# Senses and Sensitivity

THE 5TH INTERNATIONAL COS HEIDELBERG SYMPOSIUM

JUNE 21-22 2017 Im Neuenheimer Feld 230 - Heidelberg, Germany



**PROGRAM AND ABSTRACTS** 





Centre for Organismal Studies Heidelberg

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#### June 21 (Day 1)

09.00 - 09.15: Welcome note

Session 1: "Sensory machineries"

- 09.15 10.15 **The EMBO Keynote Lecture** Jonathan Jones (The Sainsbury laboratory, Norwich) Plant immune receptors; dissection, diversity and deployment
- 10.15 11.00 **Lucia Prieto-Godino** (University of Lausanne) Evolution of olfactory circuits in Drosophila: zombie genes and other surprises
- 11.00 11.45 **Chris Bowler** (École Normale Supérieure, Paris) Epigenetic phenomena in response to environmental signals in plants and marine diatoms

in life sciences

Coffee Break (11.45 - 12.15)

- 12.15 13.00 **Charalambos Kyriacou** (University of Leicester) Molecular bases of rhythmic behaviors in animals
- 13.00 13.30 Erika Tsingos (Centre for Organismal Studies, Heidelberg) (Speaker selected from abstract) On growth and form in a complex organ: The neural retina drives postembryonic eye morphogenesis in fish

Lunch and **Poster session 1** (13.30 - 15.30)

#### Session 2: "Processing and decoding"

- 15.30 16.15 **Jürgen Gross** (Julius Kühn-Institut, Dossenheim) Chemical communication between phytopathogens, their host plants and vector insects – from basic research to applications in plant protection
- 16.15 17.00 Anne Pfeiffer (Centre for Organismal Studies, Heidelberg) Influence of light on shoot stem cell regulation in plants

#### June 22 (Day 2)

#### Session 2: "Processing and decoding"

- 09.00 09.45 **Michael Brecht** (Bernstein Center for Computational Neuroscience Berlin) Social Touch - the cortical neurobiology of physical contact
- 9.45 10.30 **Marieke Essers** (Deutsches Krebsforschungszentrum / University of Heidelberg) Hematopoietic stem cells and their niche under inflammatory stress

Coffee break (10.30 -11.00)

#### Session 3: "Behavior and phenotypic plasticity"

- 11.00 11.45 **Rainer Hedrich** (University of Würzburg) Venus flytrap - a plant on animal diet
- 11.45 12.15 **Ruediger Hell** (Centre for Organismal Studies, Heidelberg) Schmeil Award ceremony for the best PhD in organismal biology at the COS

#### Lunch and Poster session 2 (12.15 - 14.15)

- 14.15 15.00 **Aurelio Teleman** (Deutsches Krebsforschungszentrum / University of Heidelberg) Regulation of mitochondrial function via a dietary lipid
- 15.00 15.30 **Eva-Sophie Wallner** (Centre for Organismal Studies, Heidelberg) (Speaker selected from abstracts) Strigolactone and karrikin-independent SMXL proteins are central regulators of phloem formation

Coffee break (15.30 -16.00)

- 16.00 16.45 **Anke Steppuhn** (Freie Universität Berlin) Plants use insect eggs as telltale signals for an upcoming herbivory
- 16.45 17.30 **Nick Foulkes** (Karlsruhe Institute of Technology/Centre for Organismal Studies, Heidelberg)

Food, light and the evolution of the circadian timing system

#### Announcement of the poster prize

Grill party in front of the COS INF 230 Building (17.45 -)

The EMBO Keynote Lecture



Plant immune receptors; dissection, diversity and deployment

Jonathan D G Jones\*, Zane Duxbury, Yan Ma, Panagiotis Sarris, Sung Un Huh, Kee Sohn, Kamil Witek, Hari Karki and Oliver Furzer

Sainsbury Lab, Norwich Research Park, Colney Lane, Norwich, UK \*EMBO Member

Diverse microbes cause plant disease, and plants have evolved a robust innate immune system that recognizes pathogen molecules and then activates defense. Immunity involves cell surface receptors and also intracellular Nucleotide-binding, Leucine-rich Repeat (NLR) immune receptors, encoded by *Resistance* (R) genes.

Some resistances require two co-functioning NLR proteins. The adjacent, divergently transcribed, Arabidopsis RPS4 and RRS1 genes, encoding TIR-NLR proteins, are both required for resistance to bacteria that deliver AvrRps4 or PopP2 effectors, and for resistance to certain *Colletotrichum* strains. RRS1 carries a C- terminal WRKY domain targeted by AvrRps4 and PopP2, suggesting these effectors target WRKY domains. We investigate how the RPS4/ RRS1 complex activates defense upon effector recognition.

*R* gene enrichment sequencing, "RenSeq", involves sequence capture to enrich for NLR genes prior to sequencing (Jupe et al, 2013). We use RenSeq to discover new *R* genes in diverse *Solanum* sp against *Phytophthora infestans* that could provide useful protection against blight in potato. We also use RenSeq to define new *R* genes against various pathogens of the *Brassicaceae*, in particular the "White Rust"-causing *Albugo* species, and to investigate NLR gene diversity in Arabidopsis. NLR gene clusters are complex and difficult to resolve using Illumina reads, but PacBio enables us to define the diversity of NLR genes in *Arabidopsis*, and to understand better the evolutionary history of these fascinating genes (Witek et al 2016).

Jupe et al (2013) Plant Journal 76, 530–544 Williams et al. Science (2014) vol. 344 (6181) pp. 299-303 Sohn et al. PLOS Genetics doi: 10.1371/journal.pgen.1004655 Saucet et al (2015) Nat. Comms 6 pp. 6338 Jupe et al (2013) TPJ 76, 530–544 Sarris et al (2015) Cell 161 pp. 1089-1100 Witek et al (2016) Nat Biotech 6:656-660

# Evolution of olfactory circuits in Drosophila: zombie genes and other surprises

# Lucia Prieto-Godino

University of Lausanne, Faculty of Biology and Medicine (FBM), Lausanne, Switzerland

Animals adapt their behaviors to specific ecological niches, but the underlying genetic and cellular basis of nervous system evolution is poorly understood. We have compared the olfactory circuits of the specialist fly species *Drosophila sechellia*, which feeds and breeds exclusively on the acid-rich fruit of *Morinda citrifolia*, with its generalist cousins D. melanogaster and D. simulans, which are associated with a wide range of fermenting fruits. We have identified both loss and gain of sensory responses to acids in D. sechellia and link these to single nucleotide differences within a tandem cluster of olfactory receptor genes. Unexpectedly, we find that one of these receptors bears a premature stop codon (PTC), and yet encodes a functional receptor, due to efficient neuron specific translational read-through of the PTC. Importantly we show that this is a widespread phenomenon. Through comparative analysis and protein homology modeling of the ligand binding domain of these receptors we were able to delineate their evolutionary trajectory. These peripheral functional differences are accompanied by regulatory and developmental modifications that shape the species-specific neuroanatomical organization of acid-sensing pathways. Our work links chemosensory ecology to genetic changes influencing nervous system structure and function across evolutionary time.

Epigenetic phenomena in response to environmental signals in plants and marine diatoms

# **Chris Bowler**

Ecology and Evolutionary Biology Section, Institut de Biologie de l'Ecole Normale Supérieure (IBENS), Ecole Normale Supérieure, Paris, France

The ability to respond appropriately to a variable environment is essential for the survival of photosynthetic organisms in both terrestrial and aquatic environments. The mechanisms by which the environment can influence genome structure and dynamics are also likely to be important in driving evolution. In order to investigate these processes, we use *Arabidopsis thaliana* as a higher plant model, and the diatom *Phaeodactylum tricornutum* as a model marine phytoplankton.

In *Arabidopsis*, we are examining the influence of light on chromatin-level regulation. We are exploring the changes in genome structure mediated by morphogenic light signals during the dark to light transition in young seedlings. The team investigates the impact of chromatin organization and composition dynamics on the regulation of gene expression. This is achieved by integrating spatial and temporal chromatin dynamics with transcriptional reprogramming in response to light signals. Our work has focused on histone H2B monoubiquitination (H2Bub), which has enabled us to identify a series of light-regulated genes whose upregulation following light perception is optimized by rapid chromatin state changes. This gene list comprises several master regulators of plant light responses and circadian clock function.

