t1r1a1c1t1i1n1g1 1m1u1c1h1 1a1t1t1e1n1t1i1o1n1 1a1s1 1a1 1m1o1d1e1l1 1s1y1s1t1e1m1 1f1o1r1 1p1l1a1n1t1 1m1o1r1p1h1o1g1e1n1e1s1i1s1,1 1t1h1e1 1p1r1e1c1i1s1e1 1o1r1c1h1e1s1t1r1a1t1i1o1n1 1o1f1 1m1o1l1e1c1u1l1a1r1 1a1n1d1 1c1e1l1l1 1b1i1o1l1o1g1i1c1a1l1 1e1v1e1n1t1s1 1l1e1a1d1i1n1g1 1t1o1 1h1i1g1h1l1y1 1c1o1m1p1l1e1x1 1c1e1l1l1 1s1h1a1p1e1s1 1i1n1 1c1o1t1e1l1y1d1o1n1s1 1a1r1e1 1s1t1i1l1l1 1l1e1s1s1 1w1e1l1l1 1u1n1d1e1r1s1t1o1o1d1.1 1W1e1 1u1s1e1d1 1l1o1n1g1-1t1e1r1m1 1l1i1v1e1-1c1e1l1l1 1i1m1a1g1i1n1g1 1a1l1o1n1g1 1w1i1t1h1 1g1e1n1e1t1i1c1a1l1l1y1 1e1n1c1o1d1e1d1 1m1i1c1r1o1t1u1b1u1l1e1 1a1n1d1 1c1e1l1l1u1l1o1s1e1 1s1y1n1t1h1a1s1e1 1m1a1r1k1

P-49 | **Characterization of cambium stem cell activity during radial plant growth**

Shi D., Jouannet J., Lopez V., Lebovka I. and Greb T.

Radial growth of plant shoots and roots is a stem cell-driven process fundamental for the mechanical and physiological support of enlarging plant bodies. In most dicotyledonous species, the underlying stem cell niche, the cambium, generates xylem toward the organ center and phloem toward the organ periphery. Despite its importance and intriguing dynamics, however, the functional characterization of cambium stem cells is hampered by the lack of experimental tools for accessing distinct cambium subdomains and for demonstrating stemness. Here, we used the hypocotyl of Arabidopsis thaliana to identify stem cell activity in the proliferating cambium. Combining pulse-labeling using the thymidine analogue EdU (5-ethynyl-2'deoxyuridine) with genetically encoded lineage tracing, we found that a single bifacial stem cell generates xylem and phloem cell lineages. This stem cell is characterized by the combined activity of PHLOEM INTERCALATED WITH XYLEM / TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTOR RECEPTOR (PXY/TDR), SUPPRESSOR OF MAX2 1-LIKE PROTEIN 5 (SMXL5) and WUSCHEL HOMEOBOX RELATED 4 (WOX4) genes. Furthermore, in contrast to other plant stem cell niches, we observed that predominantly stem cells divide and not tissue-specific progenitors. To characterize the different cell types in the vascular system, we generated a tissue-specific gene expression atlas, based on tissue-specific promoters, fluorescence activated nuclear sorting (FANS) and RNAseq analysis. Our analysis provides a cellular and molecular fate map of vascular development, and provides comprehensive insight into patterns of plant growth regulation.

P-50 | **Behaviour of plant single cell nucleus under mechanical stress**

Singh G., RICK C., Michaël R., Atef A., and Chaboute M.E.

Plant developments are linked to internal cellular changes which controlled by several factors of signalling pathways. Environmental factors including biotic and abiotic signal has been already discussed to regulate the genetic program during the plant development. Perception of mechanical cues is also a major but unrevealed factor that control morphogenesis by transducing signals and changing cellular shape in plants. In the current study, we have studied the behaviour of nucleus in root hair under control and mechanical stress conditions which concerns potential changes in the shape, size and movement during root hair growth. In order to address the question, we have investigated our study in Col-0 Arabidopsis line expressing the nuclear envelope marker SUN1-GFP. To analyse the root hair nucleus under normal and stress conditions, we used microfluidic setup for live cell imaging. Under control condition, the shape of the nucleus is elongated and shows oscillating and displacement movement in the root hair. An experimental mechanical stress, induced by hyperosmotic stress using mannitol, seems to affect the displacement of the nucleus in the root tip. The results will be discussed in regards of the cytoskeleton and nuclear envelope network.

