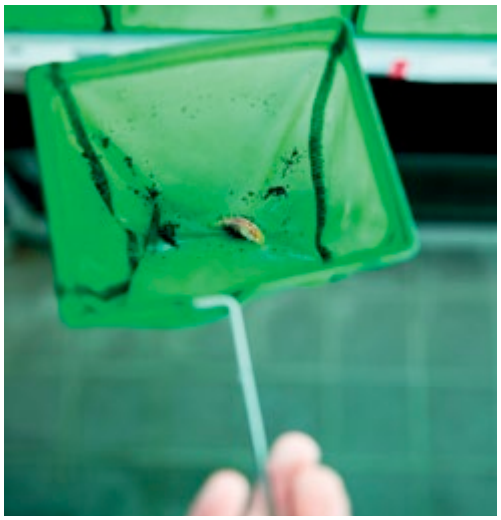
Centre for Organismal Studies Heidelberg

UNIVERSITÄT HEIDELBERG
ZUKUNFT SEIT 1386

REPORT 2014-2017








CONTENT

INTRO-DUCTION

1

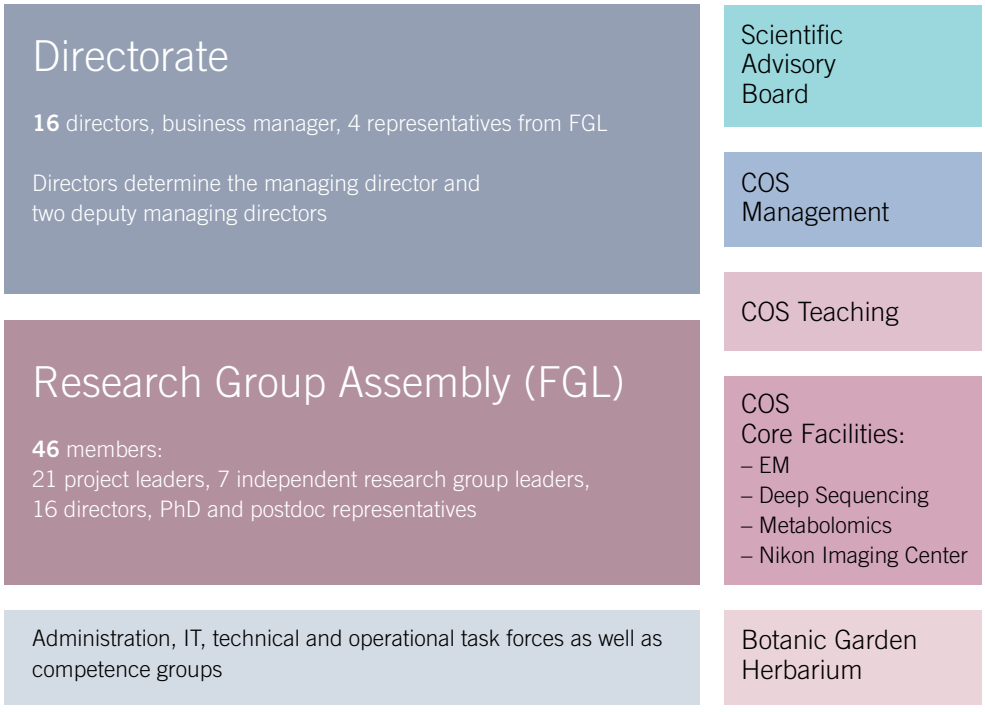
1.1 PREFACE

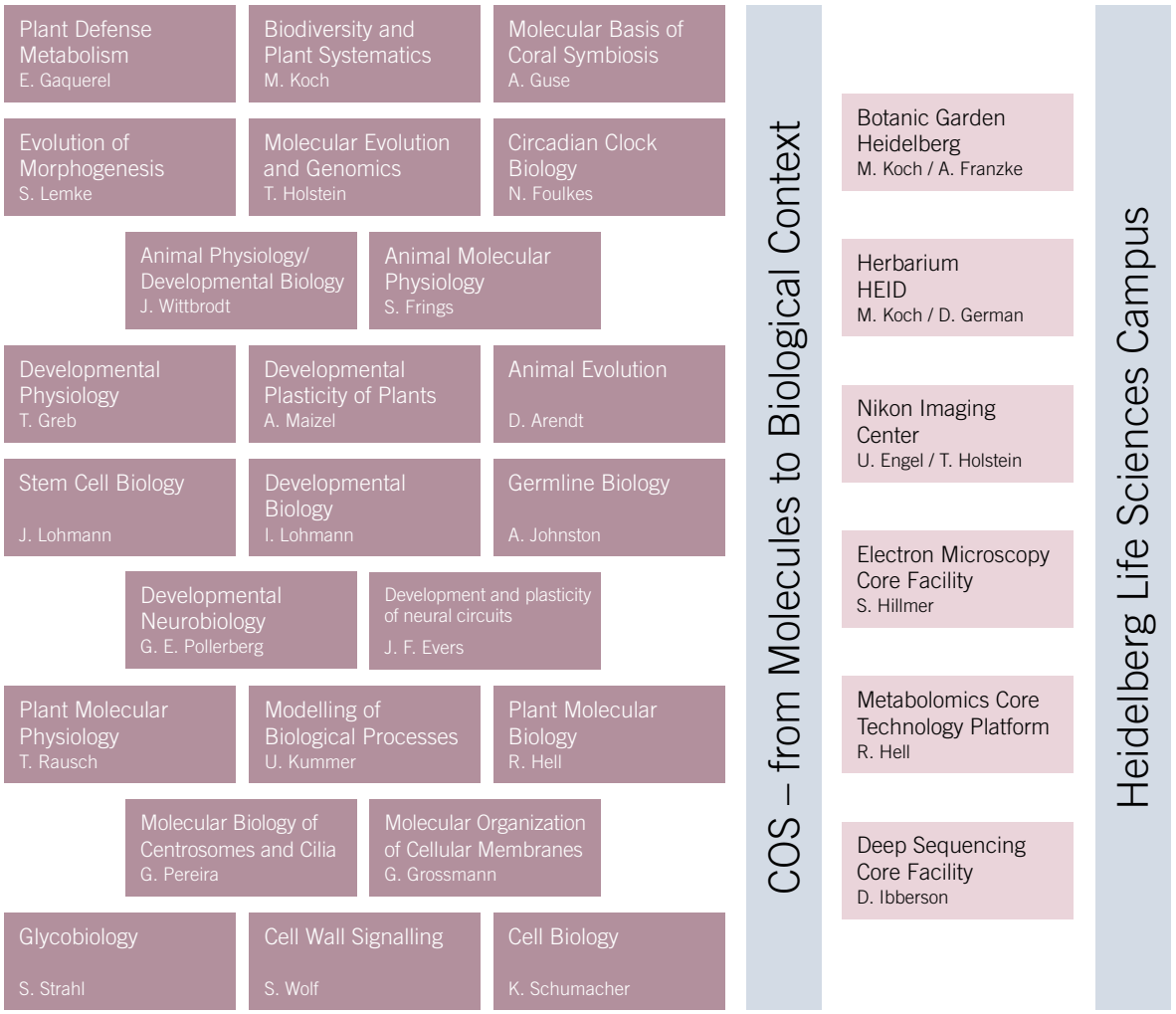
The Centre for Organismal Studies (COS) was established in 2010 with the goal to revive organismal biology by bridging the gap between molecules and living systems. Research at COS thus aims at a mechanistic and causal understanding of biological processes at all scales, from molecules and cells to organs and whole organisms across kingdoms and in the context of their native environments. With this second report covering the years 2014-2017 we hope to convince you that COS has lived up to the expectations. COS does not only provide an excellent platform for research and teaching in organismal biology, it has created synergies and interactions across campus and has thus established itself as a major player in the Heidelberg Life Sciences.

1.2 DEVELOPMENT AND STRUCTURE OF COS

Since COS was founded by a merger of the former Heidelberg Institutes for Plant Science (HIP) and Zoology (HIZ) it has grown substantially and with a total of 24 independent research groups has reached, given the currently available space, full capacity. The senior faculty of COS consists of 16 permanent professorships beloning to the Faculty of Biosciences. Each professor serves as head of a department (director) that can consist of several research groups. In addition to the permanent faculty, independent junior groups are a key element of our strategy to create a dynamic and innovative research environment.

So far COS has recruited 10 young scientists funded among others by the Emmy Noether program of the DFG (Annika Guse, Amal Johnston, Steffen Lemke, Sebastian Wolf), the Chica and Heinz Schaller foundation (Alexis Maizel) or the Excellence Initiative (Emmanuel Gaquerel, Guido Grossmann). Our junior faculty has significantly contributed to the development of COS and in turn COS has made substantial efforts to support their scientific careers. In the reporting period, COS has made a clear commitment to provide a two-year extension of the group leader position in addition to the usual five-year tenure of junior programs and thus support the successful transition to a professorship or any other permanent research position. Exceeding our own standards, we were able to provide non-permanent professorships for a maximum of six years for Alexis Maizel (2015), and Steffen Lemke (2017) with generous help from the University. In addition to providing an excellent environment for junior group leaders, COS has attracted two outstanding mid-career scientists via the DFG sponsored Heisenberg program. Importantly, COS was able to secure a permanent career perspective in both cases. Thomas Greb will take over the W3 position of Thomas Rausch after his retirement in 2020, the W3 professorship for Gislene Perreira is provided by the ZMBH and Heidelberg University to create a bridge between COS and ZMBH and to further promote the interactions within the Heidelberg Life Sciences.




Plant Defense Metabolism
E. Gaquerel
Biodiversity and Plant Systematics
M. Koch
Molecular Basis of Coral Symbiosis
A. Guse
Evolution of Morphogenesis
S. Lemke
Molecular Evolution and Genomics
T. Holstein
Circadian Clock Biology
N. Foulkes
Animal Physiology/ Developmental Biology
J. Wittbrodt
Animal Molecular Physiology
S. Frings
Developmental Physiology
T. Greb
Developmental Plasticity of Plants
A. Maizel
Animal Evolution
D. Arendt
Stem Cell Biology
J. Lohmann
Developmental Biology
I. Lohmann
Germline Biology
A. Johnston
Developmental Neurobiology
G. E. Pollerberg
Development and plasticity of neural circuits
J. F. Evers
Plant Molecular Physiology
T. Rausch
Modelling of Biological Processes
U. Kummer
Plant Molecular Biology
R. Hell
Molecular Biology of Centrosomes and Cilia
G. Pereira
Molecular Organization of Cellular Membranes
G. Grossmann
Glycobiology
S. Strahl
Cell Wall Signalling
S. Wolf
Cell Biology
K. Schumacher
COS – from Molecules to Biological Context
Botanic Garden Heidelberg
M. Koch / A. Franzke
Herbarium HEID
M. Koch / D. German
Nikon Imaging Center
U. Engel / T. Holstein
Electron Microscopy Core Facility
S. Hillmer
Metabolomics Core Technology Platform
R. Hell
Deep Sequencing Core Facility
D. Ibberson
Heidelberg Life Sciences Campus

1.3 RESEARCH AT COS

With its more than 50 PIs COS covers a wide range of excellent and innovative research leading to highly recognized and cited publications in top tier journals (e. g. Cell, Cell Stem Cell, Current Biology, Developmental Cell, Genes & Development, Nature, Nature Genetics, Plant Cell, PLOS Biology, PNAS, Science). After three successful COS faculty member applications for ERC grants (Detlev Arendt, Jan Lohmann, Joachim Wittbrodt) until 2013, in 2015 Thomas Greb and in 2016/2017 Annika Guse were able to win further prestigious ERC grants.

Generally, the individual researchers are very successful with funding from DFG, EU, BMBF, Federal State, and from various foundations. During 2013-2017 more than 100 individual grants from a diverse range of biological disciplines were successfully applied for. These very active and constant activities on high level are complemented by a number of collaborative research activities often co-initiated and/or led by COS PIs (SFB873, Jan Lohmann; SFB1324, Thomas Holstein; FOR2509, Sabine Strahl; FOR2581, Alexis Maizel; SPP1529, Marcus Koch) or which involve participation (SFB1036, SFB1101, SPP1710, others).

Apart from publications in internationally leading journals, research at COS is made visible with our international COS Symposia Series, which features COS researchers and top-level scientists from around the world working in forefront topics of organismal biology. The Symposia are held bi-annually and typically attract more than 300 attendees. Particular attention is given to the selection of speakers, who are not only recognized leaders and pioneers in their fields but also enthusiastic presenters, and we aim for a balanced mix of international and local. The topics from 2013 (Building beauty – from genes to shape), 2015 (Darwin 2.0 – new tools to go through time) and 2017 (Senses and sensitivity) do reflect core interests of COS combined with pressing topics in modern organismal biology. COS symposia have served as platforms for career development and brainstorming for new COS activities such as the state funded graduate school focusing on »Evolutionary novelty and adaptation – from molecules to organisms«, coordinated by Steffen Lemke.

Publications, grants and awards reflect the performance of our researchers and are listed in the sections presented by the individual PIs. Importantly, COS has been highly successful to generate substantial synergy between PIs and, therefore, emerging collaborative activities that have been particularly stimulated by the founding of COS are highlighted in the following:

Physiology remains one of the key connecting topics within COS. Physiological studies that integrate genomics, proteomics and metabolomics can provide a more comprehensive picture of how the organism deals with the given environmental conditions. Combining state of the art metabolite analysis with the development and application of genetically encoded metabolite sensors will facilitate physiological measurements at the level of cells, tissues, organs and organisms. Through the initiation of a metabolomics core technology platform we have followed our successful model and have placed a nucleation point together with a metabolomics junior group (Emmanuel Gaquerel). Both, the platform and the junior group, which are funded by the excellence initiative, established themselves not only as a technology transfer unit, but also serve as an important hub to bridge many disciplines, ranging from evolutionary biology to medical research. Along these lines, a grant on metabolism and development within the CellNetworks Cluster of Excellence »Emerging Collaborative Topic (EcTop)« funding line was one by a COS led consortium (Gaquerel, Greb, Großmann, Hell, Kummer, I. Lohmann, J. Lohmann, Maizel, Schumacher). This activity has successfully brought together physiologists with cell- and developmental-biologists and has not only laid the foundation for further collaborations, but also has yielded a number of joint publications.

An important topic that serves as a scientific anchor for a diverse range of COS PIs are stem cells and developmental processes. Although this may seem a rather generic topic, focus within COS is on evolution, adaptation and environmental interactions rather than regenerative medicine. PIs at COS work on stem cell related questions from plants to animals and from cells to organs to ultimately identify common rules for the response of stem cells to growth and differentiation stimuli. Many aspects of stem cell biology are highly relevant for our understanding of organismal evolution and adaptation, ultimately contributing to ecological interactions. Stem cells are thus an ideal system for studies across scales, from molecules to living systems and the SFB 873 »Maintenance and Differentiation of Stem Cells in Development and Disease« includes seven out of seventeen projects led by COS PIs. The focus on development has been recently strengthened by establishing the SFB 1324 »Mechanisms and functions of WNT signalling« at COS, a pathway controlling central processes of embryonic development cell differentiation. There is no doubt that stem cell biology will continue to be one of the important and active fields of research.