In parallel we are using *Phaeodactylum* to explore the role of epigenetic phenomena in regulating phytoplankton life histories, in particular during the rise and fall of seasonal blooms. Most recently, we have generated epi-genome maps of this diatom that include DNA methylation data as well as selected histone mark distributions. Over recent years, we have also used genome-enabled approaches to better understand diatom responses to other environmental signals, such as light and nutrients, including nitrogen and iron.

# Molecular bases of rhythmic behaviors in animals

# Charalambos Kyriacou

University of Leicester, Department of Genetics

Biological rhythms span a number of time domains but the best studied are the circadian 24 h cycles of behaviour and physiology that infiltrate almost every biological process in higher organisms and some bacteria. The molecular components underpinning circadian clocks have been identified but their possible roles in seasonal or lunar related cycles have only recently been investigated. Using flies and crustacea, the molecular genetic basis of seasonal and tidal timing will be explored.

Influence of light on shoot stem cell regulation in plants

# Anne Pfeiffer

Centre for Organismal Studies, University of Heidelberg

Plants are sessile organisms and therefore forced to adapt their development and growth behavior to the environment. The main source of energy for plants is light and therefore it is also one of the most important environmental stimuli influencing a plant's life. Equipped with a range of photoreceptors plants can perceive changes of the ambient light conditions, e.g. day length or shading through neighboring plants, which consequently leads to distinct morphological changes in plant architecture and also development.

Since the entire above ground tissue originates from stem cells embedded in the shoot apical meristem (SAM), information about the ambient light conditions have to be transduced to and integrated at the SAM in order to affect plant development and morphology.

Tracing cell behavior in the SAM by live cell imaging allowed us to identify light signaling but also nutrient sensing as two independent regulators of the shoot stem cell niche. Both signals are transduced to the SAM through activation of the TARGET OF RAPAMYCIN (TOR) kinase, which is known to be an evolutionary highly conserved nutrient sensor and potent regulator of cell cycle and metabolism.

Social Touch - the Cortical Neurobiology of Physical Contact

# Michael Brecht

Humboldt-Universitaet zu Berlin, Institut fuer Biologie, Bernstein Center for Computational Neuroscience Berlin

The cerebral cortex is the largest brain structure in mammalian brains. While we know much about cortical responses to controlled, experimenter imposed sensory stimuli, we have only limited understanding of cortical responses evoked by complex social interactions. In my lecture I will focus on response patterns evoked by social touch in somatosensory cortex in interacting rats. We find that social touch evokes stronger responses than object touch or free whisking. Moreover, we find prominent sex differences in responsiveness. We observe a modulation of cortical activity with estrus cycle in females, and in particular a modulation of fast-spiking interneurons by estrogens. The prominent sex differences are unexpected, since the somatosensory cortex is not anatomically sexually dimorphic. Recently, we confirmed the absence of anatomical sex differences in somatosensory cortex by an analysis of somatosensory representations of genitals. Despite the marked external sexual dimorphism of genitals, we observed a stunning similarity of the cortical maps representing the clitoris and penises, respectively. In the final part of my presentation I will discuss the involvement of somatosensory cortex in ticklishness. In these experiments we habituated rats to be tickled and found that animals respond to such stimulation with vocalizations. Importantly, rats seem to enjoy tickling and seek out such tactile contacts. In the physiology we observed in trunk somatosensory cortex numerous cells that were either inhibited or excited by tickling. Most interestingly, excitatory or inhibitory responses to tickling predicted excitatory or inhibitory cortical response patterns during play behavior. Microstimulation of deep layer neurons in the somatosensory cortex evoked vocalizations similar to those evoked by tickling. Thus, stimulation and recording data suggest a critical role of somatosensory cortex in mediating ticklishness and the control of playful behaviors.

Hematopoietic stem cells and their niche under inflammatory stress

# Marieke Essers<sup>1,2</sup>

<sup>1</sup>German Cancer Research Center (DKFZ), Heidelberg, Germany <sup>2</sup>HI-STEM - Heidelberg Institute for Stem Cell Technologies and Experimental Medicine gGmbH, Germany

Infections are associated with extensive consumption of differentiated hematopoietic cells, representing a high risk for health. However, the mechanism coordinating the rapid and efficient regeneration of these differentiated cells during such stress conditions remains unclear. Recently, we have reported that the phenotypic hematopoietic stem cell (HSC) compartment contains stem-like megakaryocyte-committed progenitors (SL-MkPs), a cell population that shares many features with multipotent HSCs and serves as a lineage-restricted emergency pool for inflammatory insults. This study revealed an elegant emergency machinery that counteracts life-threatening platelet depletions during acute inflammation. Furthermore, these data indicated heterogeneity within the phenotypic HSC pool regarding lineage commitment. To reconstruct how individual HSCs enter lineage commitment we mapped human bone marrow haematopoiesis by quantitatively integrating flow cytometric, transcriptomic and functional lineage fate data at the single-cell level. We found that individual HSCs neither enter lineage commitment at binary branching points nor pass through discrete intermediate progenitor cell stages. In contrast, HSC lineage commitment occurs in a gradual manner best described by a continuous Waddington landscape with initially flat but progressively deepening valleys. Our data determine a detailed model of developmental trajectories within this landscape. as well as their underlying gene expression modules and biological processes. In addition to identifying how HSCs respond under inflammatory conditions, we also investigate the response of the BM niche to inflammatory stress and how different components of the BM niche support the response of quiescent HSCs to inflammatory stress in vivo.

# Venus flytrap - a plant on animal diet

# **Rainer Hedrich**

# Molecular Plant Physiology & Biophysics, Wuerzburg University, Germany

Charles Darwin over 100 years ago recognized that the Venus flytrap *Dionaea muscipula* living on nutrient poor soil is capturing animals. When small animals visit the trap surface and touch the trigger hairs the trap gets excited and after firing two action potentials closes. Trying to escape the encaged prey keeps on exciting the capture organ and thereby glands covering the inner trap surface trigger secretion of a digestive fluid. During prey decomposition the animal-derived nutrients are ingested. Although the concept of botanical carnivory is known since Darwin's time, due to the entire lack of genomic information, the molecular processes providing for animal feeding remain still unknown.

To bridge that gap, we sequenced the genome together with transcriptome expressed in different organs of *Dionaea* and assembled a backbone transcriptome of the carnivorous plant. Given that with *Dionaea* leaves only the bi-lobed tip but not the petiole develop into a sophisticated capture organ, we focused on trap genes that become active upon contact with the animal victim. Special attention we gave to trigger hairs and glands engaged with i) generation of the action potential, ii) secretion of hydrolases, and iii) uptake of nutrients extracted from the digested animal. Serving the latter function we spotted ion channels and transporter. Following expression of the Dionaea gland-expressed nutrient transporter genes in *Xenopus* oocytes, ion selective voltage changes and currents were recorded. Our studies indicate that Dionaea glands operate selective, high capacity channels and transporters to provide nutrients and osmotic potential while the feeding on a decomposing victim.

During the seminar the molecular nature and mechanism of the hunting cycle of the most exciting green carnivore will be discussed.

Regulation of mitochondrial function via a dietary lipid

# Aurelio Teleman

Signal Transduction in Cancer and Metabolism, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 580, 69120 Heidelberg

Signaling pathways and metabolic pathways are interconnected. Signaling pathways regulate metabolic pathways, and the other way around. I will present data showing that dietary levels of a lipid metabolite are sensed by cells and lead to signaling changes and eventually altered mitochondrial function in animals.

Plants use insect eggs as telltale signals for an upcoming herbivory

# Anke Steppuhn

Freie Universität Berlin, Institue of Biology, Molecular Ecology, Berlin, Germany

Insect oviposition on plants often precedes larval herbivory. We investigate whether and how plants use oviposition by herbivorous insects as signals to increase their plant defence. Some plants respond strongly at the sites of egg deposition including eggkilling responses. Others show no obvious effects on the leaf tissue beneath the eggs nor on egg survival. Independent of that, oviposition experiences increase the resistance of several plants to the feeding larvae. We identified feeding-induced defence traits that are systemically primed by oviposition and linked these traits with the increased resistance to larval feeding of oviposited plants. The primed defence traits can affect lepidopteran larvae of divergent host plant ranges very differently and not only reduce their growth and survival but also their immune parameter. Moreover, the known species-specificity of plant responses to different lepidopteran larvae seems to be primed already with the oviposition by the moth. Thus, despite insects may profit from hatching on their host plant, insect oviposition is also used by plants to optimize their anti-herbivore defence.