P-51 | **Examining biological hotspots and enzyme action on cell growth using a continuum modelling approach**

Smithers ET. and Dyson RJ

Cell growth is regulated through rearrangement of the cell wall network, which consists of oriented cellulose microfibres embedded within a ground matrix incorporating pectin and hemicellulose components. However, there are still many unknowns related to how this rearrangement occurs, and how it is regulated. Evidence has shown the cellulose reorients in cell walls as the cell expands with recent opinion turning to the idea that growth is controlled by distinct collections of hemicellulose which join the cellulose molecule together. The enzymes expansin and Cel12A have both been shown to induce growth of the cell wall. However, whilst Cel12A reduces cell wall strength, expansin has been shown to increase the strength of the cell wall. In contrast, members of the XTH enzyme family hydrolyse hemicellulose but do not appear to cause creep. This experimental behaviour still awaits a full explanation. We derive and analyse a simple mathematical model for the effective mechanical properties of the evolving cell wall network, incorporating cellulose microfibres, which reorient with cell growth and are linked via biological “hotspots” made up of regions of crosslinking hemicellulose. Assuming a visco-elastic response for the cell wall and using a continuum approach we calculate the total stress resultant of the cell wall for a given overall growth rate. By changing appropriate parameters effecting breakage rate and viscous properties we can test the theorised enzyme action. Through assuming the biological hotspot hypothesis, we begin to develop an understanding of the mechanistic behaviour as to how these enzymes work.

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P-52 | **A phosphoinositide map at the shoot apical meristem in Arabidopsis thaliana**

Frank Ackermann & Thomas Stanislas

The shoot apical meristem (SAM) has 2 main functions, the production of all aerial organs and selF-0maintenance, allowing the production of organs during the entire post-embryonic life of the plant. Biochemical signals and structural elements have been involved in meristem function. Whereas phosphatidylinositol phosphates (PIPs) have been involved in almost all biological functions, including stem cell maintenance and organogenesis in animals, the processes in meristem biology to which PIPs contribute still need to be delineated. Using specific PIPs biosensors for PI4P and PI(4,5)P2, the two most abundant PIPs at the plasma membrane, we reveal that meristem functions are associated with a stereotypical PIP tissue-scale pattern. Interestingly, this pattern echoes that of cortical microtubules and stress anisotropy at the meristem. While other cues are very likely to contribute to the final PIP pattern, we provide evidence that the patterns of PIP, cortical microtubules and mechanical stress are positively correlated, suggesting that the PIP pattern, and its reproducibility, relies at least in part on the mechanical status of the SAM. Given these observations, we are now in a unique position to address the following questions:1) What is the contribution of the PIP pattern to SAM function? 2) What are the functions of PIPs in mechanotransduction? 3) What is the molecular relation between the PM and mechano-responsive elements?

P-53 | **Non-canonical Cytokinin Signaling Controls Auxin-regulated Exploratory Root Circumnutation in Rice**

Taylor I., Lehner K., McCaskey E., Nirmal N., Jain R., Ronald P., Goldman D., Benfey P.

The helical growth of the tips of plant organs is known as circumnutation, a term coined by Darwin, who recorded its occurrence in the shoots and roots of dozens of plant species. Since then a large number of studies have examined circumnutation in shoots, but our understanding of the molecular mechanisms and function of circumnutation in roots has remained limited. Rice roots, which undergo pronounced circumnutation driven by a strongly oscillatory bending of the root tip, are a useful yet largely unstudied model for this process. Using high-resolution timelapse imaging of growing rice plants, we have recently identified a mutant in which roots display a dramatic defect in root circumnutation. The mutated gene, Oryza sativa HISTIDINE KINASE-1/OsHK1, encodes a protein with homology to cytokinin receptors. Functional analysis and gene expression studies indicate HK1 activates canonical downstream cytokinin signaling by an atypical, cytokinin-independent mechanism. Chemical and genetic evidence links HK1-regulated signaling to control of auxin transport. Polar auxin transport is well known to regulate root bending during gravitropic response, and we have found evidence that an analogous mechanism operates to induce oscillatory root bending during circumnutation. Our evidence further suggests that in the hk1 mutant, this auxin transport process is disrupted primarily by a reduction in auxin influx capacity in the root tip. Analysis of root growth indicates the mutant has reduced exploratory ability when encountering high-impedance substrates, suggesting that the HK1-regulated auxin transport processes underlying circumnutation are critical determinants of root exploration. Overall this work establishes a mechanistic framework to guide further study of the regulation and function of root circumnutation.