An emerging field of interest is the interaction of the environment with biological processes from the molecular to the cellular and organismal level. Understanding the mechanistic and evolutionary basis of adaptation represents the next challenge in organismal biology and is the basis for systematic approaches. While biology has had tremendous success in shaping our mechanistic understanding of how organisms develop and thrive in a given standardized stable environment, less is known about the mechanisms, causes and consequences of adaptation when environmental parameters are changing. The study of these Organisms X Environment interactions is an emerging cross-disciplinary field and central to our strategy.

Our independent research groups are central for the successful development of COS and our future concepts. However, most of the currently established collaborative funding schemes (DFG, BMBF, EU) require a rather lengthy application procedure which often

precludes group leaders with non-permanent positions from participating. To at least partially overcome this problem we are currently following two lines: we actively promote applications of small networks to funding schemes of the Excellence Initiative (CellNetworks EcTops, Frontiers Program) or the Federal State (LGFG graduate programs, see above). Senior groups participating in these programs do complement the scientific portfolio, without requesting additional funds and thus enhance the chances for funding success of the junior faculty. In both funding lines, our independent group leaders are well represented and we can expect further successful applications in the future.

Research at COS starts with our students, and, therefore, we actively participate and develop exchange programs with international partners to foster scientific exchange at early stages of the career to seed networks for collaborative research. Here, a balanced mix of established and independent groups is applying for fellowships in international consortia with complementary research interests such as the regularly organized Kyoto-Bristol-Heidelberg Plant Science Workshop involving top partner universities of our Ruperto Carola. This strengthens tripartite partnerships between the University of Bristol, Heidelberg University and Kyoto University, all of them are world-leading institutions in research and education.

During the reporting period COS researchers were awarded prestigious appointments and prizes, such as Marcus Koch, who was inaugurated in 2016 as permanent guest professor and full faculty member at Praque university to bridge teaching and scientific exchange between faculties and institutes. In 2015 Detlev Arendt and Jan Lohmann were elected for EMBO membership, and Thomas Holstein received the HMLS prize.

These are just some few examples of COS driven activities aiming to connect COS groups along major topic lines, integrating PhD students, non-permanent PIs as well as international collaborators, and reflects the COS spirit to bridge people and scientific fields.

1.4 TEACHING AT COS

COS is the biggest contributor to teaching of biosciences at the Heidelberg University. This is true both at the BSc and the MSc level. COS provides around 80 % of compulsory practical courses, and more than 60 % of compulsory lectures during bachelor studies (for details see Appendix COS-Teaching). Five of the eight majors offered within the MSc »Molecular Biosciences« are predominantly or exclusively coordinated and run by COS lecturers (Developmental and Stem Cell Biology, Molecular Plant Sciences, Evolution and Ecology, Neuroscience, Systems Biology). With its highly appreciated and evaluated teaching, the entire faculty of COS is actively contributing to the international recognition of life sciences in Heidelberg. In addition, these efforts help to build up an active, highly motivated community of young scientists and help us to recruit top talent for master and Ph. D. theses.

In line with the organismal perspective of COS, we further developed our teaching programs to match our research interests: COS teaching ranges from fundamental fields, such as molecular biology, or biochemistry, to the central principles of development, physiology and neurobiology all the way to ecology and evolution. At all of these levels, we aim to teach biological mechanisms in the organismal context to allow the students to appreciate the importance of individual processes, to intellectually connect apparently diverse fields, as well as to highlight fundamental and re-occurring biological principles. Therefore, we have developed an integrated, research oriented teaching concept to stimulate our students to learning facts by addressing and solving questions, starting in the first semester. We are convinced that this will benefit students even with career choices outside of science, such as administration, teaching, or industry, since the increasing complexity in all fields demands the ability to handle complexity across all scales, from the precise details to the big picture.

Teaching at German high schools is struggling to implement comparable concepts due to the immanent lack of adequate research opportunities. In close collaboration with more than 40 high schools in the Rhein-Neckar area we are providing first insight into the basic concepts of up to date, cutting edge research for high school students in Bertalanffy lectures twice a year. To this end we invite world leading experts in exciting fields of biology to present their research, but also their career path to high school students. In a full afternoon with keynote presentation, tutorials and plenary discussions interested high school students (200-400, matched by 30-100 university students and tutors) and their teachers get a real life impression of science at work. For a selection of motivated students we offer hands-on experience in a two weeks practical course. This serves as one example of several innovative teaching concepts that even go to the preschool level (for details see Appendix COS-Events) developed and actively pursued at COS.

1.5 INTEGRATION IN THE LIFE AND NATURAL SCIENCES ON CAMPUS

COS is deeply embedded into the Heidelberg life science and natural science campus. The integration process on campus was massively fostered during the two rounds of the German Excellence Initiative in 2007-2012 and 2012-2018.

German excellence initiative: The structural elements that emerged across the Heidelberg life science campus were the excellence cluster CellNetworks and the Graduate School HBIGS. From the beginning, COS members played an essential role in both activities during the application process, but also as members of the respective steering committees, as coordinator of the Graduate School HBIGS and as cluster co-coordinator of CellNetworks.

Core Facilities: One of the keys for success of the CellNetworks cluster was the understanding that the impact of the initiative will be highest when a broad community of active participants can profit. This is elegantly achieved by installing core facilities that provide high end technology and user support to the entire campus. The core facility concept in Heidelberg was pioneered at EMBL and soon adapted by one of the founding partners of COS, the Heidelberg Institute for Zoology (HIZ). Thomas Holstein succeeded in attracting and securing support by Nikon for establishing the first core facility on campus, one of the two Nikon Imaging Centers worldwide at this time. The Nikon Imaging Center (NIC) in Heidelberg is sustained by three main sources, COS (personnel and running costs), Nikon (Instruments, part of personnel) and the CellNetworks cluster (personnel). It fostered numerous campus-wide collaborations and interactions and is the Heidelberg role model for a successful core facility. It should also be stressed that meanwhile nine NICs have

been successfully established in renowned research institutions worldwide. By analogy, new core facilities were established all aiming to generate technology hubs, to provide technology and expertise and, last but not least, cross-connect expertise with questions across the Heidelberg life science campus in an easy and systematic manner.

COS is now providing equipment and/or personnel for three additional core facilities: The CellNetworks deep sequencing core facility, the Electron Microscopy core facility and most recently, the Metabolomics core technology platform. The availability of cutting edge technology and support has been repeatedly voiced as one reason why Heidelberg and COS in particular are so attractive for PIs running their first independent research group. Even though the core facilities can be accessed by any scientist on the Heidelberg life science campus, they are financially supported by individual COS PIs by providing positions for technical personnel and staff scientists. While this has proven to be a successful short and medium term strategy, it does not provide the necessary long term stability. Thus, COS works together with other institutions on campus and the university to further professionalize the core facility landscape with the hope to harmonize user access and financing across diverse facilities.

National and international research collaborations: Research at Heidelberg University is also strongly interconnected with national and international research activities. COS therefore has excellent links to the scientific infrastructure at other research institutions both on the national and international level. One of the directors is appointed at the European Molecular Biology laboratory (EMBL) (Detlev Arendt), another at the Karlsruhe Institute of Technology (KIT) (N. S. Foulkes). Another program that was established in the context of the excellence initiative and is now maintained independently is the Heidelberg Karlsruhe research partnership (HEIKA). Thomas Holstein was involved in heading one of the five HEIKA research bridges (Synthetic Biology). Thomas Holstein is also heading the

Heidelberg Academy of Sciences, one of the eight German academies of sciences and humanities with elected scholars, all of whom are outstanding representatives of their research fields both nationally and internationally.

COS PIs also contributed to the German-Japanese University Consortium HeKKSaGOn. This research bridge was founded in 2010 as an association of three German and three Japanese leading universities (Göttingen, Heidelberg, Karlsruhe/KIT, Kyoto, Osaka, and Tohoku) to intensify their transnational collaboration.

DFG–SFB and –FOR: COS PIs are also heading four important research initiatives, the two newly funded DFG research units FOR 2509 on »The Concert of Dolichol-based Glycosylation: From Molecules to Disease Models« (Sabine Strahl) and FOR 2581 on »Quantitative Morphodynamics of Plants« (Alexis Maizel) as well as the two DFG Collaborative Research Centres SFB 873 on »Maintenance and Differentiation of Stem Cells in Development und Disease« (Jan Lohmann) and SFB 1324 (newly funded) on »Mechanisms and functions of WNT signalling« (Thomas Holstein). All research initiatives include not only COS members but also members from other institutes and faculties on the campus, as well as from other German research institutions. In addition, many COS PIs were members in SFBs or FORs that were coordinated by other universities, thus further strengthening our integration with national centres of excellent research.

Scientific training and education: COS PIs are actively contributing to and continuously developing HBIGS (Hartmut Hoffmann-Berling International Graduate School), the Heidelberg life science graduate school, successfully funded in both rounds of the Excellence Initiative. Since the very beginning, two of the five elected members of the HBIGS steering committee are COS PIs (T. Rausch/R. Hell, J. Wittbrodt, A. Maizel) which is headed since 2016 by J. Wittbrodt. The traditional COS PhD program initially ran in parallel for some years, but since most of the COS PhD students were members of HBIGS, we further developed our curriculum to become an integral part of HBIGS. The HBIGS PhD program is providing a structured PhD education with a tailored scientific core course, regular seminars, retreats, international meeting participation and annual TAC (thesis advisory committee) reports and meetings to guide both PhD student and supervising PI. The selection of PhD students via HBIGS and the structured education has crucially contributed to an increase of scientific exchange within COS (via seminars and TAC meetings) and across the campus. The program is highly popular among the PhD students who are making an effort to get selected for HBIGS (written exam and panel interview). With the COS program as part of the HBIGS school we have contributed to extending the initially molecular and cell biological focus to the broader organismal spectrum of COS.

Within context of the LGFG research training program a program on »Evolutionary novelty and adaptation – from molecules to organisms« (Steffen Lemke) is funded by the State of Baden-Württemberg. It unites nine research groups on the campus with complementary expertise in evolutionary biology, developmental biology, and ecology employing state-of-the-art molecular tools and imaging technology as well as field research in diverse habitats such as desert caves, coral reefs, and alpine systems.

1.6 FUTURE PERSPECTIVES AND CHALLENGES

COS is a young institution, having been founded in 2010 only, and consequently is still developing rapidly.

The number of departments (»Abteilungen«) increased from 13 to 16. At the same time the number of independent research groups remained constant with eight groups. We do see maintaining such a high level as good evidence that we successfully provide a highly permissive and stimulating environment. For the future, we now aim at a steady state of six to ten independent research groups at COS, as well as non-permanent professors to allow for flexible extensions. To create a solid legal and administrative framework for this consolidated setup of COS, we have updated and improved our VBO (Internal regulations and administrative system) in 2017. However, COS currently has no long-term core funding for supporting these independent research groups and thus we have to seek for individual solutions case by case. This not only places a heavy burden on the budget, but also creates inequality among the junior groups, since some will benefit from additional support by the university and other may not. Consequently, we have identified this problem as one of the major future challenges for COS and hope to develop adequate possibilities to further support junior groups in a fair and transparent fashion. In addition, we currently do not enjoy the flexibility to implement reward structures for active and innovative members of COS on any level. All of these measures will be instrumental for attracting novel and complementary expertise through creative and innovative new colleagues dedicated to excellence in teaching and research.

COS firmly believes that teaching is an integral part of science and that investment into teaching and training will ultimately benefit our research. Consequently, we aim to continuously improve our teaching program and the first successes of these activities have become visible. Importantly, the structures implemented to maintain a dynamic development over time: friendly competition for the best students based on creativity and originality of the teachers rather than on the technological setup of the teaching facilities. We strongly believe that learning by doing and learning to ask the right questions are among the keys to success, not only in science. However, to continuously develop teaching programs and to offer training in intense, research oriented modules for small cohorts of students requires dedicated and highly trained personnel, up to-date facilities, and access to cutting edge technology and expensive supplies. Unfortunately, our commitment to excellence in teaching is challenged by limitation in resources at all these levels. These limitations have a strong effect on the capacity of COS to attract and retain the best students and ultimately to recruit them for our research projects. While we even offer highly attractive seminars and practicals for high school students, the number of applications to the bioscience curriculum has dropped. Similarly, despite the innovative and research oriented curricula in the BSc and MSc programs, less than half of the students remain in Heidelberg for their next career step. Thus, one of our major future challenges is to communicate more with suitable candidates and to make the local transition from one level of training to the next more attractive. To this end it will be essential to maintain specialization in MSc education by the several Major programmes headed by COS (Development, Evolution & Ecology, Molecular Plant Sciences, Neurobiology, System Biology) but at the same time allow and enable the students within the study programme to deepen expertise and skills in any of those disciplines to broaden their mind and cross-discipline skills, which is need for any future modern biology. Another important measure will be to further align BSc, MSc and PhD training to create an even more attractive and transparent career path for students and thus to ensure a healthy influx of skilled and dedicated young scientist into our labs.

Our core facilities have been a crucial motor for success in the past. To keep this engine running in times of limited resources we will critically review their performance and adapt them to the needs of the entire Heidelberg life science campus. Since user fees can only cover running costs and do not even allow us to hire additional personnel, core facilities can only grow if new resources can be acquired. The necessary purchase of new and up to date equipment poses a big challenge to the success model of the entire campus. Individual instrumentation grants can only partially cover the needs of the coming years. Strategic partnerships with industry, as successfully established for the Nikon Imaging Center, only work in exceptional cases. Thus, COS will work closely with other institutions and the University to develop strategies that will allow long term success of our core facilities. This likely will include central ordering of supplies, handling of user requests and data, as well as billing. For the Botanic Garden and Herbarium, serving as a renowned and internationally widely recognized resource for scientific material and knowledge, the renewal of a nearly 100 years old building infrastructure is on the agenda. During the past years all scientific and building concepts have been developed and approved by university and Federal State and need immediate prioritisation to be realized in due time.

A substantial barrier to realizing the true potential of COS remains the spatial fragmentation. Currently, COS is housed in five buildings spread around campus. Together with the University, we are currently developing the concept to unite most of COS at one single site.

The merger of plant and animal sciences has provided an interface that has stimulated and developed COS immensely in various new directions. We are approaching continuously more complex levels of organismal organisation. And consequently, key terms such as phenotypic and genotypic plasticity, phenotype-environment interactions, holistic approaches using any »omics« technology (genomics, epigenomics, transcriptomics, metabolomics, proteomics, and taxonomics) and thereby bridging disciplines via technology, and finally »evolution in its broadest sense« become central to COS and its scientific mission.

RESEARCH GROUPS

2

2.1 PROF. DR. DETLEV ARENDT
ANIMAL EVOLUTION

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Fields of Interest
animal evolution, evo-devo, marine developmental biology, comparative neurobiology, central nervous system evolution, eye evolution, origin and evolution of cell types

Brief summary of work since 2013
Since the last review, we have focused much effort on studying development, cell type complement and function of the annelid brain. This has allowed us to partially reconstruct the larval and adult brains of urbilaterian ancestors. »BrainEvoDevo« has also been the title and subject of our Advanced ERC grant (with focus on the annelid chemosensory system).