Food, light and the evolution of the circadian timing system

# Nicholas S. Foulkes

Karlsruhe Institute of Technology, Eggenstein, Germany, Centre for Organismal Studies, University of Heidelberg, Heidelberg, Germany

The circadian clock dominates most aspects of physiology, allowing animals and plants to anticipate and thereby to adapt to the environmental changes that constitute the daynight cycle. A characteristic feature of the clock mechanism is its ability to respond to a range of "timing" signals including light, temperature and food availability, and thereby to adjust the phase of the clock rhythm to maintain its synchrony with the 24 hours environmental cycle. Therefore the clock represents a key point of interaction between organismal biology and the environment.

For some time we have studied the mechanisms whereby clocks sense environmental signals and in turn how clocks are coupled with various cellular processes. In our work, we have exploited a range of fish species as models, notably including the genetic models zebrafish and medaka as well as fish that inhabit extreme habitats, such as blind cavefish. Comparative and mechanistic studies involving these species as well as comparison with findings from mammalian models such as the mouse, provide us with a unique glimpse of how this central timing mechanism has evolved in vertebrates in response to changes in their environment. Recently we have revealed how light and food availability are integrated by the circadian clock and in turn differentially control cycling levels of many metabolites in the zebrafish liver in a manner quite different from what has been previously described in the mouse, a nocturnal rodent. Furthermore, in certain species of blind cavefish which have evolved for millions of years in an extreme, perpetually dark environment, clocks are no longer regulated by light, but instead have become extremely responsive to the time of food availability. This striking shift in clock regulation may well reflect the unique ecology of these cave animals. Thus our findings point to an inherent flexibility in clock regulatory pathways during evolution which may enable the circadian timing system to optimally respond to changing combinations of environmental factors as animals adapt to new habitats.

# Short talk

# Erika Tsingos

Centre for Organismal Studies, University of Heidelberg

Fish grow throughout their lives, providing an excellent model system to study mechanisms of growth regulation in the context of functioning organs. The eye consists of many anatomically distinct tissues; i.e. cells in one tissue do not contribute new cells to other tissues in the organ. The growth rate of all tissues must be precisely tuned to maintain the 3D shape crucial for visual function. If any tissue grows too much or too little, this could lead to tissue bulging and deformation of the organ shape or stretching and tearing of adjacent tissues.

The retina consists of two concentrically arranged hemispherical tissues: the neural retina (NR) and the retinal pigmented epithelium (RPE). In these tissues, cell size remains roughly constant, and cell death is negligible. Therefore, the critical parameter that needs to be tuned to adjust tissue growth rate to organ growth rate is the proliferation rate of the tissue stem cells. Using computational approaches, I explore possible scenarios to address how retinal tissues coordinate their growth rate with organ growth rate and how the shape of the eye is maintained. Comparing simulations to experimental data reveals that the NR stem cells represent the driving force and shape-giving element in postembryonic eye growth, while the RPE follows instructive external signals.

# Short talk

# Strigolactone and karrikin-independent SMXL proteins are central regulators of phloem formation

# **Eva-Sophie Wallner**

# Centre for Organismal Studies, University of Heidelberg

The body shape of multicellular organisms depends on the activity of distinct stem cell niches coordinated over long distances. Plant stem cell niches, the meristems, require long-distance transport of energy metabolites and signalling molecules along the phloem tissue. However, currently it is unclear how specification of phloem cells is controlled. Here we show that the SUPPRESSOR OF MAX2 1-LIKE3 (SMXL3), SMXL4 and SMXL5 genes act as cell-autonomous key regulators of phloem formation in Arabidopsis thaliana. The three genes form an uncharacterized subclade of the SMXL gene family which mediates strigolactone and karrikin signalling. Strigolactones are endogenous plant hormones regulating shoot and root branching whereas exogenous karrikin molecules induce germination after wildfires. Both activities depend on the F-box protein and SCF (Skp, Cullin, F-box) complex component MORE AXILLARY GROWTH2 (MAX2). Strigolactone and karrikin perception leads to MAX2-dependent degradation of distinct SMXL protein family members, which is key for mediating hormonal effects. However, the nature of events immediately downstream of SMXL protein degradation and whether all SMXL proteins mediate strigolactone or karrikin signalling is unknown.

In this study we demonstrate that, within the SMXL gene family, specifically SMXL3/4/5deficiency results in strong defects in phloem formation, altered sugar accumulation and seedling lethality. By comparing protein stabilities, we show that SMXL3/4/5 proteins function differently to canonical strigolactone and karrikin signalling mediators, although being functionally interchangeable with those. Independence of SMXL3, SMXL4 and SMXL5 from strigolactone/karrikin signalling may be crucial for a robust formation of long-distance transport capacities and, consequently, plant vitality.

# Poster session 1

Poster number	First Name	Last Name	Email address	Poster title
1-1	Christophe	Gaillochet	christophe.gaillochet@cos.uni- heidelberg.de	Control of plant cell fate transitions by transcriptional and hormonal signals
1-2	Bérénice	Ziegler	Berenice.Ziegler@cos.uni-heidelberg.de	Mechanosensitivity: Function of TRP channels and mechanoreception in Hydra
1-3	Jana Christin	Askani	jana.askani@cos.uni-heidelberg.de	Function of HOPS/CORVET membrane tethering complexes in microtubule arrangement?
1-4	Ivan David	Mendez Gonzalez	ivan.mendez@cos.uni-heidelebrg.de	Evo-Devo of convergent extension in flies
1-5	Vadir / Ann- Kathrin	Lopez- Salmeron / Schürholz	vadir.lopez-salmeron@cos.uni- heidelberg.de / ann- kathrin.schuerholz@cos.uni-heidelberg.de	Dexamethasone-inducible reporter lines for a spatio-temporal cell-type specific expression in <i>A. thaliana</i>
1-6	Marie	Jacobovitz	jacobovitz@stud.uni-heidelberg.de	Uncovering the mechanisms of symbiont phagocytosis and intracellular maintenance using the endosymbiosis model Aiptasia sp.
1-7	Theresa	Schlamp	theresa.schlamp@cos.uni-heidelberg.de	Brillouin Imaging of plant cell wall
1-8	Victor	Jones	victor.jones@cos.uni-heidelberg.de	Unraveling the mechanisms of partner recognition in the coral-algal symbiosis
1-9	Rik	Brugman	rik.brugman@cos.uni-heidelberg.de	Multi-parameter analysis of calcium signal propagation in roots to differentiate signaling mechanisms in response to biotic and abiotic stresses.
1-10	Diana- Patricia	Danciu	dpdanciu@math.uni-heidelberg.de	Defining numbers and functional heterogeneities among stem cells during organ growth
1-11	David	Schiel	david.schiel@cos.uni-heidelberg.de	Cross-talk between the energy-sensing SnRK1 kinase and the cystein synthase complex protein OAS-TL A in the stress response
1-12	Francesca	Caroti	francesca.caroti@cos.uni-heidelberg.de	Study of Megaselia dynamics: yes we can and it looks beautiful!
1-13	Ali	Seleit	ali.seleit@cos.uni-heidelberg.de	Post-embryonic stem cells in Medaka