P-54 | **Quantitative analysis of cellular growth patterns in integument morphogenesis and ovule curvature in Arabidopsis thaliana**

Tofanelli R., Vijayan A., and Schneitz K.

How the Gestalt of a plant tissue is generated remains an open question in plant developmental biology. Ovule curvature represents a unique phenomenon in plant tissue morphogenesis and invites interesting questions regarding the cellular, molecular, and mechanical mechanisms involved in the formation of this characteristic shape. The ovule of Arabidopsis thaliana represents a nice model system to address this question. At maturity, the ovule exhibits a distinctive curved shape such that the micropyle is placed next to the base of the funiculus, resulting in a 180° bending (anatropy). A qualitative description of ovule development in A. thaliana has been established, as has been a staging system based on morphological features. Ovules originate from placental tissue of the gynoecium as protrusions and three morphological pattern elements are recognized along the proximal-distal (PD) axis: funiculus, chalaza, and nucellus. A. thaliana carries bitegmic ovules as the chalaza originates two integuments, precursors of the seed coat. It has been postulated that differential growth of the outer integument contributes to curvature. Comparative evolutionary studies suggested that the presence of an outer integument is crucial for anatropy in bitegmic ovules. Moreover, genetics provided evidence on the central role of the outer integument, through the identification of mutants carrying ovules with a defect in outer integument development. We have performed a quantitative analysis of the cellular growth patterns of the integuments in wild-type and mutants at different developmental stages. Our pipeline consisted of 3D image acquisition of fixed samples at cellular resolution followed by image processing and analysis in MorphographX. The results indicate that the outer layer of the outer integument is a central regulator of ovule curvature. Furthermore, they validate our quantitative approach as a promising strategy to explore the cellular mechanisms underlying ovule curvature.

P-55 | **Does the spikelet development program influence the shape and spikelet abortion of a spike?**

Venkatasubbu T., Rutten T. and Schnurbusch T.

Improving grain yield is a major objective of crop breeding, and one promising avenue is increasing grain number through enhanced spikelet survival. Grain crops like barley and maize form indeterminate inflorescence meristems and develop spikelets in acropetal succession. The grain-bearing structures of these crops exhibit spikelet abortion following a basipetal gradient even in optimal conditions. In unfavorable conditions, spikelet abortion becomes more severe and sharply reduces the yield. Though several hypotheses, such as competition for assimilates, the position of the spikelets and pollination time gap, were proposed for the cause of spikelet abortion, the mechanism of spikelet abortion is still elusive. From our comprehensive studies on barley spikelet development, we found that during the spikelet development phase, the inflorescence meristem and the initiated spikelets show high mitotic activity. However, before the end of the spikelet development phase, mitosis is arrested in the cells surrounding the inflorescence meristem, and the arrest front starts moving basipetally to the developed spikelets. Interestingly, the mitotic arrest front is followed by the abortion of spikelets in the same basipetal pattern. Since Goethe's path-breaking finding in 1790, we already knew that floral parts (inflorescence) are nothing else but modified leaves. By applying his conception to our observed pattern of spikelet abortion, we hypothesize that it resembles the movement of the cell cycle arrest front followed by programmed cell death in the leaf growth and senescence program. Also, near the end of the spikelet development phase, the rate of spikelet development is significantly reduced, which impacts the shape of a spike apex. We, therefore, hypothesize that the rate of spikelet development influences the shape of a spike and drives the spikelet abortion front. These intriguing ideas may open up new avenues in improving the grain number and yield.