Our research strategy has roughly followed three steps. First, we have investigated the subdivision of the body into developmental regions, by the differential activity of gene regulatory networks. These highly conserved »developmental regions« give rise to different parts of the nervous system result and provide a valuable spatial framework for comparisons across phyla. Second, we have characterized the cell types that develop from distinct regions in different animals, combining high-end microscopy, expression screening and single-cell sequencing. From this we have successfully inferred cell type interrelationships and their step-wise diversification in the diverging lineages. Finally, having determined the cell types that were present in remote ancestors, our next step has been to determine their ancestral function in a comparative neurobiological-behavioural approach.

Most prominently, this strategy has allowed us to unravel the evolution of the apical nervous system and its significance in the control of larval swimming in marine zooplankton and to identify the axochord in invertebrate bilaterians as an evolutionary precursor and homolog of the chordate notochord. In addition, we have focussed our effort in establishing single-cell sequencing as a new research direction for the field of evolution and development.

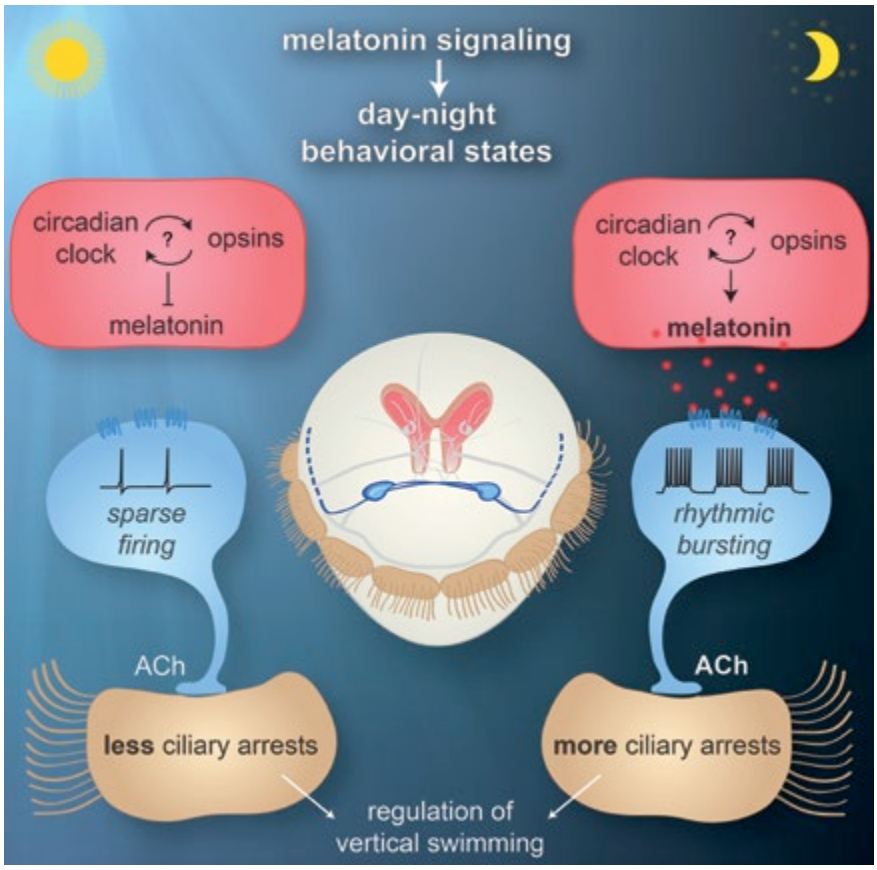

Figure 1
Cell types constituting the *Platynereis* melatonin system

Major contributions since 2013

The apical nervous system: control of circadian swimming behavior in marine zooplankton (Tosches et al., 2014)
The evolutionary origin of opsin-based light perception and melatonin signalling has so far been elusive. Melatonin has a well-established role in the control of physiology and behavior in vertebrates; downstream of the circadian clock, it reduces locomotor activity and induces sleep.

Our study of the role of the melatonin system in annelid larvae has linked the evolution of opsin phototransduction and melatonin signaling to the upwards and downwards swimming of marine plankton at dusk and dawn in the ocean. This phenomenon is referred to as diel vertical migration (DVM) and represents the biggest transport of biomass on earth.

We found that, in striking resemblance to the pineal organ of lower vertebrates, opsin phototransduction, melatonin release and the clock are situated in the same cells of the *Platynereis* zooplankton larva and are functionally coupled. Probing the effects of melatonin release on larval behavior, we have shown that nocturnal melatonin signaling reduces the activity of locomotor cilia in the »ciliary bands« used for swimming – so that the larvae sink down.

To explore the neurobiology underlying these effects, we have successfully established GCaMP Ca^{2+} imaging and used electrophysiology in *Platynereis*. We found that melatonin signaling alone is sufficient to establish and maintain a »nocturnal behavioral state«, characterized by the constant and rhythmic burst firing of the cholinergic motor neurons that innervate the ciliated cells, which results in enhanced ciliary arrests.

Development of the annelid axochord: insights into notochord evolution (Lauri, Brunet et al., 2014)

The evolution of the chordate notochord has remained elusive. By chance, we have identified a structure in annelid worms that, by several criteria, qualifies as a notochord homolog. It is a ventromedial longitudinal muscle running directly underneath the neural midline that we have termed »axochord«. In *Platynereis*, the axochord develops by convergence of mesodermal cells from left and right body sides as two adjacent bands of muscle fibres along the ventral midline and expresses a combination of transcription factors, signalling molecules and axon guidance factors that are characteristic for the notochord in vertebrates. Similar to the notochord, the differentiated axochord is positioned between the central nervous system and the axial blood vessel and is surrounded by a strong collagenous extracellular matrix. Ancestral state reconstruction suggests that an axochord (as a ventromedial longitudinal muscle) existed in bilaterian ancestors. We propose that, due to dorsoventral inversion in chordates, the axochord came to lie on the dorsal body side. Its constituent cells gradually stiffened by enhanced secretion of hydrated material, thus giving rise to the notochord. This was concurrent with the evolution of swimming movements.

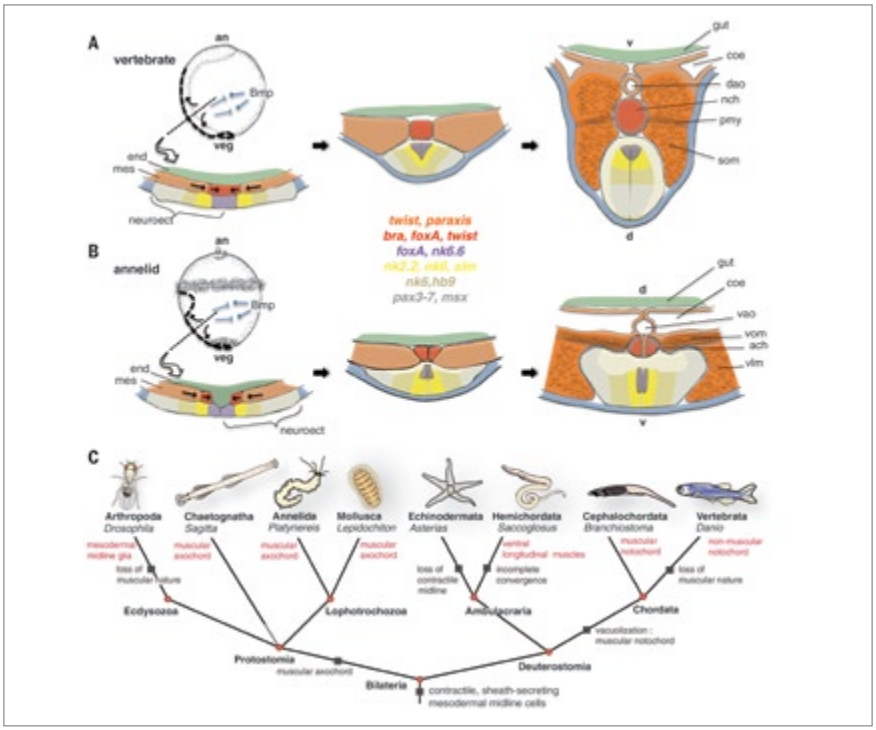

Figure 2
Comparison of notochord and axochord development.
(A) Notochord development schematized for zebrafish at 90 % epiboly, 14 hpf/neural keel, and 30 hpf/ organogenesis stages.
(B) Axochord development schematized for Platynereis at 34 hpf, 72 hpf, and 2 months of development.

Animal bodies comprise a diverse array of tissues and cells. To characterise cellular identities across an entire body, we have compared the transcriptomes of single cells randomly picked from dissociated whole larvae of the marine annelid Platynereis dumerilii. For this, we have developed a high-throughput protocol to identify the spatial origin of cells assayed by single-cell RNA-sequencing within a tissue of interest, based on positional gene expression profiles derived from a gene expression atlas. Using clustering algorithms, we identify five transcriptionally distinct groups of differentiated cells that are spatially coherent, as revealed by spatial mapping. Besides somatic musculature, ciliary bands and midgut, we find a group of cells located at the apical tip of the animal, comprising sensory-peptidergic neurons, and another group composed of non-apical neural and epidermal cells covering the rest of the body. With these data we have established a basic subdivision of the larval body surface into molecularly defined apical versus non-apical tissues, and supported the evolutionary conservation of the apical nervous system as a distinct part of the bilaterian brain.

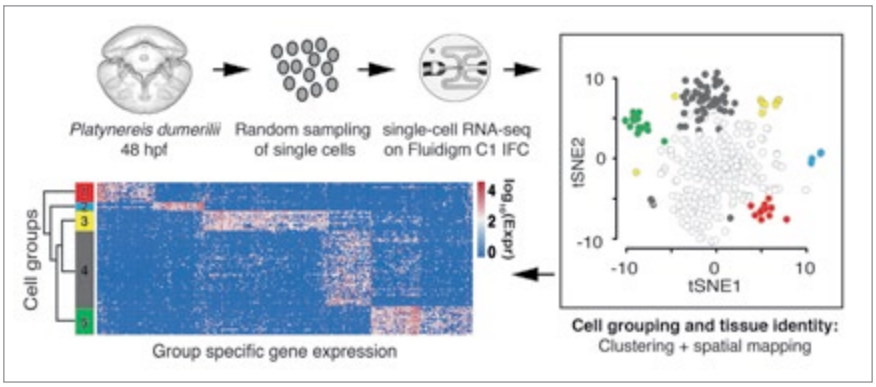

Figure 3
Summary scheme for single-cell sequencing in *Platynereis dumerilii*

Planned research and new directions

In the next years, we want to unravel major lineages of the cell type tree in animals. In particular, we want to find out how the nervous system came into place at the cellular level. What were the first cells that functioned in the context of a nervous system and what was their function? How did they diversify further? For illustration (Figure), if we assume that the single black line at the bottom of panel B (*) represents the first neuron, this would have been inherited and differentially diversified in the cell type tree (embedded in big species tree). And each cell type diversification would involve differential inheritance of cellular modules as shown in the enlarged detail in panel C.

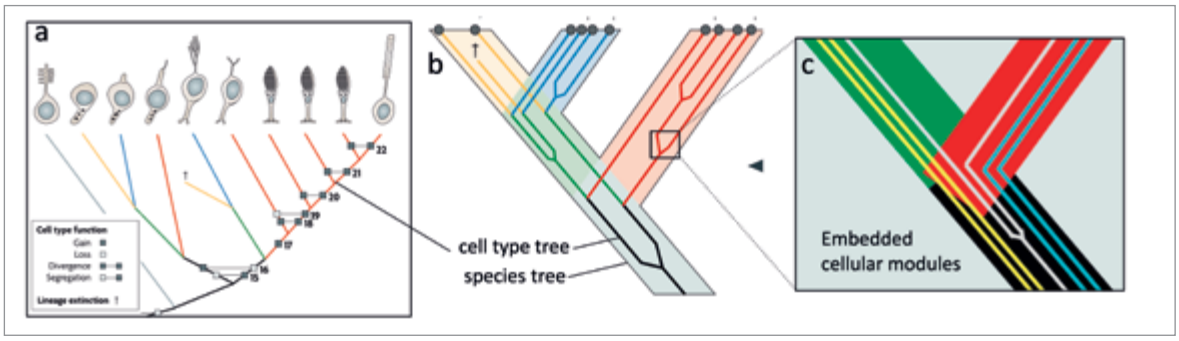

Figure 4
Evolution of cell types. A, an example tree illustrating the evolution of photoreceptors from (Arendt, 2008). B, a cell type tree embedded in a species tree. C, detail of the cell type tree with embedded cellular modules

Cell type evolution can be addressed by comparisons between groups, but also by resolving sister cell types and their functions within a given group (e. g., within the red lineage of panel B). We plan to resolve cell type interrelationships for several animal models including our main model system, the annelid *Platynereis*, in several projects focussing on the central and peripheral nervous system. To elucidate the very beginnings of nervous system evolution, we will also investigate cell type interrelationships for our latest model species acquisition, the demosponge *Tethya wilhelma*. (Notably, these approaches will be mutually informative, as the fundamental subdivisions of the sponge cell typogenetic tree should re-occur at the base of the annelid cell type tree.)

For *Platynereis*, this will involve extensive sc-RNAseq and subsequent mapping of randomly picked cells into cellular atlases, for several larval stages. We will then investigate the distribution of neural modules (sensory receptors, signalling cascades, ion channels of various kinds, transmitter synthesis, release, reception, etc.) over individual cells and cell types. These efforts will be combined with a systematic phenotypic characterization (e. g., cellular morphology and axonal connectivity) and by functional studies of key genes (encoding transcription factors or neural effectors) in the context of behavioural assays.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 31, number of citations 2013-2017: 580, h-index (2013-2017): 10, total h-index: 40 (according to Thomson Reuters).

Vergara, H.M., Bertucci, P.Y., Hantz, P., Tosches. M.A., Achim, K., Vopalensky, P., Arendt, D. (2017) A whole-organism cellular gene expression atlas reveals conserved cell types in the ventral nerve cord of Platynereis dumerili. Proc Nat Acad Sci, 114(23):5878-5885

Arendt, D., Musser, J.M., Baker, C.V.H., Bergman, A., Cepko, C., Erwin, D.H., Pavlicev, M., Schlosser, G., Widder, S., Laubichler, M.D., Wagner, G.P. (2016) The origin and evolution of cell types. Nat Rev Genet. 17(12):744-757

Achim, K., Pettit, J.-B., Saraiva, L. R., Gavriouchkina, D., Larsson, T., Arendt, D.*, Marioni, J. C.* (2015) Single-cell expression profiling and spatial mapping into tissue of origin. Nat Biotechnol. 33(5):503-9

Tosches, M. A., Bucher, D., Vopalensky, P., Arendt, D. (2014) Melatonin signaling controls circadian swimming behavior in marine zooplankton Cell 159(1):46-57

Lauri, A., Brunet, T., Handberg-Thorsager, M., Fischer, A. H., Simakov, O., Steinmetz, P. R., Tomer, R., Keller, P. J., Arendt, D. (2014) Development of the annelid axochord: insights into notochord evolution. Science 345(6202):1365-8


ZEISS

2.2 DR. JAN FELIX EVERS
INDEPENDENT RESEARCH GROUP
NEURAL NETWORK DEVELOPMENT GROUP

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Fields of Interest
Molecular and developmental genetics, development and differentiation, neuronal networks, synapse molecular physiology, optics and microscope engineering, computational image analysis

Brief summary of work since 2013
The nervous system is built from a large number of diverse neuron types, and the connectivity between these neurons determine the way how organisms are able to perceive, and interact with their environment. Using the motor system of *Drosophila* as a model, we investigate how synaptic connectivity patterns emerge in the CNS during embryogenesis, and how they are adapted to changing environmental and behavioral requirements during postembryonic development.