# Poster session 2

Poster number	First Name	Last Name	Email address	Poster title
2-1	Naima	Ruhland	naima.ruhland@cos.uni- heidelberg.de	Linking mesoderm internalization and ectoderm closure in flies
2-2	Meliha Görkem	Patir Nebioglu	gorkem.patir@cos.uni- heidelberg.de	Two is company, three is a crowd? - Vacuolar proton pumps during cold acclimation.
2-3	Linda	Manhart	linda.manhart@cos.uni- heidelberg.de	Visualization of endogenous protein expression, localization and turnover in single cells
2-4	Aura	Navarro- Quezada	aura.navarro@cos.uni- heidelberg.de	Non-stochastic homoleog gene expression reshuffling shapes defense metabolism strategies to insect herbivory in <i>Nicotiana</i> allopolyploids
2-5	Everardo	González	everardo.gonzalez@cos.uni- heidelberg.de	Artificial Sensitivity: Deep Artificial Neural Networks for 3D Image Segmentation
2-6	Zhenni	Li	zhenni.li@cos.uni-heidelberg.de	The role of cell identity in the response to cell wall perturbation in Arabidopsis thaliana root
2-7	Lucas	Schütz	lucas.schuetz@cos.uni- heidelberg.de	Transient folds as key partners of Germband extension
2-8	Michael	Stitz	michael.stitz@cos.uni- heidelberg.de	Arabidopsis Target of Rapamycin (TOR) acts as gatekeeper for Auxin-dependent Lateral root formation
2-9	Phil-Alan	Gärtig	phil-alan.gaertig@cos.uni- heidelberg.de	Anterograde, trans-synaptic Alk / Jelly Belly signaling allows neurons to sense their environment and induces dendritic branching
2-10	Nina	Tonn	nina.tonn@cos.uni- heidelberg.de	Characterising the regulatory role of SMXL3/4/5 during phloem development in Arabidopsis thaliana
2-11	Viola	Noeske	viola.noeske@cos.uni- heidelberg.de	Two new genes provide evolutionary switch for tall blastoderm cells in flies
2-12	Virginie	Jouannet	virginie.jouannet@cos.uni- heidelberg.de	The transcription factor WOX4 acts as an hormonal hub to control stem cell activity during plant secondary growth.
2-13	Yingxue	Yang		Exploring the role of plant glutamylcysteine ligase (GCL), a redox-sensitive switch in glutathione (GSH) biosynthesis

# Control of plant cell fate transitions by transcriptional and hormonal signals

# Christophe Gaillochet

# Centre for Organismal Studies, University of Heidelberg

Plant meristems carry pools of continuously active stem cells, whose activity is controlled by developmental and environmental signals. After stem cell division, daughter cells that exit the stem cell domain acquire transit amplifying cell identity before they are incorporated into organs and differentiate. In this study, we used an integrated approach to elucidate the role of HECATE (HEC) genes in regulating developmental trajectories of shoot stem cells in Arabidopsis thaliana. Our work reveals that HEC function stabilizes cell fate in distinct zones of the shoot meristem thereby controlling the spatio-temporal dynamics of stem cell differentiation. Importantly, this activity is concomitant with the local modulation of cellular responses to cytokinin and auxin, two key phytohormones regulating cell behaviour. Mechanistically, we show that HEC factors transcriptionally control and physically interact with MONOPTEROS (MP), a key regulator of auxin signalling, thereby modulating the autocatalytic stabilization of auxin signalling.

# Mechanosensitivity: Function of TRP channels and mechanoreception in Hydra

# Bérénice Ziegler

Centre for Organismal Studies, University of Heidelberg

# Function of HOPS/CORVET membrane tethering complexes in microtubule arrangement?

Jana Christin Askani, Falco Krüger and Karin Schumacher

Centre for Organismal Studies, University of Heidelberg

Vacuoles are essential for the proper functionality of plant cells and thereby contributing to the success of land plants. Hence the biogenesis of vacuoles and the endomembrane trafficking towards the vacuole is of general interest. Previous work of our lab identified a Golgi-independent trafficking route from the Endoplasmic Reticulum (ER) directly to the vacuole via provacuoles in meristematic root cells (Viotti and Krüger et al., 2013). This trafficking pathway bypasses also the trans-Golgi network / early endosome (TGN/EE) and multivesicular bodies / late endosomes (MVB/LE).

In order to elucidate this novel trafficking pathway in more detail, we wanted to know whether the HOPS membrane tethering complex described in yeast and mammals is involved in vacuolar membrane fusion of provacuoles and vacuoles in plants.

While investigating the involvement of the HOPS complex in the trafficking towards the vacuole, we also found that the microtubule cytoskeleton is affected when the membrane tethering function of the HOPS complex is impaired. Our current research aims to answer the question whether the HOPS complex might also plays a role in shaping the microtubule cytoskeleton in addition to its function in promoting vacuolar membrane fusion.

# Evo-Devo of convergent extension in flies

# Ivan David Mendez Gonzalez

# Centre for Organismal Studies, University of Heidelberg

The origin and development of morphological characteristics is controlled by developmental gene regulatory networks, and specific changes in these networks can lead to the modification and innovation in morphogenesis. During germband extension in the *Drosophila melanogaster* embryo, cells from the ectoderm converge dorsoventrally and extend anterioposteriorly, causing the germband to more than double its length along its anterior-posterior axis. Convergent extension of the ectoderm depends on the rearrangement of cellular junctions initiated by the expression of the pair-rule genes eve and runt in evenly spaced transvere stripes along the anetrio-to-posterior axis of the embryo. This transcriptional input activates the combinatorial expression of Toll receptors in overlapping transverse stripes and ultimately results in a coordinated tissue level behavior. The conserved expression of eve, runt and Toll receptors genes in different arthropod species and the functional validation of Toll genes during convergent extension in both beetle Tribolium castaneum and the spider Parasteatoda tepidariorum suggest that early embryonic convergent extension is built on an ancient and conserved system in all arthropods. However, different species seem to employ a different number and/or set of Toll receptors to control convergent extension. The morphogenetic consequences of these differences are currently unknown. To address a putative role of the Toll gene divergence in the evolution of convergent extension in insect embryogenesis we are using D. melanogaster as a reference organism and compare its convergent extension during germband elongation with two emergent fly models, Chironomus riparius and Clogmia albipunctata. Using recently developed molecular characterizing the differences in cell tools for non-model species we are rearrangements during convergent extension among these fly species and identifying coinciding genetic differences. With our approach, we aim to identify the evolutionary mechanisms and possible constraints for the functional divergence of this fundamental morphogenetic process.

Dexamethasone-inducible reporter lines for a spatio-temporal cell-type specific expression in *A. thaliana* 

Vadir Lopez Salmeron, Ann-Kathrin Schürholz, Zhenni Li, Thomas Greb and Sebastian Wolf

Center for Organismal Studies (COS). Im Neuenheimer Feld 360, 69120 Heidelberg (Germany).

The introduction of transgenes in the early 80s opened the door to scientists for genetic engineering. The usage of this had substantially contributed to our understanding on the mechanisms that occur during plant development. It has been extensively used generalpurpose constitutively promoters to drive the expression of transgenes. But this often can lead to an out of context functions or for certain genes it could even compromise the plant viability. Therefore, it has been of increasingly importance to study gene function in its natural context by the use of its own promoters. Additionally, gene function can be different depending on its spatial or temporal distribution, for example, interacting partners present only in a specific cell-type or those that require an external cue to be expressed. Therefore, the use of a conditional and cell-type specific control of transgene expression can provide a detailed spatial-temporal resolution to study gene function. The use of the pOp/LhGR system provides a stringent dexamethasone-inducible system for Arabidopsis and has proven to be more reliable than other inducible systems (Craft et. al., 2005). Also, neither dexamethasone nor activated LhGR-N adversely affects Arabidopsis seedling development (Craft et. al., 2005). This system has been proven successfully, for example, to understand that geometric edges of cells represent an additional and important spatial domain, specified by a plant Rab GTPase activity that is essential for morphogenesis (Kirchhelle et. al., 2016) or as tool for a real time genetic manipulation in field studies (Schaefer et. al., 2013). Here we present a toolbox of 31 promoters to study gene function in a spatio-temporal resolution that comprehensibly cover most of Arabidopsis tissues. Stable transgenic lines where generated with a cassette that comprises of a cell-type specific promoter that drives the expression of the LhGR (DRIVER lines) and four or six copies of the pOp (p6xOP) promoter that drive the expression of the mTurquoise2 fluorophore to monitor the spatio-temporal specificity of the induction. With this lines as tools, by introducing a transgene that carries the p6xOP with any gene (EFFECTOR), it can be investigated the gene function or its contribution in a specific tissue to overall plant architecture.