P-56 | **Quantitative analysis of cellular growth patterns in the Arabidopsis ovule**

Athul R Vijayan, Rachele Tofanelli and Kay Schneitz

The cellular and molecular mechanisms underlying tissue morphogenesis in plants remain poorly understood. The Arabidopsis ovule, with its small size and characteristic curvature, represents an interesting model to address this topic. Development of the Arabidopsis thaliana ovule has been qualitatively well described in past years and a staging system has been proposed that is based on the appearance of characteristic morphological features within the organ. Ovule arises as a finger-like protrusion from the placental tissue of the gynoecium, at maturity, the ovule exhibits a distinct 180° curvature (anatropy). Although genetic and molecular mechanisms controlling the outgrowth and patterning of the ovule are known in detail, a quantitative analysis of the cellular growth patterns that contribute to the final three-dimensional shape of the ovule is still lacking. Our approach for the extraction of quantitative information of ovule growth consists of 3D image acquisition of fixed samples at full cellular resolution using confocal microscopy. The obtained z-stacks are processed using a convolutional neural network and cells are segmented and analyzed using MorphographX. From the 3D computational reconstruction of 150 ovules, covering all developmental stages, we have obtained information on cell number, cell size and shape, and spatial distribution of cell divisions. Additionally, cell type annotation has been performed using 3D cell atlas tools. The 3D digital models allow to explore the contribution of the different tissues to the overall growth of the organ. We will discuss our present findings. Our quantitative cellular analysis represents a novel approach in the study of ovule morphogenesis and will lay the foundation for further investigations aimed at understanding how molecular, cellular, and mechanical mechanisms are translated into final ovule shape.

P-57 | **Cytoskeleton dynamics are necessary for early events of lateral root initiation in Arabidopsis**

Vilches Barro A., Stöckle D., Thellmann M., Ruiz-Duarte P., Bald L., Louveaux M., von Born P., Denninger P., Goh T., Fukaki H., Vermeer J. and Maizel A.

How plant cells re-establish differential growth to initiate organs is poorly understood. Morphogenesis of lateral roots relies on the asymmetric cell division of initially symmetric founder cells. This division is preceded by the tightly controlled asymmetric radial expansion of these cells. The cellular mechanisms that license and ensure the coordination of these events are unknown. Here, we quantitatively analyse microtubule and F-0actin dynamics during lateral root initiation. Using mutants, pharmacological and tissue-specific genetic perturbations, we show that dynamic reorganisation of both microtubule and F-0actin networks is necessary for the asymmetric expansion of the founder cells. This cytoskeleton remodelling intertwines with auxin signalling in the pericycle and endodermis in order for founder cells to acquire a basic polarity required for initiating lateral root development. Our results reveal the conservation of cell remodelling and polarisation strategies between the Arabidopsis zygote and lateral root founder cells. We propose that coordinated, auxin driven reorganisation of the cytoskeleton licenses asymmetric cell growth and divisions during embryonic and post-embryonic organogenesis.

P-58 | **Role of actin in shoot apical meristem growth**

Wang Y., Strauss S., Smith. R., Sampathkumar A.

Plant cell directional growth and cell division plays crucial roles in plant morphogenesis. These two processes have been shown to be closely related with cell wall synthesis and modification. It is well established that the microtubule cytoskeleton in plants read and respond to mechanical cues regulating growth direction and cell division plane orientation. Apart from microtubules, actin cytoskeleton in plants have also been shown to play important roles in plant growth and development. However, much less is understood about the relation between actin filaments, mechanics and morphology. Here we undertake an interdisciplinary approach that combines quantitative live cell imaging, molecular perturbation and micromechanical manipulations along with computational modeling to understand the role of actin in directional growth and cell division in Arabidopsis shoot apical meristem.