In the last years we generated a powerful set of genetic tools and imaging techniques that allow effective visualization of neuronal structure, circuit connectivity and molecular composition of individual synaptic contacts throughout embryonic and larval development in the intact animal with time-lapse microscopy, and to visualize the molecular ultra-structure of pre- and postsynaptic specialisations using expansion microscopy.

We show that neuronal growth in the embryo is geared to occupy synaptic territory; the emergent neuronal architecture then adapts to an increasing body size during post-embryonic animal growth, adding dendritic branches and synaptic connections while maintaining the same synaptic territory that has been established in the embryo. During this time synapses not only increase in number, but also change their molecular composition with increasing developmental age: individual release sites accumulate more of the scaffold protein Bruchpilot (Brp), which likely subserves an increase in release probability and therefore gives rise to stronger and more reliable synaptic connectivity. Using pulse-chase induction of Brp labeling at the endogenous genomic locus, we now have evidence that Brp is synthesized in the axon proximal to the synaptic release sites by local translation from long-lived mRNA.

Major contributions since 2013

We have shown that at the cellular level, motoneuron dendrites, and the amount and distribution of presynaptic sites are variable when networks become first functional at the end of embryogenesis, suggesting that there is not a hard-wired program of connectivity. Motor neurons continue to grow during larval development, and as their dendrites enlarge, they form more connections with presynaptic partners, leading to greater levels of synaptic input. Interestingly, we find substantial variability in the quantity and quality of synapses that individual pairs of inter- and motoneurons make, between segments of the same nervous system, and between animals. Together with our previous data this demonstrates that circuit formation in the motor system of *Drosophila* is plastic and acquires functional patterns of connectivity in an activity dependent manner.(Zwart et al. 2013; Couton et al. 2015)

In the embryo, neuronal circuits emerge de novo and assume function only by the end of embryogenesis; larval circuits however need to maintain function while continuously expanding in support a growing body size. To compare the different mechanisms of initial circuit formation and circuit expansion we developed techniques to visualize neuronal growth of identified motor neurons in the intact animal with time-lapse microscopy. Using these techniques, we find that motorneuron postsynaptic arbors (dendrites) grow in a highly dynamic fashion during embryogenesis: while the dendrite is invading its respective synaptic territory in the neuropil, it shows a high turnover of filopodial protrusions that serves to explore the surrounding neuropil for synaptic partner neurons. Dendritic growth during larval development, however, is characterized by much more static growth: previously established parts of the dendrites expand, and most newly added dendritic branches are long term stable beyond 2 days of imaging time. We find that this switch in growth mode coincides with the developmental onset of the presynaptically released protein jelly belly (Jeb) and its postsynaptic receptor tyrosine kinase Anaplastic Lymphoma Kinase (Alk). Both, knockdown of Alk and removal of Jeb from all neurons lead to an increase in filopodial protrusions on axonal (premotor interneurons) and dendritic (motorneuron) arbors. Targeted knockdown of Alk signaling in individual motorneurons, however, results in reduced formation of new dendritic protrusions, and increased branch stability. This finding can be best explained by a competition of postsynaptic neuronal arbors for secreted Jeb, and that cell-autonomous loss of Alk results in a reduction of fitness in the competition for synaptic connectivity.

We are currently preparing these findings for publication.

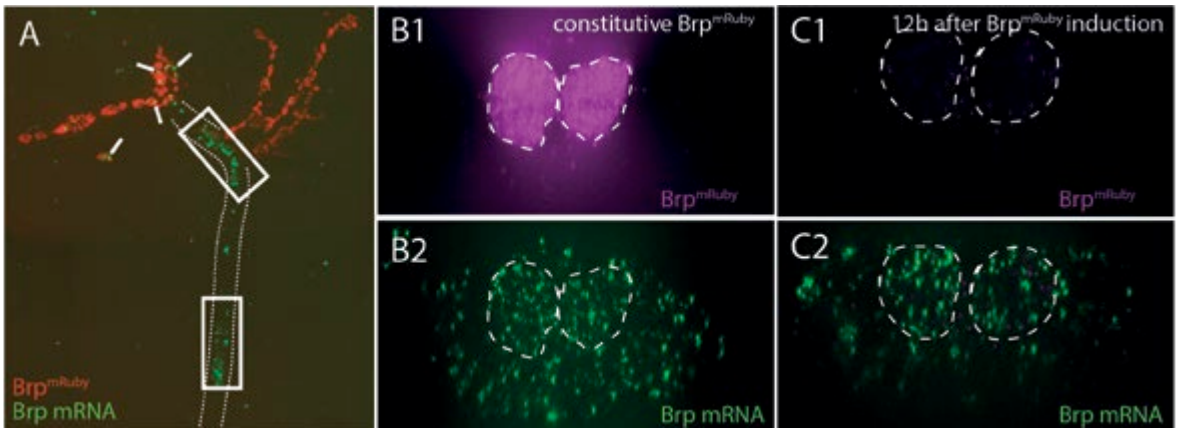

Figure 1
The mRNA for the active zone scaffold protein Bruchpilot (Brp) localizes effectively towards presynaptic release sites. (A) Brp mRNA accumulates in patches within motorneuron axons proximal to the neuromuscular junction (boxes), and single mRNA are detectable close to presynaptic release sites (arrows) (B) in the CNS, constitutively labeled Brp^{mRuby} (B1) is detectable only in the neuropile (outlined by dashed line); (B2) mRNA of Brp^{mRuby} is detectable in neuronal cell bodies, and also strongly accumulates in the neuropile (C) Brp^{mRuby} and its mRNA is imaged 12h after induction. (C1) shows Brp^{mRuby} protein at low expression strength; (C2) mRNA of Brp^{mRuby} is effectively trafficked to the neuropile where it accumulates prior to Brp^{mRuby} protein buildup.

Synaptic coupling between neurons not only depends on the numbers of individual synaptic sites, but can also be tuned by changing the molecular composition of both presynaptic release machinery, and the postsynaptic receptor field. On the postsynaptic site local protein synthesis has been demonstrated and was shown to be crucial for specific modulations of synaptic efficacy. To study whether presynaptic plasticity might be regulated by local translation we focused on Bruchpilot (Brp), a protein that has been shown to correlate with the efficacy of synaptic transmission.

To minimize experimental artifacts we developed a technique to conditionally tag proteins at their genomic locus upon recombinase expression (dFLEx). Using dFLEx, we address the developmental dynamics of Brp protein localization and find that amounts of Brp at individual presynaptic release sites increase with developmental age until third instar stage.

Using a pulse-chase paradigm with the induction of dFLEx labeled Brp molecules, we determine the lifetime of Brp proteins to be 3 days. FRAP experiments of Brp at the NMJ show that recovery to half maximum intensities takes 12 hours, irrespective whether individual active zones, boutons or entire axonal branches were bleached. This suggests a common pool of Brp protein that serves all active zones. Indeed, we find an extra-synaptic pool of Brp protein in motoneuron axons proximal to the NMJ. Using fluorescent in-situ hybridization targeted to the inducible fluorescent tag of the dFLEx system we find accumulations of mRNA in distinct patches overlapping this axonal Brp protein pool. In the CNS, Brp mRNA signal is detectable in cell bodies and close to presynaptic release sites. Pulse-chase activation of the dFLEx cassette reveals effective transport of mRNA into the neuropil and along motor axons in advance of detectable Brp protein.

In summary, we show that Brp amounts are actively regulated at the presynaptic site, and that protein synthesis and degradation reach steady state by third instar larva, likely fed by local translation. These data are currently prepared for publication.

Planned research and new directions
Based on our previous results we will now investigate: 1) The developmental progression of the molecular composition of nascent synaptic sites in the CNS; 2) the mechanisms of Brp mRNA transport to and translational regulation at presynaptic site; 3) the effect of circuit activity on the assembly of circuit and synapse architecture during a critical period in the embryo.

1) The developmental timeline of molecular maturation of presynaptic sites has been thoroughly studied at the NMJ. Much less is known about how synapses mature in the central nervous system, particularly how the postsynaptic site assembles. We have generated a rich set of dFLEx tagged synaptic molecules that we will now use to trace synaptogenesis between individual partner neurons. The main aims in this part are a) to compare how synapses of different transmitter type develop; b) to establish the role of individual molecular players in coordinating the orderly assembly of pre- and postsynaptic sites.

2) We have shown that Brp mRNA is effectively transported towards axonal terminals where it co-localizes with an extrasynaptic pool of Brp protein. We will now investigate the mechanisms that direct Brp mRNA to the axon, and what impact an abrogation of Brp mRNA transport has on the physiology and plasticity of synaptic transmission.

3) We have previously shown that in the Drosophila motor system, emerging neuronal circuits undergo a phase of activity dependent refinement during embryogenesis. Others have shown that this developmental time window coincides precisely with a critical period in which experimentally altered neural activity potently induces motor circuits that are prone to epileptiform seizures. To uncover the morphological and molecular alterations that discriminate healthy from epileptic circuits we will investgate neuronal structure, the balance of excitatory to inhibitory synapses and the molecular composition of synaptic sites in normal brains and how this is altered when activity levels during embryogenesis are increased or decreased experimentally.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 6, number of citations 2013-2017: 247, h-index (2013-2017): 4, total h-index: 11 (according to Thomson Reuters).

Gjorgjieva, J., Evers, J. F., & Eglen, S. J. (2016). Homeostatic Activity-Dependent Tuning of Recurrent Networks for Robust Propagation of Activity. The Journal of Neuroscience: the Official Journal of the Society for Neuroscience, 36(13), 3722–3734.

Chmielewski, A. K., Kyrsting, A., Mahou, P., Wayland, M. T., Muresan, L., Evers, J. F., & Kaminski, C. F. (2015). Fast imaging of live organisms with sculpted light sheets. Scientific Reports, 5, 9385.

Couton, L., Mauss, A. S., Yunusov, T., Diegelmann, S., Evers, J. F., & Landgraf, M. (2015). Development of connectivity in a motoneuronal network in Drosophila larvae. Current Biology: CB, 25(5), 568–576.

Gjorgjieva, J., Berni, J., Evers, J. F., & Eglen, S. J. (2013). Neural circuits for peristaltic wave propagation in crawling Drosophila larvae: analysis and modeling. Frontiers in Computational Neuroscience, 7, 24.

Zwart, M. F., Randlett, O., Evers, J. F., & Landgraf, M. (2013). Dendritic growth gated by a steroid hormone receptor underlies increases in activity in the developing Drosophila locomotor system. Proceedings of the National Academy of Sciences of the United States of America, 110(40), E3878–87.


52725

2.3 PROF. DR. NICHOLAS S. FOULKES
CIRCADIAN CLOCK BIOLOGY

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Fields of Interest
The regulation, function and evolution of the circadian timing system. Light dependent control of gene expression. How environmental signals differentially regulate metabolism via the circadian clock. Regulation and evolution of DNA damage repair systems.

Brief summary of work since 2013
The circadian clock is a key biological timing mechanism which temporally coordinates most aspects of plant and animal biology, as an adaptation to the environmental day-night cycle. Central to its function is its daily resetting by environmental signals, (so called »zeitgebers«), such as light and temperature which are indicators of the time of day. This ensures that the endogenous clock time remains tightly synchronised with the external day-night cycle. In turn, a wide range of systemic and cell autonomous mechanisms relay timing information from the clock to its regulatory targets. We have used a complementary set of fish model species including zebrafish, medaka and blind cavefish as well as fish cell lines to tackle fundamental questions concerning circadian clock biology in vertebrates. Over the past 4 years, we have placed particular attention on understanding the signal transduction pathways whereby light exposure regulates the clock via triggering changes in gene expression in fish cells. We have also studied the evolution of elements of light sensing mechanisms under extreme environmental conditions of constant darkness. More recently, by a comparative study involving zebrafish and blind cavefish, we have explored how light and food differentially regulate circadian rhythms in metabolism via the clock. Furthermore, we have studied how light shapes the function of DNA repair mechanisms not only over the timescale of minutes and hours, but also during evolution spanning millions of years.

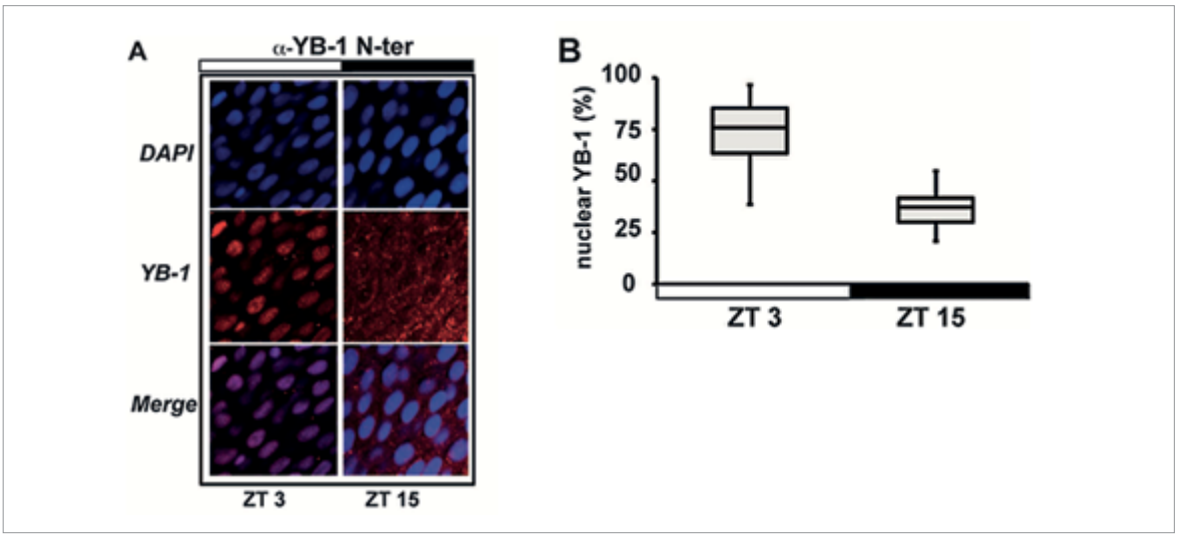


Figure 1
Differential regulation by blue and red light is mediated through D-box enhancer elements. Real time bioluminescence assays of transfected PAC-2 cells. Cells were transfected with (Left panel): multimerized copies of the D-box from the period 2 clock gene promoter or (Right panel): multimerized copies of the D-box from the Cryptochrome 1a promoter. In each panel relative bioluminescence is plotted on the y- axis and time (hrs) on the x-axis. Each time-point represents the mean of three independent experiments +/2 SD. Red, blue and black bars above each panel represent the red light, blue light and dark periods, respectively. For clarity, blue, red and white background shading also indicates the blue, red and dark periods respectively.