Uncovering the mechanisms of symbiont phagocytosis and intracellular maintenance using the endosymbiosis model Aiptasia sp.

# Marie Jacobovitz

Centre for Organismal Studies Heidelberg, University of Heidelberg

The mutualistic endosymbiosis between cnidarians and unicellular algae of the genus Symbiodinium is essential for the viability and success of coral reefs, which are of tremendous ecological and economic value. The symbiotic algae are intracellularized by the host's endodermal cells via phagocytosis and are maintained in a specialized organelle, the symbiosome. In contrast to other phagocytosed particles, which are typically digested by animal cells, it is known that the symbionts avoid destruction to allow bidirectional nutrient transfer between the partners; however, the underlying mechanisms are unclear. We utilize the marine sea anemone Aiptasia sp. as a model to investigate symbiont phagocytosis and intracellular maintenance. We found that, unlike Symbiodinium spp, algae of the genus Nannochloropsis are phagocytosed by Aiptasia endodermal cells, but are subsequently lost over time. To better understand how symbionts are stably integrated into host cell function, we have taken a comparative approach using Symbiodinium and Nannochloropsis. We are currently developing protocols to compare gene expression in cells containing symbionts to cells containing non-symbiotic algae by single cell transcriptomics. Using confocal microscopy of fixed specimens as well as live imaging, we aim to discern whether non-symbiotic algae are eliminated via digestion or expulsion by the host. Furthermore, we will use various phagolysosomal markers to compare the molecular composition of a symbiosome to the Nannochloropsis-containing phagolysosome. Finally, we plan to test whether or not symbiotic and non-symbiotic algae affect host physiology and thus, long-term survival, by providing nutrition to the host. Our comparative analysis will allow us to examine symbiosis establishment and maintenance from the organismal and cellular levels down to the molecular mechanisms underlying these processes. We expect to provide novel contributions to the field, which motivates research efforts in understanding this complex and delicate relationship that allows coral reef ecosystems to thrive.

# Brillouin Imaging of plant cell wall

**Theresa Schlamp**<sup>1</sup>, Kareem Elsayad<sup>2</sup>, Stephanie Werner<sup>3</sup>, Youssef Belkhadir<sup>3</sup>, Thomas Greb<sup>1</sup>

<sup>1</sup>Centre for Organismal Studies (COS), Heidelberg University, Heidelberg, Germany <sup>2</sup>CSF, Vienna BioCenter (VBC), Vienna, Austria 3Gregor Mendel Institute, Vienna Biocenter, Vienna, Austria

Unlike in animals, the extracellular matrix of plants is made of a rigid cell wall that fixes the position of plant cells relative to each other. The presence of this cell wall makes the cells susceptible to mechanical forces. Cell wall remodeling is an essential aspect of plant growth and results from internal and external stimuli. In this context, lateral plant growth is a remarkable process. Lateral growth expands the plant body laterally in a strictly postembryonic growth process which is based on the activity of a lateral stem cell niche, called the cambium. However the sequence of events that contribute to plant cell wall remodeling during cambium development remains unknown. One of the reasons is the lack of methods to monitor and follow mechanical properties of biological structures. Monitoring these properties will help us to decipher what changes in cellular mechanics occur during lateral growth. To be able to access mechanics on a subcellular level, we have initiated a project to combine the use of the Brillouin light scattering imaging with the study of lateral growth in Arabidopsis thaliana. Brillouin imaging is a non-invasive microscopy method that allows us to detect mechanical properties in a certain depth of the investigated tissues. As a proof of principle, this method has revealed that cell walls with different orientation are showing also different elasticity moduli. With the help of this innovative technique we will be able to gather information about the change of cell wall mechanical properties during developmental processes in plants.

# Unraveling the mechanisms of partner recognition in the coral-algal symbiosis

# Victor Jones

Centre for Organismal Studies, University of Heidelberg

The productivity and biodiversity of tropical reefs is underpinned by the growth of corals that rely for survival on an obligate symbiosis with intracellular photosynthetic symbionts, dinoflagellate algae of the genus Symbiodinium, that transfer fixed carbon to the host. Host larvae ingest symbionts, which are phagocytosed by endodermal cells lining the gastric cavity and proliferate throughout the endoderm.

In order to dissect the processes that underlie this symbiosis we are developing the larva of the sea anemone Aiptasia as a tractable laboratory model. To allow functional molecular studies to be undertaken we have established protocols for the controlled production of gametes, in vitro fertilization, and protein and mRNA microinjection. I am currently working to develop approaches for transient expression and transgenesis.

In future work I aim to elucidate the first steps in the establishment of a stable endosymbiosis, identifying molecular mechanisms by which Symbiodinium cells are recognized and phagocytosed by cnidarian endodermal cells, and what symbiontderived molecules are recognized. To find the host cells that take up algae I plan to use the transcriptomes of single endodermal cells (sequencing to be done by Philipp Voss) to classify larval endodermal cell types, map them in intact larvae, determine which are associated with symbiont uptake, and identify candidate host receptors that mediate phagocytosis, to be tested in later functional studies. In parallel I am developing a phagocytosis assay to test the ability of algal cell wall components to enhance the uptake of coated microbeads. Preliminary results indicate that cell wall proteins from a compatible Symbiodinium strain promote phagocytosis; in future experiments I plan to test for differences between compatible and incompatible strains, and to identify active fractions from the total protein extract with the aim of finding algal ligands recognized by the host cell during phagocytosis. These experiments should provide the first detailed molecular insights into the mechanisms that initiate this ecologically crucial endosymbiosis.

# Defining numbers and functional heterogeneities among stem cells during organ growth

**Diana-Patricia Danciu**, Julian Stolper, Elizabeth Ambrosio, Lázaro Centanin, Anna Marciniak-Czochra

Centre for Organismal Studies, University of Heidelberg

Determining stem cell numbers during organ growth in organ-specific biological systems has proven very difficult to tackle, not only due to technical issues but also to heterogeneity within the systems and to absence of structural organization.

The model system proposed for approaching the question of "How many stem cells are needed to build an organ?" is a quantifiable, modular organ - the respiratory gill of the Medaka fish, which due to its stereotypic spatio-temporal organization is accessible to study towards our goal.

Following clones via lineage tracing, we formulated the hypothesis that few stem cells coordinating their division are sufficient to give rise to such a "module". Furthermore, the need for functional heterogeneity among the stem cells was established. Subsequently, with the help of stochastic simulations and parameter estimation, the hypothesis was confirmed, and in addition heterogeneity in the division rates of stem cells were quantified.

In conclusion, using lineage tracing and mathematical modeling of a novel modular spatio-temporally structured model, we proved that a small number of functionally heterogeneous active stem cells is sufficient for building and maintaining an organ such as the Medaka fish gill.

Cross-talk between the energy-sensing SnRK1 kinase and the cystein synthase complex protein OAS-TL A in the stress response

**David Schiel** 

Centre for Organismal Studies, University of Heidelberg

Sulfur represents a vital macronutrient for all living organisms since it is a constituent of the two proteinogenic amino acids cysteine and methionine. Primary metabolism of sulfur in plants centers on the assimilation of reduced sulfate (sulfide) that is ultimately incorporated into cysteine. Regulated uptake of sulfate is implemented by high and low affinity sulfate transporters (Sultr) that are located in the plasmalemma of root cells. Reduction of sulfate is restricted to the plastids of root and leaf cells whereas cysteine biosynthesis occurs in the plastids, cytosol and mitochondria of plant cells. Generation of cysteine is catalyzed by the hetero-oligomeric cystein synthase complex (CSC) that is formed reversibly by serine-acteyltransferase (SAT) hexamers and O-acetylserine (thiol)-lyase (OAS-TL) dimers. Remarkably, cellular sulfur homeostasis is regulated by the CSC itself. In my PhD project I want to further elucidate the role of OASTL-A and SAT5 as regulatory proteins in the sulfur metabolism pathway and beyond. Especially, a new link has been found between the Sucrose non-fermenting kinase 1 (SnRK1) which is a master regulator in the sugar starvation response and CSC signalling.