P-59 | **Genetic control of cell morphologies in the shoot apical meristem**

Christian Wenzl, Jan U. Lohmann

The shoot apical meristem enables plants to form new lateral organs throughout their lives. This requires the coordination of cell division and cell growth as well as the control of cell fate transitions in a complex tissue build by small, undifferentiated and uniformly shaped cells. However we have observed slight variations in cell morphologies at different developmental stages or under different environmental conditions. How such changes in SAM cell morphology can effect SAM function is largely unknown. Here we identified a new recessive mutation that leads to altered cell morphologies specifically in the inflorescence shoot apical meristem. The mutation effects cell size, shape and cell number throughout the SAM with particularly strong effects in the epidermal cells of the L1 layer. Polarized auxin transport across L1 cell membranes leads to the periodic formation of local auxin maxima that define sites of organ initiation and ultimately results in a rhythmic pattern of lateral organ formation known as phyllotaxis. The formation of auxin maxima is unaffected in the mutant but the timing of organ initiation is disrupted and can lead to simultaneous outgrowth and sometimes even to fusion of organ primordia. Despite its effects on SAM cell morphology the overall phenotype of mutant plants appears to be very similar to wildtype. We could map the mutation to the top arm of chromosome 3. To our surprise the most likely candidate is a small in frame deletion of rrp5 an essential gene that is involved in ribosomal RNA processing and we are currently investigating how rRNA processing and cell morphogenesis in the SAM could be linked.

P-60 | **A morpohodynamic study of Cardamine hirsuta CUP-SHAPED COTYLEDON1 (CUC1) role in leaf development**

Wilson-Sanchez D., Hu Z., Runions A., Rast-Somssich M. and Tsiantis M.

Leaves are the main photosynthetic organs of seed plants and show considerable variation in their shape in part owing to different growth patterns at their margins (Bilsborough et al., 2011; Kierzkowski et al., 2019). PIN-FORMED 1 (PIN1), CUP-SHAPED COTYLEDON2 (CUC2) and the hormone auxin are part of the regulatory network that controls margin patterning and growth in both Cardamine hirsuta, which has dissected leaves divided to leaflets, and in the simple leafed Arabidopsis thaliana (Bilsborough et al., 2011; Rast-Somssich et al., 2016). This raises the question of how this network was modified to produce different margin configurations. Recently, we found that regulatory diversification of CUC1 results in ChCUC1-only expression in leaves, and that interspecific gene transfer from C. hirsuta into A. thaliana is sufficient to increase A. thaliana leaf complexity. Conversely, previous observations indicate that ChCUC1 is redundantly required with ChCUC2 and its paralogue ChCUC3 for leaflet formation (Blein et al., 2008). By combining time-lapse imaging and growth tracking at cell resolution level, we are exploring how ChCUC1 influences growth to promote formation of leaflets instead of lobes. We are also investigating cell level processes via which CUC1 affects growth. The resulting information will be used to generate physically based models that conceptualize the role of CUC1 in leaf development and diversity.

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Rast-Somssich, M.I., et al. (2015). Genes Dev. 29, 2391–2404.
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P-61 | **Auxin promotes periderm development in Arabidopsis**

Xiao W., Wunderling A., Molina D., Vermeer J., Maizel A., Ragni L.

Secondary growth, the increase the girth of plant organs, occurs in most dicots and gymnosperm and it is mainly driven by the vascular cambium. The phellogen, is another lateral meristem, which contributes to the radial thickening of plant organs and give origin to the periderm. The periderm during secondary growth replaced the epidermis as a barrier against biotic and abiotic stresses. Despite the economic importance of the periderm as a source of cork, few regulators controlling phellogen activity have been identified and the network underlying periderm development is largely unknown. Here, using the Arabidopsis root as a model, we investigated the role of auxin during periderm development. Auxin accumulates in the phellogen and many AUXIN RESPONSIVE FACTORS (ARFs) and IAAs are expressed thorough periderm development. Exogenous application of auxin promotes periderm development, whereas several iaa gain-oF-0function mutants shows defects in periderm growth. To assess the specific contribution of auxin in phellogen establishment, phellogen maintenance and phellem differentiation, we engineered plants to block auxin signaling in a spatio-temporal controlled manner. Impairing auxin signaling during the early steps of periderm growth results in a delay of periderm formation and in a completely disorganized periderm, whereas blocking auxin in the phellogen leads to its consumption. By contrast preventing auxin signaling in the phellem has no effect, suggesting that auxin does not act during phellem differentiation. In summary, our work highlights that auxin is required for proper phellogen establishment and maintenance.

P-63 | **The Plant cell wall as an actor of organ morphogenesis during radial plant growth**

Zakieva A., Qi J., Weber P., López-Salmerón L. and Greb T.