Major contributions since 2013

Our previous work with blind cavefish had demonstrated that non-visual opsins serve as photoreceptors for the light input pathway of the circadian clock in fish peripheral tissues and cells. During this review period, we have logically extended this analysis to study which signal transduction pathways and transcription control mechanisms relay light-derived signals from these photoreceptors to changes in gene expression and thereby enable the adjustment of the phase of the clock mechanism. Bioinformatics analysis and functional testing of the promoters of batteries of light regulated genes have both pinpointed the D-box enhancer element as the primary light responsive promoter enhancer element in fish (Ben-Moshe et al., 2014) (Figure 1). Interestingly, this differs from the situation in mammals where the D-box instead serves as a clock regulatory target. Thus, it seems that during vertebrate evolution, the role of the D-box enhancer has changed considerably. We have also revealed that the MAPK signaling pathway plays an important regulatory role upstream of the bZip transcription factors which bind to D-boxes (Mracek et al., 2013). Specifically, activation of ERK / MEK signaling results in down regulation of light driven D-box enhancer function and so serves to limit the duration of light induced gene expression.

We have continued to study our blind cavefish model (the Somalian blind cavefish, *Phreatichthys andruzzii*) in order to gain mechanistic insight into how light normally regulates the clock as well as a view of the evolution of light dependent mechanisms under extreme photic conditions. Of course, the most striking phenotype related to light sensing in cavefish is the loss of eyes and visual photoreception. Characteristically, in most cavefish studied to date, during early embryonic development a complete eye is formed but is subsequently lost. The mechanisms underlying this eye loss have been the subject of many studies in the Mexican cavefish *Astyanax*. In this species, eye loss has been attributed to interference during eye field patterning (Yamamoto et al., 2004)[1]. Furthermore, lens apoptosis appears to play a coordinating role. In the case of *P. andruzzii*, in close collaboration with Jochen Wittbrodt's lab (COS), we have studied the expression of marker genes involved in eye patterning, morphogenesis, differentiation and maintenance (Stemmer et al., 2015). In contrast to *Astyanax*, eye field patterning and evagination of the optic vesicles appears to proceed normally. However, the subsequent differentiation of retinal cell types is arrested during generation of retinal ganglion cells, which also fail to project correctly to the optic tectum of the brain. Retinal apoptosis progresses in a wave-like manner and eliminates progenitor cells that fail to differentiate. Thus, evolution has targeted late retinal differentiation events, indicating that there are several ways to discontinue the development and maintenance of an eye. We have also investigated the molecular evolution of the nonvisual photoreceptor melanopsin opn4m2, whose mutation contributes to the »blind« circadian clock phenotype of *P. andruzzii* (Calderoni et al.,

1 Yamamoto Y, Stock DW, Jeffery WR. Hedgehog signalling controls eye degeneration in blind cavefish. Nature (2004) 431: 844-7.

2016). Intra- and inter-species analyses suggest that the »blind« clock in *P. andruzzii* evolved because of the loss of selective constraints on a trait that was no longer adaptive. Based on this change in selective regime, we estimate that the functional constraint on cavefish opn4m2 was relaxed at ~ 5.3 Myr. This implies a long subterranean history, about half in complete isolation from the surface. The visual photoreceptor rhodopsin, expressed in the brain and implicated in photophobic behavior, shows similar evolutionary patterns, suggesting that extreme isolation in darkness led to a general weakening of evolutionary constraints on light-responsive mechanisms. In contrast, the same genes are still conserved in *Garra barreimiae*, a cavefish from Oman, that independently and more recently colonized subterranean waters and evolved troglomorphic traits.

Our work has also addressed the nature of clock output pathways. Specifically, during the review period we have collaborated with the Calabrò group (University of Naples, Italy), to identify the mechanisms whereby the circadian clock directs circadian rhythms of cell proliferation (Pagano et al., 2016). We revealed that the subcellular localization of the tumor associated, Y-box binding protein (YB-1) is tightly controlled by the circadian clock, via a circadian rhythm in SUMOylation (Figure 2). Accumulation of YB-1 in the nucleus at the beginning of the light phase serves to downregulate transcription of Cyclin A2 via direct interaction with the cyclin gene promoter. The clock control of YB-1 might thereby serves as part of a more general mechanism to restrict DNA replication to a specific temporal window to minimize the impact of sunlight-induced DNA damage.

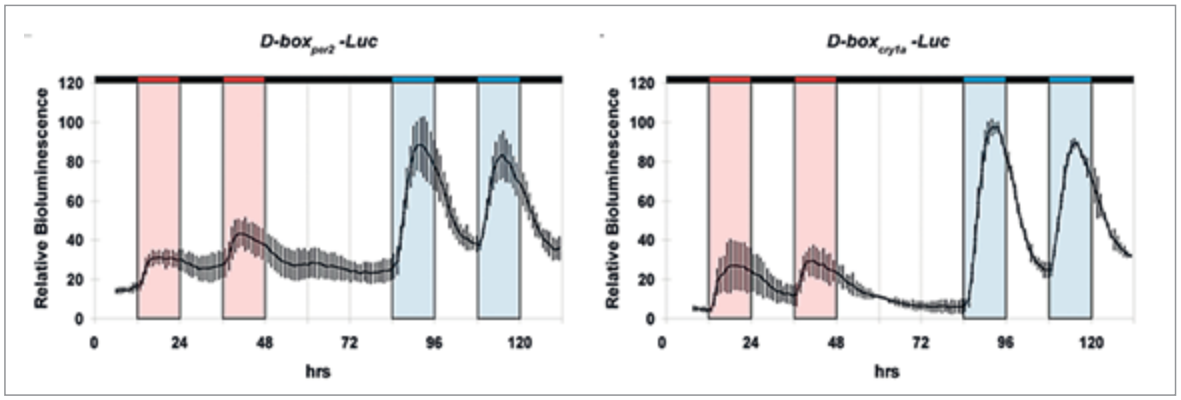


Figure 2
zfYB-1 cellular localization in zebrafish caudal fins.
(A) Immunofluorescence analysis of zfYB-1 protein in the caudal fin at ZT3 (light phase) and ZT15 (dark phase) using α-YB-1 N-ter antibody. Panels also show DAPI staining and Merge, which combines both the DAPI and YB-1 signals. White and black bars indicate the corresponding lighting conditions.
(**B**) Quantification of the panel A.

Planned research and new directions

One exciting new topic concerns how signals from the environment and the endogenous circadian clock are integrated to direct day night rhythms in metabolism. To date, this issue has been almost exclusively addressed in the mouse and has led to the conclusion that circadian metabolic rhythms in the liver are driven locally by the classical circadian clock mechanism via its core transcription translation feedback loop. This liver peripheral clock is synchronized with other peripheral clocks by the central clock in the suprachiasmatic nucleus (SCN) but can also be set independently of the SCN via feeding activity. However, this begs the obvious question, do the metabolic control systems identified in a nocturnal rodent operate in all vertebrates? If not, then how does evolution shape the regulation of metabolic pathways according to the particular needs of each organism? Using zebrafish as well as our cavefish model *P. andruzzii*, we are embarking on a metabolomic and transcriptomic analysis of the differential contribution of light and regular feeding time to setting the circadian metabolome of the liver. Our initial results have revealed that, in contrast to the mouse, light and feeding time differentially regulate metabolite rhythms in the fish liver. Thus, for example, while the phase of cycling non-essential amino acids is set by the timing of the light cycle and is unaffected by regular feeding time, essential amino acid cycling is set exclusively by the feeding time. Indeed, our results point to the existence of distinct food and light regulated clock mechanisms. Using the genetic tools available for the zebrafish and exploiting the absence of a light regulated clock in the blind cavefish, our goal is to explore the molecular mechanisms underlying the food regulated clock and how it regulates metabolism.

In a second project, again exploiting our blind cavefish model, we are exploring the interaction between light and DNA repair systems. We have identified multiple mutations in *P. andruzzii* which lead to loss of function of key elements of the mechanisms which repair UV damaged DNA. Interestingly, other DNA repair elements are highly conserved and show an upregulated expression. We plan to characterize the DNA repair systems in various species of cavefish, as a novel strategy to explore how light modulates DNA repair and how DNA repair mechanisms can be shaped by evolution in response to the environment.

Selected publications since 2013
Number of peer-reviewed articles 2013-2017: 11, number of citations 2013-2017: 94, h-index (2013-2017): 7, total h-index: 40 (according to Thomson Reuters).

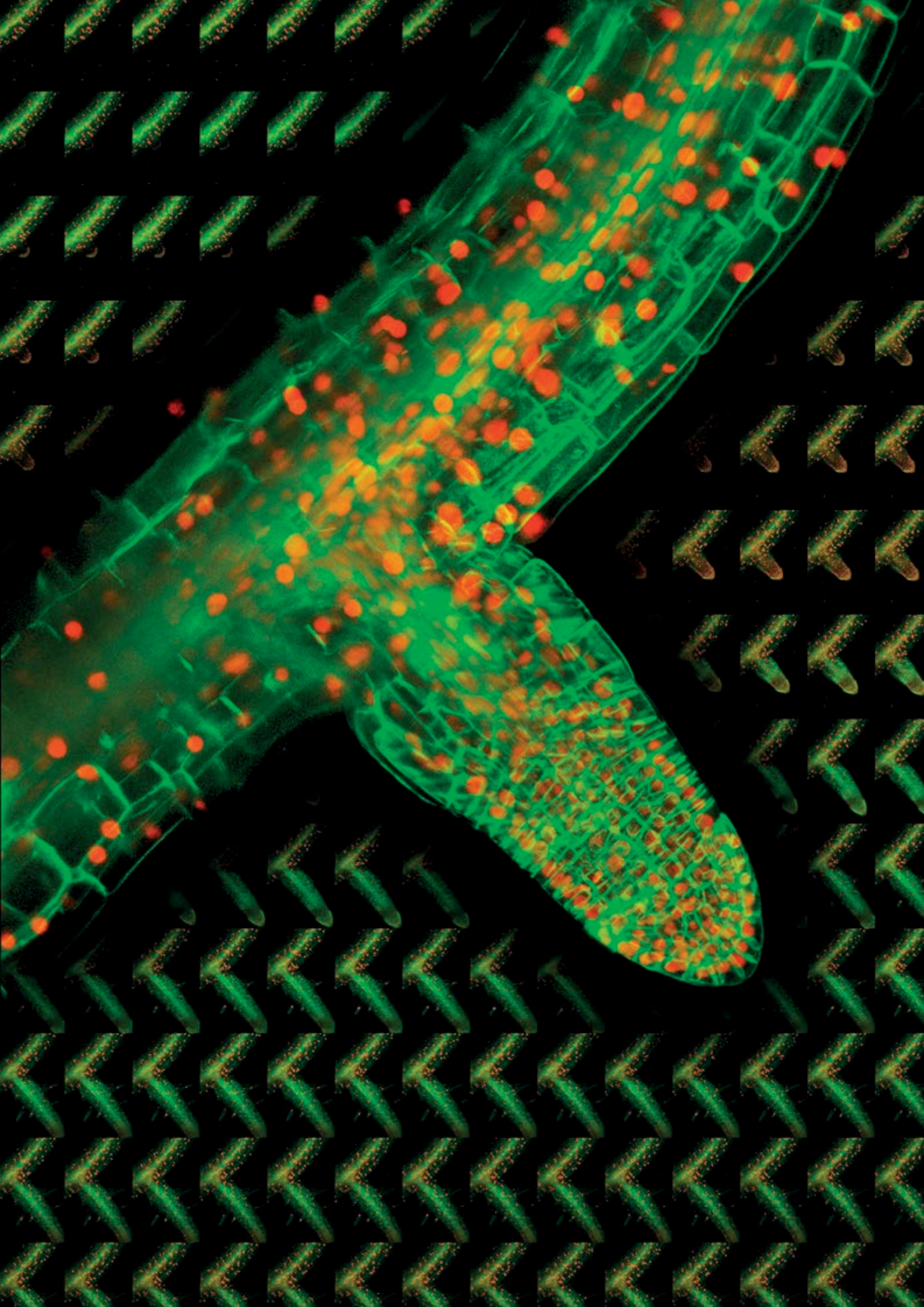
Pagano C., Di Martino O., Ruggiero G., Guarino A.M., Mueller N., Siauciunaite R., Reischl M., Foulkes N.S., Vallone D. and Calabrò V. (2017) The tumor-associated YB-1 protein: new player in the circadian control of cell proliferation. Oncotarget 8: 6193-205.

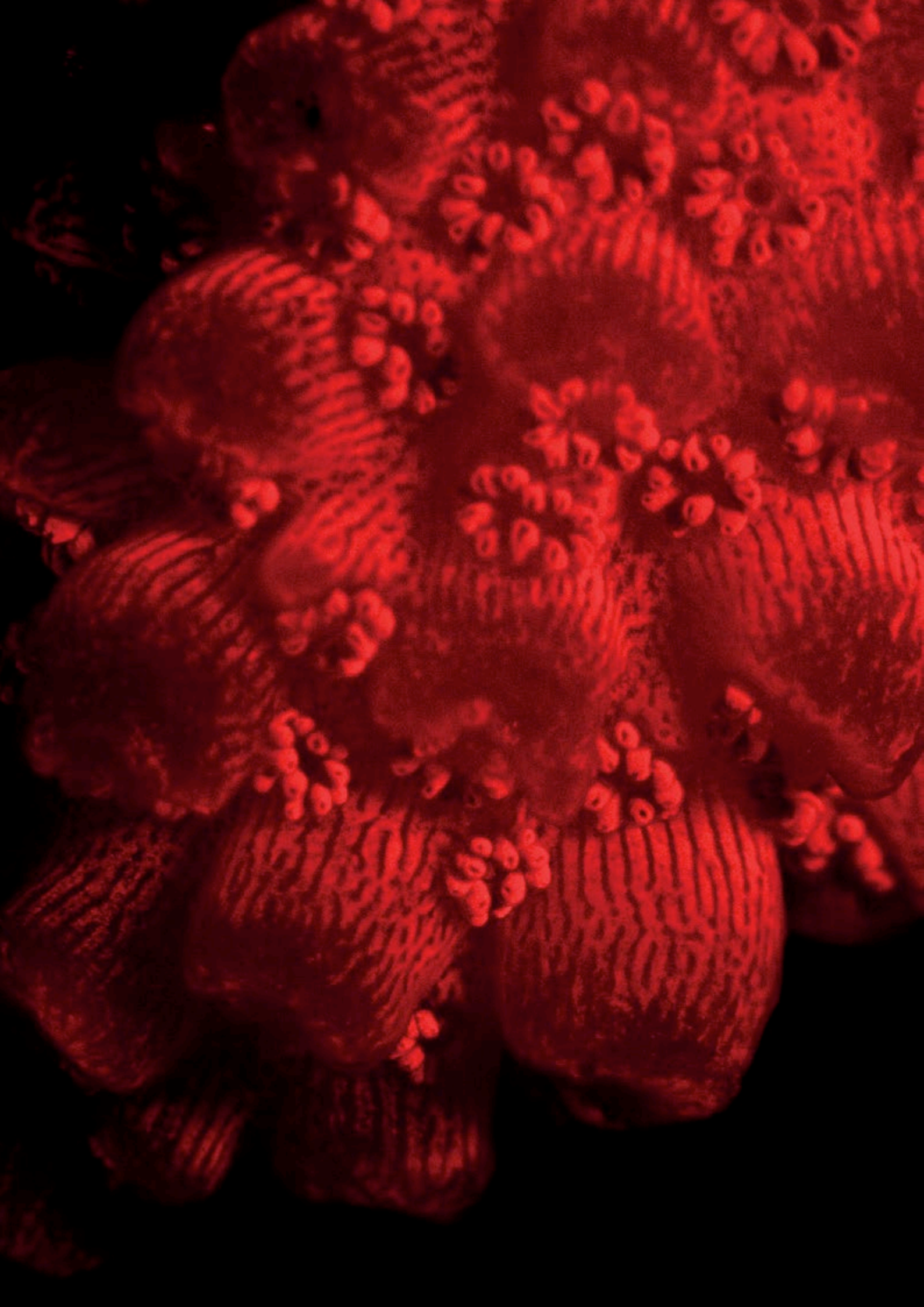
Calderoni L., Rota-Stabelli O., Frigato E., Panziera A., Kirchner S., Foulkes N.S., Kruckenhauser L., Bertolucci C. and Fuselli S. (2016) Relaxed selective constraints drove functional modifications in peripheral photoreception of the cavefish P. andruzzii and provide insight into the time of cave colonization. Heredity. 117:383-392.