# Study of Megaselia dynamics: yes we can and it looks beautiful!

F. Caroti, E. Gonzalez, P. Gonzalez, D. Kromm, S. Lemke

Centre for Organismal Studies, University of Heidelberg

Embryonic development in many animals is protected and assisted by extraembryonic epithelia, which aid building the embryo without itself becoming a part of it. In most insects, extraembryonic epithelia include two distinct tissues, the serosa and the amnion. Despite their importance, extraembryonic development is significantly reduced in a subgroup of insects, including the fruit fly Drosophila melanogaster. In D. *melanogaster* only a single tissue, the amnioserosa, takes over all extraembryonic tasks in embryonic development. How exactly the extraembryonic morphology changed from the ancestral two-tissues topology to the single-tissue topology known from D. melanogaster, and how exactly the amnioserosa is related to and fulfills functions of both amnion and serosa, is still under debate. Here we characterize amnion and serosa of the scuttle fly *Megaselia abdita* and compare it to the amnioserosa of D. melanogaster. Our quantitative analyses of cell and tissue dynamics using in toto timelapse recordings of wildtype and RNAi embryos, allow us to identify features of these tissues and distinguish them without cell fate markers. Throughout its development, we find that distinct regions of the amnioserosa behave and function similar as the serosa and amnion in *M. abdita*, raising the possibility that the amnioserosa has remained characters of both extraembryonic tissue types and evolved by a loss of amnion/serosa disjunction. Consistent with this interpretation, we find that modulation of cell-ECM adhesion affect serosa detachment in *M. abdita*, giving rise to an amnioserosa-like morphology.

# Post-embryonic stem cells in Medaka

# Ali Seleit

Centre for Organismal Studies, University of Heidelberg

Adult stem cells have been discovered in a variety of different organs in vertebrates. They are responsible for maintaining organ homeostasis and in cases of injury have been shown to contribute to the regenerative response with different efficacies depending on species, age and tissue composition. Adult stem cells are invariably located in specialized compartments termed niches. Stem cell niches have been shown to play critical roles in regulating and maintaining stem cell function in different tissues. It is, however, unclear whether the niche is a preformed structure which stem cells lodge into or whether stem cells can induce the de novo formation of their own niches. Here we make use of the morphogenesis of neuromast organs during the embryonic development and post-embryonic growth of Medaka fish to answer this question. Using a combination of novel tissue specific transgenic lines, long-term live-imaging and 2-photon laser ablations we reveal the location and action of neuromast stem cells under homeostatic and regenerative conditions. In addition, we report that these neural stem cells are able to induce a stable fate change in the surrounding epithelium, demonstrating the in vivo formation of a life-long stem cell niche in a vertebrate model.

# Linking mesoderm internalization and ectoderm closure in flies

Naima Ruhland, Paula González, Steffen Lemke

Centre for Organismal Studies, University of Heidelberg

Mesoderm formation is one the first morphogenetic processes during fly gastrulation, and it coordinates two epithelial transformations. One the one hand, the future mesoderm is brought into the inside of the embryo, on the other hand the adjacent ectoderm fuses after mesoderm internalization and thus closes the hole left by the internalizing mesoderm cells on the ventral side of the embryo.

Work in our lab has shown that the process of mesoderm formation can be achieved by very distinct modi. In mosquitoes and midges like *Chironomus riparius* (*C. riparius*), the presumptive mesodermal cells move inwards in an individual, stochastic manner (ingression). Similar modes of mesoderm internalization have been observed in beetles and other insects, and it is considered to represent the ancestral mode of mesoderm formation in arthropods. During the course of fly evolution, this process has been optimized to a coordinated, coherent tissue movement (invagination), as can be seen in the fruit fly Drosophila melanogaster. Our lab could previously show that the switch between ingression and invagination can be facilitated by a simple change in expression levels of two factors, Fog and T48. In fog overexpressing *C. riparius* embryos, mesodermal cells get internalized in a process resembling the tissue wide movement in D. melanogaster.

The coordinated cell behavior has been shown to speed up and increase robustness of mesoderm internalization. It is currently unclear, however, how changes in the mode of mesoderm internalization have affected associated morphogenetic processes such as ectoderm fusion and hole closing. For the ectoderm to close properly, force needs to be built up to drive the two lateral sides of the embryo towards each other. In particular, it is unclear whether the ectoderm pushes towards the ventral midline, or whether the mesoderm is pulling the ectoderm towards each other. By taking advantage of *C. riparius* as experimental system in which the two possibilities can be genetically induced and tested, I aim at identifying the genetic basis of ventral closure and how it was affected by the evolution of coordinated mesoderm invagination.

# Meliha Görkem Patir Nebioglu

Centre for Organismal Studies, University of Heidelberg

Properly functioning vacuole is a prerequisite for the plant to survive in a constantly changing environment and to optimize its cellular metabolism. All vacuolar functions require massive fluxes of ions and metabolites, most of the time against their concentration gradient, which require constant energy supply. Two tonoplast localized proton pumps; V-ATPase and V-PPase, fulfill this task by creating a proton motive force across the vacuolar membrane[1]. Although the V-ATPase is the main proton pump under normal conditions, evidence suggests that the V-PPase can become the major pump depending on the developmental stage and proton environmental conditions[2],[3],[4]. Our objective is to determine how much each proton pump contributes to the acidification of vacuole and to find out whether there is a cooparation between them under normal and cold conditions. For that purpose we used two V-PPase mutants (vhp1-1, fugu5-1), one V-PPase overexpression line (UBQ:AVP1) and one V-ATPase mutant (vha-a2 vha-a3) as well as wild type (Col-0) of Arabidopsis thaliana. We could point out the importance of V-PPase as a proton pump in stress conditions by showing that V-PPase overexpression lines outperform the wild type upon cold exposure and reveal possible crosstalk between two proton pumps. Lastly, we investigate the action of a P-type ATPase (Autoinhibited H+-ATPase isoform 10 (AHA10)), which has been shown to be residing on the tonoplast [5]. Normally, AHA10 expression is confined to seed coat where its action is important for filling the central vacuole with proanthocyanidins [5],[6] Since, our aim is to explore whether it can complement the defects in vha-a2 vha-a3 phenotype and/or vacuolar acidification, we had created overexpression lines. With that in hand, we can overcome the expression limitations and clarify its functionality. Moreover, this lines may reveal defects of vha-a2 vha-a3 that are unrelated to vacuolar acidification which can in turn help in understanding functions of V-ATPase other than proton pumping.

Visualization of endogenous protein expression, localization and turnover in single cells

Linda-Joel Manhart<sup>1</sup>, Aaron Ostrovsky<sup>1</sup>, Astrid Petzold<sup>2</sup>, Tatjana Kovacevic<sup>1</sup>, Sebastian Cachero<sup>3</sup>, Stephan Sigrist<sup>2</sup>, Jan Felix viola

<sup>1</sup>University of Heidelberg, Centre for Organismal Studies, Im Neuenheimer Feld 230, 69120 Heidelberg, Germany <sup>2</sup>Neurogenetik, Institut für Biologie, Freie Universität Berlin, 14195 Berlin, Germany<sup>3</sup>Neurobiology Division, MRC Laboratory of Molecular Biology, Cambridge CB2 0QH, UK

The formation of the nervous system is a complex process where neurons make connections with a number of partners in order to integrate sensory input and compute it into a behavioural output. Contacts between neurons are synapses, where many proteins from pre- and postsynaptic cells appose each other and orchestrate communication between the partners. We are interested in synapses, how they form and how they are fine-tuned during animal life. To study synapse development, we visualize synaptic proteins using acutely dissected preparations and antibody stainings to determine their characteristics. One powerful technique for visualizing proteins is overexpression of tagged isoforms. Unfortunately, overexpression of synaptic proteins has been shown to induce artefacts in protein trafficking and the development of normal synapses. We therefore developed the dFLEx technique to conditionally label endogenous proteins in single cells, thereby avoiding introducing extra copies of our proteins of interest. We use the dFLEx label to investigate protein synthesis and turnover of the presynaptic protein Bruchpilot (Brp) in the intact nervous system. We show that the amount of Brp at individual presynaptic sites increases in the first two days of larval development until it reaches a steady state. Through pulse-chase experiments we determined the lifetime of Brp as approximately three days. To investigate if synaptic protein levels can be regulated locally at the synapse, we used fluorescent in situ hybridization and determined the localization of Brp transcripts. Here, we show that Brp mRNA is present in the synaptic neuropil and not only in the cortex. Additionally, transcripts accumulate in nerves close to neuromuscular junctions (NMJs), where Brp protein accumulations can be observed in close proximity. This suggests that Brp proteins are locally translated, providing a mechanism by which synaptic composition can be locally regulated. Together, we provide the first in vivo analysis of mRNA and protein localization and lifetime, and suggest that local translation of Brp might be a mechanism by which synapses are modified during development and maintenance of the nervous system.