Plant cell walls separate cells from each other and keep them in a fixed position. Tissue growth is thus directly dependent on cell division, expansion and differentiation. The cell wall plays a role as a matrix connecting cells and its mechanical properties vary between different tissue types. In particular, stem cells are supposed to carry flexible cell walls, while cell wall stiffening is associated with tissue differentiation. Here, tissue-specific regulation of cell wall properties is investigated during radial plant growth – a process producing wood and a large part of terrestrial biomass. The Arabidopsis hypocotyl – an organ at the intersection of the stem and root system – is a hotspot of radial growth and, thus, it is a suitable model for investigating tissue morphogenesis during this process. Cambium stem cells are the driving force of radial plant growth which proliferate and differentiate into the vascular tissues xylem and phloem. What is the role of cell wall mechanics in regulating cell fate and tissue morphogenesis during radial growth? Here, we use inducible tissue-specific expression of cell wall modifying proteins to address this question. HAE-IDA is a receptor-ligand module involved in cell wall disruption during organ abscission and lateral root emergence. Preliminary results show that continuous HAE-IDA expression in the cambium and phloem inhibits vascular tissue production. In contrast, continuous HAE-IDA expression in the cambium and xylem precursors induces morphological alterations in xylem vessels. Thus, ectopic expression of cell wall modifying proteins in vascular tissues affects cell morphogenesis and proliferation. We expect that further investigation of tissue-specific cell wall properties will shed light on the involvement of cell walls in tissue and organ morphogenesis during radial plant growth.

P-64 | **Deciphering the role of a novel player in grass subsidiary cell development**

Dan Zhang, Tiago D. G. Nunes, Heike Lindner, Michael T. Raissig

Stomata are epidermal valves that are essential for plant survival because they control the entry of carbon dioxide assimilated in photosynthesis and optimize water use efficiency. Grasses like the model system Brachypodium distachyon form faster and, thus, more water-use-efficient stomatal complexes by adding two intimately connected, lateral subsidiary cells (SCs) to the central guard cells. We previously discovered that the mobile transcription factor BdMUTE specifies subsidiary cells in Brachypodium. The mutant bdmute fails to recruit SCs and forms two-celled, eudicot-like stomata instead that are slower to open and close and fail to open as wide as four-celled wild-type stomata. Transcriptome profiling of the developmental leaf zone of both wild-type and bdmute discovered genes absent or strongly downregulated in the mutant that are potentially associated with subsidiary cell formation. A gene of unknown function shows strong expression levels in wild-type but very low expression in bdmute. CRISPR-Cas9-edited mutant lines show misoriented or even absent SC division indicating a defect in polarizing the SC precursor cell. We hypothesize that this novel player participates in SC formation and, potentially, plays a role in setting up cell polarity during SC division.

P-65 | **Protein interactions of HD-Zip class I transcription factors shaping leaves in Brassicaceae**

Zühl L., Vuolo F. and Tsiantis M.

We are studying leaf shape as a model to understand how morphological diversity arises in angiosperms. In this context we are investigating developmental processes leading to generation of simple leaves in Arabidopsis thaliana versus complex leaves in its close relative Cardamine hirsuta. Previously our group identified the paralogous HD-Zip class I homeobox transcription factors LATE MERISTEM IDENTITY 1 (LMI1) and REDUCED COMPLEXITY (RCO) as important regulators of development of distinct leaf shapes in these two species and other Brassicaceae (Saddic et al. 2007, Dev. 133; Vlad et al. 2014, Science 343). Both act as local growth repressors (Vlad et al. 2014, Science 343; Vuolo et al. 2016, GenesDev. 30; Vuolo et al. 2018, GenesDev. 32), but knowledge about co-factors and downstream components of underlying regulatory processes is limited. Therefore, regulatory hierarchies through which these proteins influence leaf shape remain poorly understood. With my PhD project I aim to clarify these hierarchies and mainly focus on the predicted interaction partners of A. thaliana LMI1 and C. hirsuta RCO. To identify interactors, I will apply affinity purification coupled with mass spectrometry approaches and subsequent targeted interaction analyses. Results will also be compared to preliminary data from a yeast-two-hybrid screen, hinting towards interaction of AtLMI1 with members of other transcription factor families. Finally, I hope to analyse the role of identified interactions for leaf shape development and their conservation in Brassicaceae.

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