Stemmer M., Schuhmacher L.N., Foulkes N.S., Bertolucci C. and Wittbrodt J. (2015) Cavefish eye loss in response to an early block in retinal differentiation progression. Development 142:743-52.

Ben-Moshe Z., Alon S., Mracek P., Faigenbloom L., Tovin A., Vatine G.D., Eisenberg, E., Foulkes N.S. and Gothilf Y. (2014) The light-induced transcriptome of the zebrafish pineal gland reveals complex regulation of the circadian clockwork by light. Nucleic Acids Res. 42: 3750-67.

Mracek P., Pagano C., Fröhlich N., Idda M.L., Cuesta I.H., Lopez-Olmeda J.F., Sánchez-Vázquez F.J., Vallone D. and Foulkes, N.S. (2013) ERK signaling regulates light-induced gene expression via D-box enhancers in a differential, wavelength-dependent manner. PLoS One. 8: e67858.





2.4 PROF. DR. STEPHAN FRINGS
ANIMAL MOLECULAR PHYSIOLOGY

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Fields of Interest
We work on molecular aspects of sensory physiology with a focus on olfaction, nociception and motor control. We examine the function of ion channels in sensory neurons, and we try to understand how these channels contribute to transduction, sensitivity and sensory performance.

Brief summary of work since 2013
Our main interest since 2013 are calcium-gated chloride channels that appear to mediate neuromodulatory functions in various neurons involved in sensory information processing. These channels belong to the anoctamin-protein family (alias TMEM16) that was discovered in 2008. We have investigated their role in olfactory transduction and in motor learning. We found that they can amplify excitatory processes and they can attenuate inhibition, depending on their expression site and on the prevailing local chloride concentration. Consequently, our research work is concerned with cellular targeting of anoctamin proteins, with the regulation of local chloride homeostasis, with electrophysiological studies of channel function, and with the behavioural effects that these channels have.

In the field of nociception, we are interested in the interactions between the olfactory system and the trigeminal nociceptive system. These two sensory pathways impact on each other. Nociceptive chemical stimuli – called irritants – change odour perception, and olfactory co-stimulation in turn alters trigeminal nociception and the perception of head ache. We are looking for points of cross-talk between the two systems in nose and brain to understand this cross-modal signal processing.

Major contributions since 2013

There are two anoctamin chloride channels operating in sensory cells, ANO1 and ANO2 (alias TMEM16A and TMEM16B). While ANO1 is expressed in somatosensory neurons, ANO2 operates in olfactory receptor neurons (ORN), in photoreceptors and in the Purkinje neurons of the cerebellum. We have started our study with the olfactory system, working out the four aspects indicated above. We found ANO2 channels to be concentrated in the chemosensory cilia of ORN, and we found that the cilia charge themselves with chloride at rest and discharge chloride during odour stimulation. We found that the chloride discharge strongly amplifies the receptor current and that mice need this amplification effect to detect unfamiliar odours at low concentrations.

In the cerebellum, we found that ANO2 channels are targeted to the dendritic tree of Purkinje neurons and that they open when the Purkinje neurons are activated by a climbing fiber. We could show that this leads to an attenuation of the inhibitory input from interneurons in such a way that the amplitudes of inhibitory postsynaptic currents decrease. This is probably the result of a diminishing local chloride gradient brought about by chloride influx through ANO2 channels. This hypothesis, however, needs to be tested. What we did find is that mice that did not express ANO2 in the cerebellum (*Ano2-/-* mice) displayed impaired motor coordination and motor learning. It thus appears that ANO2 channels are a component of cerebellar function by limiting inhibition of Purkinje neurons in the cerebellar cortex.

In our search for points of cross-talk between the olfactory and the trigeminal system, we first looked at the olfactory epithelium and the olfactory bulb. Already in these peripheral levels of signal processing, we found the two systems surprisingly entwined. Focusing first on peptidergic fibers of the trigeminal system, we found a dense innervation of the olfactory epithelium with CGRP-positive trigeminal fibers, as well as an inhibitory response of ORNs to CGRP. In the olfactory bulb, CGRP-positive fibers were present within the input circuitry, the glomerular layer, and the local circuits were inhibited by the neuropeptide. These results are in agreement with the long-standing observation that co-stimulation with irritants suppresses odour perception. Thus, we have disclosed first points of interaction between trigeminal and olfactory systems and have demonstrated their mode of interaction.

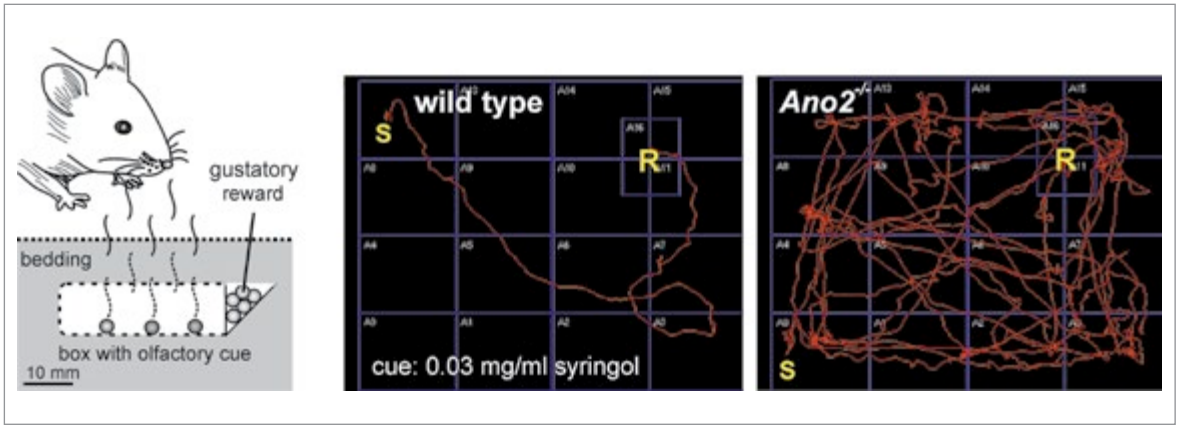

Figure 1
When confronted with an unfamiliar odor at low concentration, mice require a special signal amplification mechanism for tracking that odor. The calcium-activated chloride channel anoctamin 2 provides that amplification in olfactory receptor neurons. Anoctamin 2 knockout mice are unable to find the scented reward (R), while the tracking task is easy for wild-type mice.

Planned research and new directions

In the work on anoctamin chloride channels, we plan to complete the cerebellum project by testing our hypothesis that ANO2-mediated chloride influx attenuates network inhibition. For this we need to demonstrate that (1) the decline of inhibition correlates spatially with a local opening of ANO2 channels and (2) that the local chloride concentration near the inhibitory GABAergic synapses in the dendritic tree increases during the stimulation of climbing fibers. We have started this project in collaboration with Prof. Dr. Thomas Kuner, Medical Faculty Heidelberg, using various chloride-sensitive and pH-sensitive fluorescent dyes, brought into Purkinje cells either by internal perfusion or by viral gene transfer of appropriate GFP-variants. The goal of this project (2017–2019) is to explain the role of ANO2 in motor coordination on a molecular level.

We found ANO2 also to be expressed in the synaptic terminals of rod photoreceptors in the mouse retina. In contrast, ANO2 is absent from cone photoreceptor end feet. There is a concept for the role of calcium-gated chloride channels in the rod synapse that basically proposes a limiting function for glutamate release in the dark. We plan to investigate the role of ANO2 on the basis of this concept. Our approach is to study various aspects of scotopic vision in wild type mice and compare the relevant parameters to those obtained from *Ano2-/-* mice. This project should lead to a role of ANO2 in synaptic modulation, a new and exciting prospect for this field.

The next step in our project on olfactory/nociceptive interaction is the study of non-peptidergic trigeminal innervation of the olfactory bulb. Nonpeptidergic, sensory fibers far outnumber the peptidergic fibers in the olfactory bulb. We first need to find out, which type of activity in the bulb circuits is read out by the trigeminal system, where this information goes and, most importantly, whether it influences the signals conveyed to the second neurons in the spinal trigeminal nucleus. In collaboration with Dr. Richard Carr, Medical Faculty Mannheim, we are setting up in vivo recording from the spinal trigeminal nucleus to find out if olfactory co-stimulation affects the nociceptive signal and may hence influence the generation of headache.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 7, number of citations 2013-2017: 61, h-index (2013-2017): 4, total h-index: 37 (according to Thomson Reuters).

Genovese, F., Bauersachs, H.G., Gräßer, I., Kupke, J., Magin, L., Daiber, P., Nakajima, J., Möhrlen, F., Messlinger, K., and Frings, S. (2017) Possible role of calcitonin gene-related peptide in trigeminal modulation of glomerular microcircuits of the rodent olfactory bulb. Eur. J. Neurosci. 45, 587-600.

Genovese, F., Thews, M., Möhrlen, F., and Frings, S. (2016) Properties of an optogenetic model for olfactory stimulation. J. Physiol. 595, 3501-3516.

Zhang, W., Schmelzeisen, S., Parthier, D., Frings, S., and Möhrlen, F. (2015) Anoctamin calcium-activated chloride channels may modulate inhibitory transmission in the cerebellar cortex. PLOS ONE 10, e014216

Daiber, P., Genovese, F., Schriever, V., Hummel, T., Möhrlen, F., and Frings, S. (2013) Neuropeptide receptors provide a signalling pathway for trigeminal modulation of olfactory transduction. Eur. J. Neurosci 37, 572-582.

Dauner, K., Möbus, C., Frings, S., and Möhrlen, F. (2013) Targeted expression of anoctamin calcium-activated chloride channels in rod photoreceptor terminals of the rodent retina. Invest. Ophthal. Vis. Sci. 54, 3126-3136.

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Fields of Interest
Sensory physiology and biochemistry, signal transduction pathways in sensory neurons, ion channels

Brief summary of work since 2013
We are working on several aspects of sensory transduction and investigate the molecular components involved in the signal transduction process. We are particularly interested in ion channels of the anoctamin family of calcium-gated chloride channels. We studied the expression of anoctamin channels in the nose (Dauner et al., 2012) and in the retina (Dauner et al., 2013). We subjected the anoctamin channels to systematic mutagenesis and discovered key aspects of the channels' gating mechanism (Vocke et al., 2013). In addition, we identified anoctamins in the cerebellar cortex. Functional studies suggest that ANO2 channels are involved in a Ca^{2+}-dependent regulation of synaptic weight in GABAergic inhibition, and hence regulating ionic plasticity in the cerebellum (Zhang et al. 2015). Moreover, we could report results of behavioral studies obtained from ANO2-/- mice, a mouse line that lack this particular mode of ionic plasticity. The animals display reduced motor performance, highlighting the significance of inhibitory control for cerebellar function and introduces calcium-dependent short term ionic plasticity as an efficient control mechanism for neural inhibition (Neureither et al. 2017).

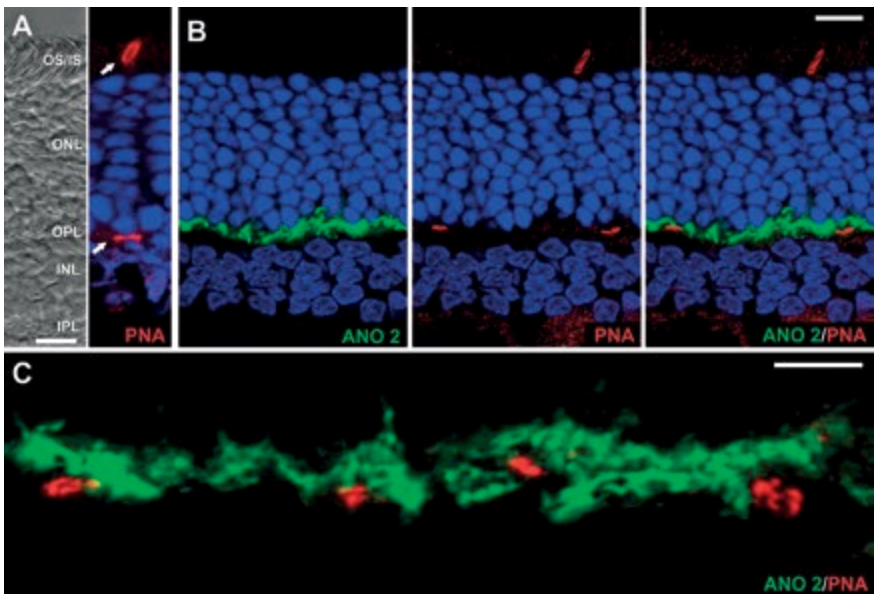

Figure 1
ANO 2 is expressed in synaptic terminals of rod spherules, but not in cone pedicles. (A) Visualization of cone photoreceptors in the rat retina with fluorescence-labeled peanut agglutinin (PNA) shows outer and inner segments (OS/IS), as well as cone pedicles in the outer plexiform layer (OPL) (B) ANO 2 immunosignals are restricted to the OPL. (C) Higher magnification reveals that PNA-positive regions in the images are ANO 2-negative, indicating that ANO 2 is absent from cone pedicles. The surrounding ANO 2-positive regions are synaptic terminals of rod spherules.

Major contributions since 2013

In collaboration with the German Cancer Research Center we have established an organellar proteome for isolated cilia of rat olfactory sensory neurons (Mayer et al., 2009). The proteome provided us with a starting point for a screening project, which leads to the molecular identification of a hitherto unknown Ca^{2+} activated Cl^- channel involved in olfactory sensory transduction (Hengl et al.2010). We studied the expression of the anoctamin channels in the nose (Dauner et al., 2012), in the retina (Dauner et al., 2013), and in various other tissues. For our studies of molecular structure-function relations in anocatamin ion channels, we combine bioinformatic analyses of domain functions with site-directed mutagenesis of proteins and biophysical studies of peptide properties. We were able to pinpoint a region in the anoctamin protein that binds calmodulin and induces inactivation (Vocke et al., 2013). This is an important finding for understanding the role of anoctamin channel in neurons.