# Non-stochastic homoleog gene expression reshuffling shapes defense metabolism strategies to insect herbivory in *Nicotiana* allopolyploids

A. Navarro-Quezada, D. Scherer, T. Naake and E. Gaquerel.

Centre for Organismal Studies, University of Heidelberg

Genome doubling, as well as rearrangements due to unequal crossing-over and retrotransposition following allopolyploidy events in plants can have important effects in gene expression and in turn affect the fitness of these organisms. We analyzed complete transcriptomes and metabolomes of five artificially generated (Nxobtusiata)and two naturally extant allopolyploids (N. clevelandii and N. quadrivalvis) stemming from two distantly related Nicotiana species (N. attenuata and N. obtusifolia). We examine the transcriptome and metabolome of allopolyploid plants treated with herbivore oral secretions of Manduca sexta, one of the main naturally occurring herbivores of these plant species. We were able to quantify parental allele specific changes in the transcribed genes of the allopolyploids involved in volatile and diterpeneglycoside (DTG) biosynthesis which correlated with directional changes of defensive pathways responsive after simulated herbivory. We notably detected different levels of expression-level dominance of the parental genomes (*N. attenuata* and *N. obtusifolia*) and found that essential reshufflings of specialized metabolism pathways (e.g. antidigestive toxins, volatile organic compounds increasing herbivore apparency to predators) modulated the overall defense inducibility by herbivory but also lead to the emergence of metabolic innovations, in particular in the terpenoids and DTG profiles.

# Artificial Sensitivity: Deep Artificial Neural Networks for 3D Image Segmentation

# Everardo González

Centre for Organismal Studies, University of Heidelberg

Artificial neural networks loosely simulate the process of sensitivity and cognition displayed by a biological brain. Here I present the application of a neural network architecture known as a multi-layer perceptron to image segmentation in selective plane illumination microscopy imagery; a heuristic approach to problem whose solution is otherwise difficult to express in computer or mathematical language.

The role of cell identity in the response to cell wall perturbation in Arabidopsis thaliana root

Zhenni Lia and Sebastian Wolfa

<sup>a</sup>Department of Developmental Plant Biology, Center for Organismal Studies, University of Heidelberg, Germany

The root is essential for plant anchoring in the soil and for the transport of nutrients and organic compounds to ensure normal plant growth. Root growth depends on tight coordination between different tissues, which involves cell elongation, proliferation and differentiation. These processes require constant cell wall biosynthesis and remodeling throughout the plant life cycle. Therefore, close surveillance of the cell wall status is essential to ensure the regular growth of the root. By using Arabidopsis thaliana seedling primary root as research model, previous studies in our lab allowed the identification of a novel signaling pathway that ensures cell wall homeostasis by monitoring the state of pectin in the cell wall and regulate root growth through receptor like protein 44 (RLP44)mediated activation of the brassinosteroid (BR) signaling pathway. We then triggered the perturbation of cell wall with mis-expression of pectin methylesterase inhibitor 5 (PMEI5) driven by a set of tissue/cell type-specific promoters in an inducible system, which allowed the investigation of the contribution of tissue/cell identity to the cell wall surveillance and the tissue/cell type-level influence of cell wall status on root development. By following responses on cellular, tissue and organ level, we observed that triggering cell wall perturbation in trichoblast cells, xylem pole pericycle cells and vasculature had the same organ morphogenesis outcome such as primary root waving. Moreover, perturbing cell wall in the cortex caused abnormal cell division and tissue organization per se and in neighboring tissues, which suggested the existence of both cell- and non cell-autonomous response. Interestingly, cell wall perturbation triggered in the cortex also resulted in a disrupted stem cell niche organization and the lost of quiescent center identity. These phenotypes did not seem to only result from the same RLP44- and BRI1-dependant regulatory mechanism, which raised the possibility that other unknown cell wall-mediated signaling pathways might also be in play.

# Transient folds as key partners of Germband extension

# Lucas Schütz

Centre for Organismal Studies, University of Heidelberg

Drosophila gastrulation is a unique concerted action of several genetically and mechanically controlled processes like ventral furrow invagination, cephalic furrow formation, germband extension, anterior and posterior midgut formation, as well as dorsal fold formation. As the embryo is a confined environment having an ellipsoidal shape where space is limited and mechanical interactions are likely between different processes, they have to be closely coordinated and synchronized to each other. Except for two processes, all of these processes contribute to tissue specification. However, the cephalic furrow and the dorsal folds do not; they invaginate during gastrulation, but the invaginated tissue unfolds during later development. Therefore two questions arise, first, why tissue invaginates when it unfolds later anyways and second, why these two invagination processes take place at a time when the embryo is involved in several other processes. I hypothesize that these furrows are needed to enable and direct germband extension in a coordinated fashion. As germband extension is one of the fastest processes, involving a few thousand cells, but not the head region it likely requires a tight control. My results suggest that part of this control is taken over by the cephalic furrow which seems to direct germband extension in posterior direction, and by the dorsal folds which seem to enhance straight germband extension. During the initial phase of germband elongation the extension seems to be almost equal in anterior and posterior direction. However anterior of the germband is the cephalic furrow, which is then pushed further anterior, but at the same time brakes the anterior extension of the germband until it comes to a full stop and extension continues only in posterior direction around the posterior pole. In contrast to the cephalic furrow which invaginates before germband extension to be ready for its blocking function, the dorsal folds invaginate during germband extension exerting their function likely by trafficking the invaginating cells from the dorsal midline towards the lateral sides. The underlying mechanism is possibly the reduction of mechanical resistance for the moving germband as cells on the dorsal midline are trafficked to the side instead of being cramped dorsally. However, I assume that this mechanism is only necessary as the mechanism of the cephalic furrow probably enables germband extension at high speeds, where the surface of the embryo is not big enough anymore to move cells fast enough from dorsal to lateral to make room for the germband. A lack of dorsal folds or small dorsal folds might result in a jammed dorsal epithelium. However as the germband is constantly propelled, it hast to find a way even if its tip is stuck which could explain the observed twists.

# Arabidopsis Target of Rapamycin (TOR) acts as gatekeeper for Auxin-dependent Lateral root formation

# **Michael Stitz**

Centre for Organismal Studies, University of Heidelberg

Root branching by formation of lateral roots is a developmental process ultimately required for the plants adaptation to its ever changing environment. As such, lateral root formation is intimately linked to the plant metabolism and most particularly to photosynthesis-derived sugars. The purpose of the current study is to investigate, whether root branching is gated by a defined metabolic state integrating shoot and root metabolic activities. Using Arabidopsis wild type and signaling mutants and by applying targeted and untargeted metabolomics, revealed a substantial up-regulation of various compounds of the plants energy metabolism only occurring during lateral root formation. We further aim to identify possible mechanistic key factors integrating the energetic state of the plant and the molecular program for lateral root initiation. In our initial screens, we further observed that plants with inducible knock-down for TARGET OF RAPAMYCIN (TOR) are incapable of forming lateral roots. The protein kinase TOR is a central sensor of cell growth in response to nutrients, the energy status, and growth factors and is highly conserved from humans to yeasts and plants. RT-g-PCR measurements revealed substantial down-regulation of lateral root specific transcripts, indicating that TOR indeed may act as gate-keeper to integrate the energetic state of the plant into the developmentally regulated formation of lateral roots. Further RNAseg and metabolomics studies will help to reveal the mechanisms by which TOR regulates lateral root formation.