We identified ANO2 channels also in the cerebellar cortex (Zhang et al. 2015). Functional studies reveal that ANO2 channels modulate the inhibitory input to cerebellar Purkinje cells. ANO2 channels attenuate GABAergic transmission by increasing the postsynaptic chloride concentration, hence reducing the driving force for chloride influx. Thus, ANO2 is involved in a calcium-dependent mode of ionic plasticity that reduces the efficacy of GABAergic synapses. This mechanism of short-term ionic plasticity was termed *depolarization-induced depression of inhibition* (DDI).

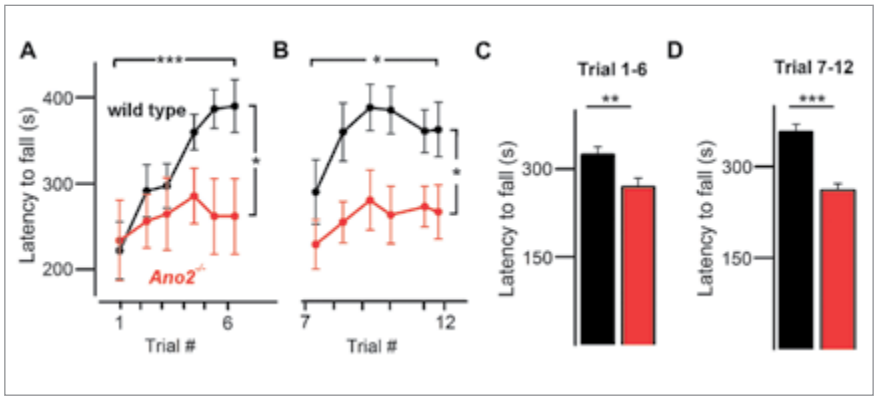

Figure 2
Impaired motor performance and learning in *Ano2-/-* mice (A) Motor performance on the accelerating rotarod increased in wild-type mice *(black)* over 6 days, while *Ano2-/-* mice *(red)* did not improve significantly (B) Wildtype mice rapidly regained motor performance levels on the rotarod after a 20-day intermission, indicating consolidated procedural memory. *Ano2-/-* mice displayed no significant motor learning ability. C) Statistical analysis of the latency to fall from rotarod (data from trials 1-6) illustrates a significantly reduced motor performance in *Ano2-/-* mice (D) In the repeated rotarod session (data from trial 7-12), a persistent difference in motor performance was seen

In a recent study (Neureither et la. 2017), we report results of behavioral studies obtained from ANO2-/- mice, a mouse line that was previously shown by us to lack this particular mode of ionic plasticity. We looked particularly for the relevance of DDI for motor control. We compared the motor performance of wildtype and ANO2-/- mice in a variety of behavioral tasks designed to specifically reveal cerebellar dysfunction. We found that ANO2-/- mice display deficiency in motor coordination and motor learning. Our results illustrate the behavioral significance of calcium-dependent modulation of inhibitory-network activity through short-term ionic plasticity, a novel pathway for controlling network function in the brain.

Planned research and new directions
In the work on anoctamin chloride channels, we plan to complete the cerebellum project by testing our hypothesis that ANO2-mediated chloride influx attenuates network inhibition. For this we need to demonstrate that (1) the decline of inhibition correlates spatially with a local opening of ANO2 channels and (2) that the local chloride concentration near the inhibitory GABAergic synapses in the dendritic tree increases during the stimulation of climbing fibers. The goal of this project (2017–2019) is to explain the role of ANO2 in motor coordination on a molecular level.

We found ANO2 also to be expressed in the synaptic terminals of rod photoreceptors in the mouse retina. In contrast, ANO2 is absent from cone photoreceptor end feet. There is a concept for the role of calcium-gated chloride channels in the rod synapse that basically proposes a limiting function for glutamate release in the dark. We plan to investigate the role of ANO2 on the basis of this concept. Our approach is to study various aspects of scotopic vision in wild type mice and compare the relevant parameters to those obtained from *Ano2-/-* mice. This project should lead to a role of ANO2 in synaptic modulation, a new and exciting prospect for this field.

Selected publications since 2013
Number of peer-reviewed articles 2013-2017: 8, number of citations 2013-2017: 57, h-index (2013-2017): 3, total h-index: 17 (according to Thomson Reuters).

Neureither F, Ziegler K, Pitzer C, Frings S, Möhrlen F. (2017). Impaired motor coordination and learning in mice lacking anoctamin 2 calcium-gated chloride channels. The Cerebellum. doi: 10.1007/s12311-017-0867-4

Genovese F, Thews M, Möhrlen F, Frings S. (2016). Properties of an optogenetic model for olfactory stimulation. J Physiol. 594(13):3501-16

Zhang W, Schmelzeisen S, Parthier D, Frings S, Möhrlen F. (2015). Anoctamin Calcium-Activated Chloride Channels May Modulate Inhibitory Transmission in the Cerebellar Cortex. PLoS One. 10(11):e0142160.

Vocke K, Dauner K, Hahn A, Ulbrich A, Broecker J, Keller S, Frings S, Möhrlen F. (2013). Calmodulin-dependent activation and inactivation of anoctamin calcium-gated chloride channels. J Gen Physiol. 142(4):381-404.

Dauner, K., Möbus, C., Frings, S., Möhrlen, F. (2013). Targeted expression of anoctamin calcium-activated chloride channels in rod photoreceptor terminals of the rodent retina. Invest Ophthalmol Vis Sci. 54: 3126-3136.


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2.5 DR. EMMANUEL GAQUEREL
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Fields of Interest
Plant Secondary Metabolism, Metabolomics, Chemical ecology (Plant-insect interactions), Computational mass spectrometry, Jasmonate signaling.

Brief summary of work since 2013
Plants adapt to their environments by diversifying in various ways. This diversification is intimately reflected in plants' fascinating capacity to evolve novel secondary metabolites. Interaction with insects is notably one of the many selection pressures thought to have sculpted plant metabolism. More generally, shedding light on the evolutionary history of plant specialized metabolism can illuminate a key question in biology: how complex phenotypic traits evolve? Eventually, the same approach can also provide molecular insights for engineering programs on plant metabolites with high economical and societal values. Since I joined the COS, my group has pursued three inter-connected research lines. First, a major focus has been dedicated to the analysis of metabolic innovations translating from allopolyploidization events in the genus Nicotiana and of their role in the arms race in which species of this genus and insect herbivores are engaged. As a case study, we delineated some of the biochemical processes linked to the innovation of highly potent insecticidal alkaloids in Nicotiana section Repandae species. Critical to this research has been the development of computational approaches to support the discovery of gene networks and metabolite identification in specialized metabolism. In particular, my lab has pioneered a BLAST-like pipeline for mass spectrometry metabolomics, analogous to that existing for genomics data, in order to accelerate the structural interpretation of previously-unknown secondary metabolites. In a third research area, my lab investigates mechanisms linking plant metabolic plasticity and core developmental processes.

Major contributions since 2013

Genomic bases to the emergence of defensive metabolic innovations
It is now clearly recognized that all modern flowering plant genomes derive from processes set in motion by a history of repeated and episodic auto- or allopolyploidy events. However, the contribution of these polyploidy events to the diversification of plant secondary metabolism and defense strategies against insects, has only rarely been examined. My lab contributed to the analysis of the reference genome of the wild tobacco species, Nicotiana attenuata, a model for plant-insect interactions. Within the consortium coordinated by Dr. Shuqing Xu (MPI, Jena), my lab conducted the evolutionary analysis of the nicotine biosynthetic pathway which emerged from tandem and whole genome duplication-derived genes from primary metabolism (Xu et al., 2017), and specific insertions of transposable elements.

Half of the species of the genus Nicotiana are allopolyploids of different ages and for some of them, the closest progenitor genomes have been mapped. We decided to focus on the analysis of »transgressive« metabolic characters that emerged from two well-mapped allopolyploidy cases in this genus. In the first one, which gave rise to the Nicotiana section Polydiclae (Figure 1), we dissected the impact that had parental gene expression reshufflings on the diversification of volatile emissions and secondary metabolism (articles in preparation). To this end, we compared NGS leaf transcriptomes and mass spectrometry (MS)-based metabolomes of the focal Polydiclae allopolyploid species and of their closest diploid progenitors, via a statistical pipeline that infers metabolic patterns innovated in allopolyploids.

In parallel to the above research, we also discovered that allopolyploid Repandae species are unique among all Nicotiana species with respect to their capacity to synthesize long chain fatty acid-based N-acyl-nornicotine compounds (NANNs) in their trichomes. We demonstrated that the fatty acid acylation of NANNs allows them to evade the resistance against nicotine and nornicotine acquired by larvae of the lepidopteran insect, Manduca sexta, and as such, that it represents a defensive gain-of-function. Using an approach combining transcriptome profiling trichome cells, as well as molecular and metabolomics methods, we identified several candidate genes recruited for NANN biosynthesis (article in preparation). Among these, NAT1, which originates from the paternal genome, shares a high amino acid homology with PUN1, the N-acyltransferase responsible for the ultimate step in the synthesis of capsaicin, the pungent metabolite present of chili peppers (Figure 1).

Computational approaches to infer evolutionary innovations at the metabolome level
Advances in the resolution and sensitivity of MS instrumentations allow the measurement of an exponentially growing volume of metabolites. Yet, interpretation of metabolite functions does not keep pace with the amount of data produced. Ideally, metabolomics data analysis should facilitate hypothesis formulation for previously unknown metabolites. With this objective in mind and using resources available at the Metabolomics Core Technology Platform (MCTP), we pioneered a »BLAST-like« pipeline through which metabolite mass spectra are aligned and their degree of similarity is scored for metabolic tree construction. This pipeline, including an interactive graphical user interface (Figure 2), is implemented in the R package MetCirc (https://bioconductor.org/packages/release/bioc/html/MetCirc.html) and a recent article (Naake et al., 2017). As illustrated in two recent research articles (Li et al., 2015 and 2016), this pipeline allows mining intra- and inter-specific variations in metabolic profiles in order to identify causal metabolic genes. A highlight of the second publication (Li et al., 2016) is the identification of UDP-glycosyltransferases responsible for the biosynthesis of flower limb-specific flavonoid glycosides.

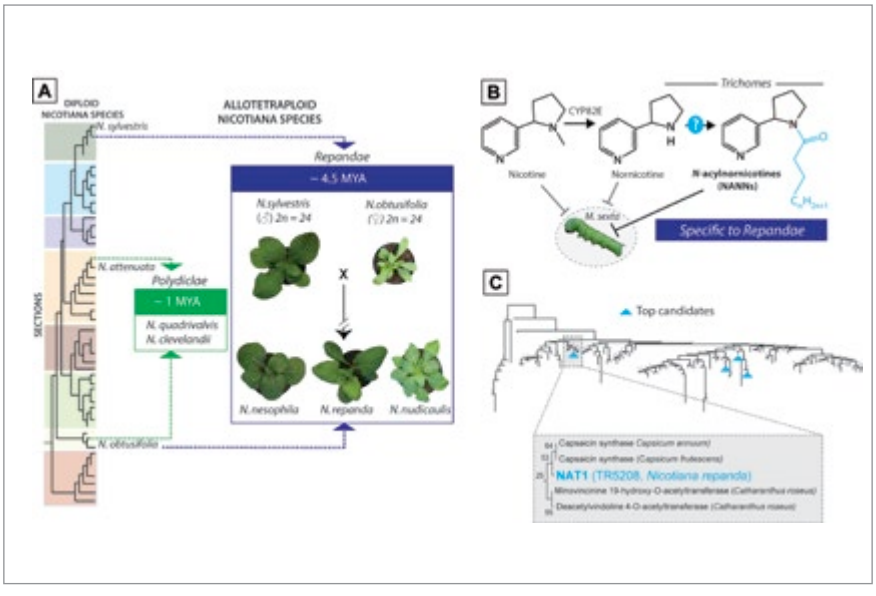

Figure 1
NAT1 is a novel *N*-acyltransferase controlling the synthesis of insecticidal alkaloids. A. Allopolyploid *Nicotiana* species studied by the research group. B. Proposed metabolic scheme for NANN biosynthesis. NANNs are superior toxins evading *M. sexta* resistance to nicotine (dashed ellipse, bioassay data not shown). C. Neighbor-joining (1000 iterations) phylogenetic reconstruction for 95 predicted BAHD acyltransferase proteins. Top candidates from our comparative NGS screen are highlighted.

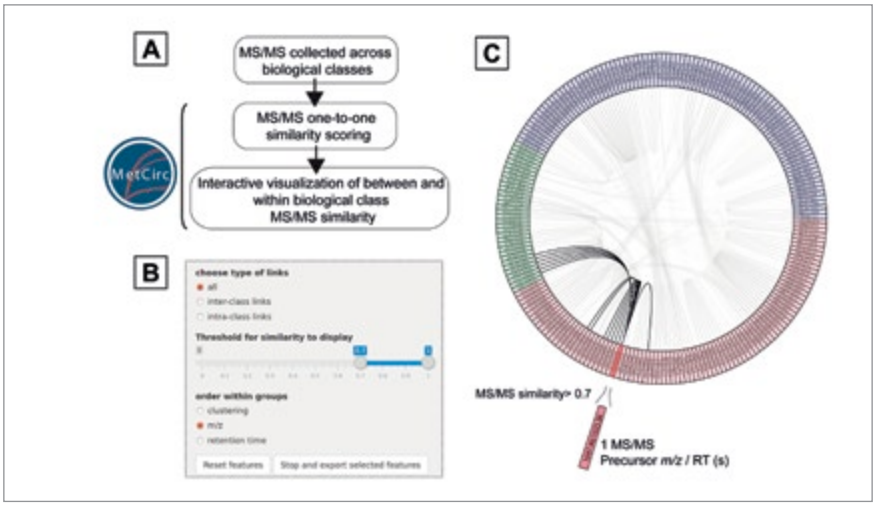

Figure 2
MetCirc, an interactive R package for phylogenetics-inspired MS metabolomics data analysis. A. Pipeline. B. Interactive interface to explore data based on metabolite-derived mass spectra (MS/MS) similarity scores or biological class assignment. C. Interactive circular visualization in which colored sectors denote for biological classes (species, tissues, etc ...), smaller features for detected compounds and edges for pairwise similarity scores for a given a user-defined threshold. This pipeline allows the rapid detection of identical and structurally-related metabolites across species/tissues.

Regulatory links between plant metabolism plasticity and development
My group has a strong interest in transposing metabolomics profiling to questions related to plant developmental processes. This interest was notably materialized through work on the jasmonate-dependent regulation of flower metabolism and opening process in tobacco species (Stitz et al., 2014). A second manuscript based on work conducted in Heidelberg is currently in preparation about the role of a COI1-like paralog, originating from a Nicotiana-specific duplication of COI1, and that acts as a sub-functionalized jasmonate receptor controlling flower metabolism (article in preparation). Finally, my lab, in collaboration with the MCTP, provides the metabolomics expertise to a collaboration project with the group of Prof. Dr. Alexis Maizel on the metabolic underpinnings for lateral root production in Arabidopsis.

Planned research and new directions
Key to the research agenda of my lab is to pursue our investigations on plant secondary metabolism. In the coming years, this perspective articulates around (i) the abovementioned research on the assembly of novel defense-related metabolic pathways in Nicotiana allopolyploids, (ii) the MetCirc method development to cross-compare plant metabolomes and (iii) newly initiated metabolomics investigations on the bioeconomy C4 grass, Miscanthus sinensis.