Anterograde, trans-synaptic Alk / Jelly Belly signaling allows neurons to sense their environment and induces dendritic branching

**Phil-Alan Gärtig**<sup>1</sup>, Aaron Ostrovsky<sup>1</sup>, Steffen Schmelzeisen<sup>1</sup>, Tatjana Kovacevic<sup>1</sup>, Barbara Chwalla<sup>2</sup>, Matthias Landgraf<sup>2</sup>, Jan Felix Evers<sup>1</sup>

<sup>1</sup>Centre for Organismal Studies, Ruprecht-Karls-Universität Heidelberg, 69120 Heidelberg, Germany <sup>2</sup>Dept of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, United Kingdom

How does a neuron establish proper morphology and connectivity in the densely packed neuropil of the central nervous system? What signals are sensed by a developing dendritic arbor to help regulate synaptogenesis and dendritic branching?

With our work, we address these questions by looking at identified synaptic partners in the motor neuropil of Drosophila larvae. Here we show that jelly belly (Jeb) is secreted at the presynaptic axonal terminal, and received by the postsynaptic receptor anaplastic lymphoma kinase (Alk), acting as a trans-synaptic regulator of neuronal growth.

Using intra-vital time lapse imaging we find that normal postsynaptic growth is characterized by exploratory extension and retraction in both embryonic and postembryonic development. Loss of Alk signaling in single cells results in reduced formation of new postsynaptic protrusions, and an increased branch stability.

Interestingly, removal of Jeb from all neurons in the CNS leads to the opposite effect, an increased number of filopodia at both postsynaptic dendrites and along presynaptic axons. Thus, we believe that Alk/Jeb allows competition between postsynaptic cells for synaptic partners by promoting the stability of connections through local feedback mechanisms.

In summary, our work demonstrates Jeb/Alk signaling as trans-synaptic regulator of neuronal growth, integrating synaptic maturation and morphological development in both pre- and postsynaptic neurons.

# Characterising the regulatory role of SMXL3/4/5 during phloem development in

# Arabidopsis thaliana

# Nina Tonn

Centre for Organismal Studies, University of Heidelberg

Members of the SUPPRESSOR OF MAX2 1 (SMAX1)-LIKE (SMXL) gene family are involved in a diversity of plant growth processes including germination, hypocotyl growth, and shoot branching. Until now, function and mode of action of sub-clade 2 members, SMXL3, 4, and 5, had been undescribed. Only recent findings in our group have identified SMXL3/4/5 as central, functionally redundant promoters of phloem formation in Arabidopsis thaliana (Wallner et al. 2017). The phloem is a vascular tissue providing long-distance transport of photo-assimilates and signalling molecules from source to sink tissues via sieve tubes built by differentiated sieve elements (SEs). In wild-type plants, SMXL3/4/5 promoters are active in SE-procambium stem cells as well as maturing phloem strands and parallelly running stem cell-like procambium strands. Loss of two members results in severely impaired phloem formation and a substantially shorter primary root including decreased root apical meristem (RAM) size. In double mutants of SMXL3/4/5 genes, transition into differentiated SEs is delayed, and numerous adventitious roots are developed. However, whether SMXL3/4/5 genes promote phloem formation by regulating gene transcription or protein activity is still unknown. Furthermore, little research has been done on potential interaction partners of SMXL3/4/5 proteins, nor on genes functionally related to SMXL3/4/5 during phloem formation. Taken together, the goal of my PhD project is to characterise the regulatory role of SMXL3/4/5 during phloem development in A. thaliana.

# Two new genes provide evolutionary switch for tall blastoderm cells in flies

Viola Noeske, Paula González, Steffen Lemke

Centre for Organismal Studies, University of Heidelberg

In insects, the fertilized egg undergoes a series of rapid nuclear divisions to amplify genetic material. These nuclei migrate to the periphery of the and, briefly before gastrulation, the cell membrane invaginates between the nuclei and a cellular blastoderm is formed. This process of cellularization has been extensively studied in *Drosophila melanogaster*, and it results in a thick columnar blastoderm. This thick blastoderm is not found among all insects and it seems that it is a relatively new feature found only in a subset of flies that comprises Drosophila and close relatives. Here we describe the process of cellularization in the midge *Chironomus riparius*, which exhibits a thin blastoderm of cuboidal cells, similar to the blastoderm of most other insects. To determine the nature of the thick blastoderm, we performed a whole-genome comparison and identified two genes, slam and dunk, that are critical for *D. melanogaster* cellularization but are absent from the genome of *C. riparius*.

These genes are important to ensure the fully emergence of the furrow canal and thus the full cell length in blastoderm cells in D. melanogaster. Both genes are known to recruit myosin to the invaginating membrane and regulate the actomyosin network. We find that early expression of either or both Drosophila genes in *C. riparius* is sufficient to invoke Drosophila-like cell elongation in *C. riparius*, suggesting that slam and dunk act as switch from cuboidal to columnar blastoderm. We propose that the two new genes were quickly incorporated into cellular blastoderm formation because their ability to recruit actin and myosin to the furrow canal provided a bona fide add-on to an ancient developmental program.

The transcription factor WOX4 acts as an hormonal hub to control stem cell activity during plant secondary growth.

Virginie Jouannet, Stephanie Suer, Thomas Greb

Centre for Organismal Studies, University of Heidelberg

Growth of multicellular organisms is a highly coordinated process. During their lifetime, plants undergo two phases of growth that can be sequential or simultaneous. Primary growth expands the plant body longitudinally. In woody plants, secondary growth arises from cell divisions in a lateral meristem, called the cambium. The activity of this stem cell niche enlarges the plant girth in stems and roots through the production of vascular tissues which provide support for the shoot system in addition to transport capacities for water and nutrients. But what controls stem cell activity in the cambium? Recently, the homeobox transcription factor WUSCHEL-RELATED HOMEOBOX4 (WOX4) has been identified as a key regulator in promoting cambium activity. WOX4, specifically expressed in cambium-related stem cells, is independently regulated by the PXY/CLE41/44 signaling module and the plant hormone auxin, placing WOX4 at the convergence point of different pathways essential for cambium regulation.

Due to the central role of WOX4 and the lack of knowledge concerning the WOX4downstream regulatory network, we have investigated how WOX4 activity is translated into cambium proliferation. By characterizing the WOX4-dependent regulatory network in the plant model *Arabidopsis thaliana* we revealed that WOX4 differentially regulates distinct hormonal signaling pathways to maintain the stem cell character. Among the hormone signaling pathways regulated by WOX4, the brassinosteroid (BR)-signaling pathway is negatively regulated while in contrast, WOX4 stimulates the strigolactone (SL)-signaling pathway. Consistently, BRs act as negative regulators of cambium activity, which is in line with their general role in promoting cell elongation and differentiation. Additionally, SL-signaling stimulates cambium activity in a cellautonomous fashion in a WOX4-dependent manner. All in all, our findings demonstrate that a single transcription factor, WOX4, functions as a central hub to maintain stem cell activity by fundamentally shifting hormonal signaling in cambium stem cells.

Exploring the role of plant glutamylcysteine ligase (GCL), a redox-sensitive switch in glutathione (GSH) biosynthesis

Yingxue Yang

Centre for Organismal Studies, University of Heidelberg

Glutathione is an universal component involved in various molecular metabolism and stress responses. It acts as an antioxidant against reactive oxygen species and detoxify heavy metals and other stress factors. My project focuses on the entry enzyme for glutathione biosynthesis, the redox-sensitive glutamylcysteine ligase (GCL). The crystallization analysis of GCL from Brassica juncea indicates that there are two disulfide bond (named CC1 and CC2) within the GCL structure. CC2 is more crucial for redox regulation than CC1. Formation of intramolecular disulfide bond followed by homodimerization is unique to redox-mediated activation of plant GCL. Reducing agents disconnect GCL dimer interface, changing the conformation from dimer to monomer with reduced enzyme activity. Except the existing knowledge of GCL regulation, it is still unclear whether forming intramolecular disulfide bond is sufficient for GCL enzyme activation or the subsequent homodimerization is also a necessary step for activation. To answer this question, amino acid residues forming the dimer interface are mutated in order to disrupt the dimerization and redox control, then we biochemically characterize the recombinant GCL activities and dimer/monomer profiles are monitored by FPLC. In summary, this project aim to elucidate the functional significance the GCL enzyme.