(i) Planned research notably consists in the analysis of structural determinants allowing plant specialized metabolites such as NANNs to evade a highly-efficient insect counter-adaptation. To this end, we are currently employing MS-metabolomics combined with stable-isotope metabolite labelling to track the post-ingestive fate of NANNs in the insect. We hypothesize that the stability of these N-amide-based derivatives to the high pH transition imposed by the ingestion process is key to the toxicity of these compounds. The concerted action of several N-acyltransferases in the biosynthesis of the NANN chemotype of Repandae species is likely to underscore intriguing analogies with the biosynthesis of other defensive metabolites within Solanaceae species. Besides, classical biochemical characterization work, comparative sequence analysis and homology-based modeling between NANN- and other N-acyltransferases will be conducted to pinpoint on key residues that are critical for long chain acylation.

(ii) In continuity with the development of MetCirc (Naake et al., 2017), our ambition is to further refine its integration with genomics data (Li et al., 2016) and its accessibility within the metabolomic community. This method development will benefit from novel MS analytical resources acquired by the MCTP platform, to conduct high-resolution metabolite fragmentation studies and alleviate some of the main bottlenecks in metabolite identification.

(iii) In the context of a PhD project (Linn Voss) within the Bioeconomy BBW graduate school and in collaboration with the groups of Prof. Dr. Thomas Rausch and Prof. Dr. Ruediger Hell, we are currently investigating lignin flux regulation in the bioeconomy C4 grass, Miscanthus sinensis. This project involves metabolomics characterization of the phenolic metabolome of this grass as well the molecular characterization of the role of a bifunctional phenylalanine/tyrosine ammonia lyase gene (PTAL), specific to monocots, in adjusting phenolic fluxes under agriculturally-relevant stress conditions.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 19, number of citations 2013-2017: 91, h-index (2013-2017): 7, total h-index: 15 (according to Thomson Reuters).

*Corresponding author

Naake, T., and Gaquerel, E*. (2017). MetCirc: Navigating mass spectral similarity in high-resolution MS/MS metabolomics data. Bioinformatics. doi: 10.1093

Gaquerel, E*., and Stitz, M. (2017). Insect resistance: An emerging molecular framework linking plant age and JA signaling. Molecular plant 10, 537-539.

Li, D., Heiling, S., Baldwin, I.T., and Gaquerel, E*. (2016). Illuminating a plant's tissue-specific metabolic diversity using computational metabolomics and information theory. Proc Natl Acad Sci U S A 113, E7610-E7618.

Li, D., Baldwin, I.T., and Gaquerel, E*. (2015). Navigating natural variation in herbivory-induced secondary metabolism in coyote tobacco populations using MS/MS structural analysis. Proc Natl Acad Sci U S A 112, E4147-4155.

Stitz, M., Hartl, M., Baldwin, I.T., and Gaquerel, E*. (2014). Jasmonoyl-L-isoleucine coordinates metabolic networks required for anthesis and floral attractant emission in wild tobacco (Nicotiana attenuata). The Plant cell 26, 3964-3983.


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2.6 PROF. DR. THOMAS GREB
DEVELOPMENTAL PHYSIOLOGY

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Fields of Interest
Our lab is interested in principles of growth and cell fate regulation in multicellular organisms. As a model we use radial plant growth which generates wood and, thus, a large proportion of terrestrial biomass.

Brief summary of work since 2013
Our lab is interested in the alignment of single cells and tissues with the requirements of the whole organism. This is a classical developmental problem and highly complex by nature. To shed light on this systemic complexity, we focus on a striking growth process found in plants: the continuous thickening of shoots and roots. This process, which is also designated as radial growth, is characterized by the constant production of vascular tissues at the periphery of organs and performed by a stem cell niche called the vascular cambium. The cambium produces two different types of vascular tissues, the xylem and the phloem, in a strictly bidirectional manner. Thereby it generates wood and a large proportion of terrestrial biomass. In our lab we take advantage of the unique features of this system and dissect the nature of cell fate decisions, intercellular communications, cambium patterning and long distance coordination of cambium activity. Since 2013 we thereby revealed a bipartite organization of the cambium-borne stem cell pool (Gursanscky et al., 2016). We discovered that the transcription factor WOX4, acting as a local promoter of stem cell attributes, balances the action of two hormones: strigolactones and brassinosteroids (Jouannet et al., in preparation). In turn, we observed that WOX4 activity is repressed by the auxin response factor ARF5 thereby stimulating cell differentiation (Brackmann et al., 2017). Moreover, we learned that phloem specification is under strict control of SMXL proteins (Wallner et al., 2017).

Major contributions since 2013
Stem cell homeostasis in shoot and root tips depends on negative regulation by ligand-receptor pairs of the CLE peptide and leucine-rich repeat receptor-like kinase (LRR-RLK) families. In a study performed by Nial Gursanscky and colleagues we showed that the LRR-RLK MORE LATERAL GROWTH1 (MOL1) is necessary for cambium homeostasis (Gursanscky et al., 2016). By employing promoter reporter lines, we revealed that MOL1 is active in a domain that is distinct from the domain of the positively acting CLE41/PHLOEM INTERCALATED WITH XYLEM (PXY) signaling module. In particular, we showed that MOL1 acts in an opposing manner to the CLE41/PXY module and that changing the domain or level of MOL1 expression both result in disturbed cambium organization. Underlining discrete roles of MOL1 and PXY, both LRR-RLKs were not able to replace each other when their expression domains were interchanged. Furthermore, MOL1 but not PXY was able to rescue CLAVATA1 (CLV1) deficiency, an LRR-RLK counterbalancing stem cell activity in the shoot apical meristem. Our findings provided evidence that common regulatory mechanisms in different plant stem cell niches are adapted to specific niche anatomies and indicated the importance of a bipartite organization of intercellular signaling cascades for a strictly bidirectional tissue production. Beyond characterizing the function

of MOL1 and its relation to the CLE41/PXY cascade, the identification of molecular markers for different cambium domains provided excellent tools for dissecting cambium organization and accessing those domains in the context of several projects.

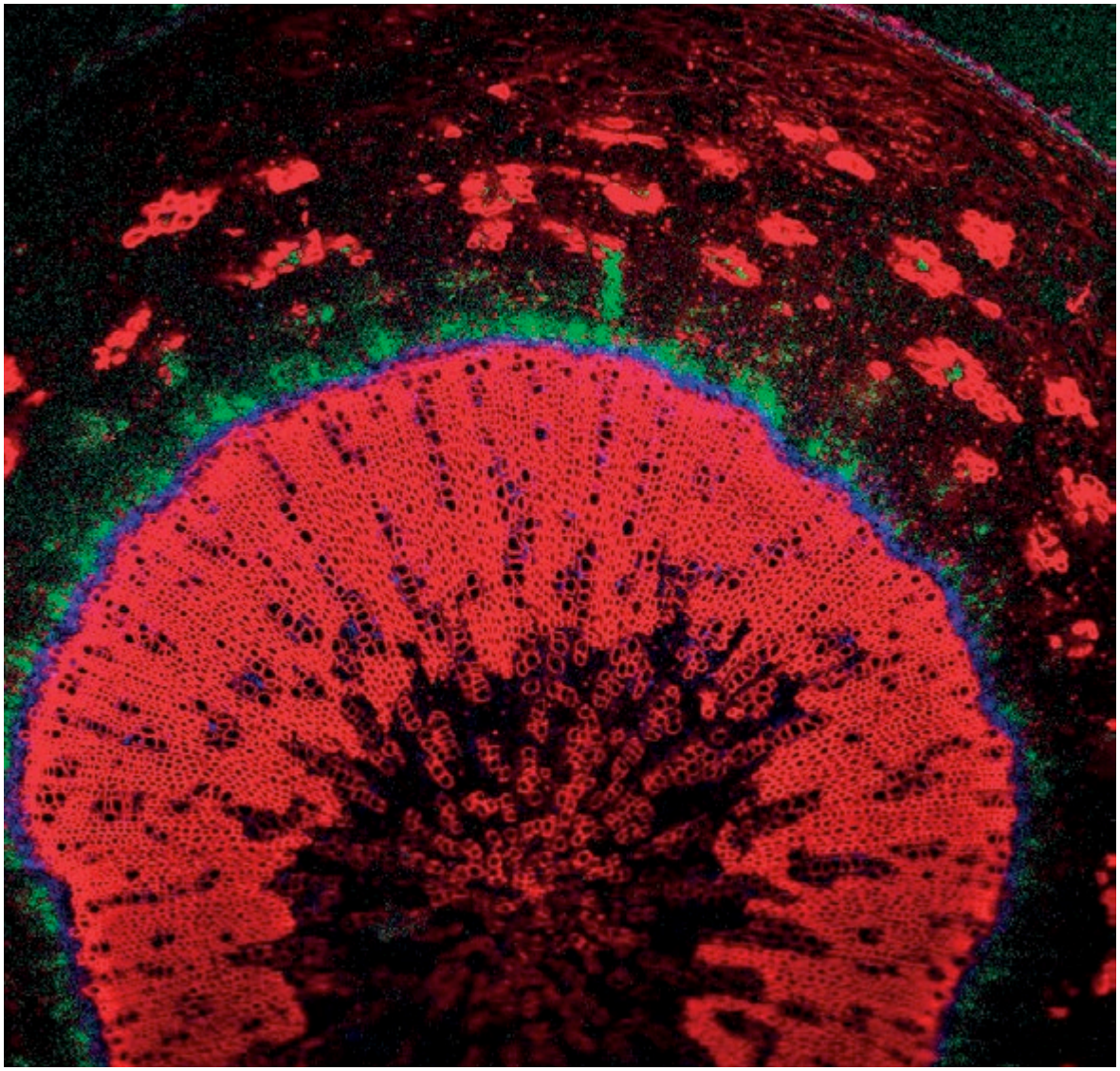

Figure 1
Cross section of an Arabidopsis hypocotyl. Various tissues are visualized in different colours. Red: xylem (central) and phloem fibres (peripheral); Blue: proximal cambium domain (highlighted by a pPXY:CFP marker); green: distal cambium domain (highlighted by a pSMXL5:YFP marker).

Fine-tuning of hormonal signaling is pivotal for the robust performance of plant stem cell systems and the coordination of cellular functions in general. The cambium fundamentally depends on auxin signaling but the function and the spatial organization of signaling domains was obscure for several decades. In a study performed by Klaus Brackmann and colleagues, we showed that, while auxin signaling levels increase in differentiating cambium descendants, a moderate level of auxin signaling in stem cells is essential for cambium activity (Brackmann et al., 2017). By local modulation of auxin signaling and the genome-wide identification of target genes, we identified the auxin-dependent transcription factor ARF5/MONOPTEROS as a promoter of cell differentiation acting directly in cambium stem cells. ARF5 fulfils its function by attenuating the activity of the stem cell-specific WUS-RELATED HOMEOBOX4 (WOX4) gene, which is central for maintaining cells in an undifferentiated state. These results revealed the influence of auxin signaling on distinct cambium features by employing specific signaling components. In a second study, Virginie Jouannet and colleagues identified direct targets of WOX4. They showed that WOX4 activates components of the strigolactone signaling pathway but represses components of the brassinosteroid pathway in a very local fashion. Through these findings we were able to enlighten the role of WOX4 as a mediator of auxin signaling in the cambium (Suer et al.,

2011) and to deepen our previous description of a positive role of SL signaling on cambium activity (Agustí et al., 2011). Overall, we identified differences and commonalities between the regulations of different plant stem cell niches allowing the conceptual integration of plant stem cell systems with distinct anatomies. This aspect is especially explored in the context of the SFB 873 »Maintenance and Differentiation of Stem Cells in Development and Disease«.

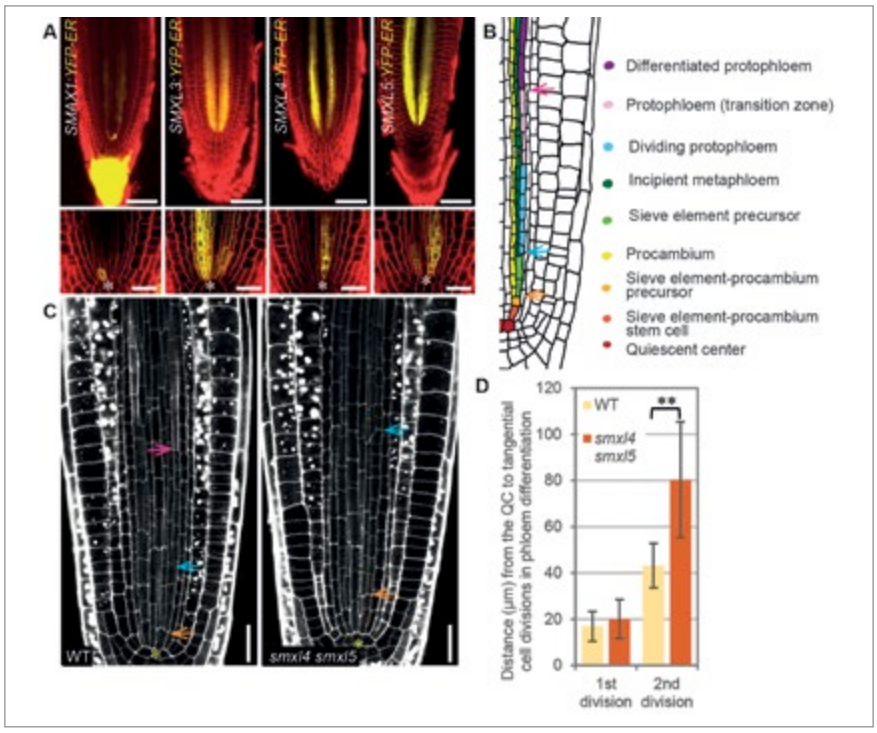

Figure 2
(A) SMXL3, 4 and 5 promoter activities in root tips of 7-day-old seedlings in comparison to the promoter of the SMXL gene SMAX1. The overlay of YFP (yellow) and FM4-64 (red)-derived signals is depicted. Scale bars represent 50 mm (top) or 20 mm(bottom). The QC is marked by white asterisks.
(B) Schematic overview of protophloem differentiation in roots. The first tangential cell division of the sieve element (SE) procambium-precursor is indicated by an orange arrow, the second tangential cell division of the SE precursor is marked by a blue arrow. The transition to differentiated protophloem SE strands is marked by a pink arrow.
(C) Disturbed phloem development in smx4;smxl5 double mutants. Tangential cell divisions in protophloem differentiation of 2-day-old roots. Arrows are described in (H). Differentiated sieve element are indicated by a pink arrow. Yellow asterisks indicate the QC. Scale bars represent 20 mm.
(D) Quantification of the distance of the first and second tangential cell division from the QC (n = 18). Welch's t test was performed.

In addition to hormonal control, plant stem cells require long-distance transport of energy metabolites along the phloem tissue. Although the phloem is the major vascular tissue produced centrifugally by the cambium, it was unclear how specification of phloem cells is controlled. In an attempt to change this situation, Eva-Sophie Wallner and colleagues showed that the genes SUPPRESSOR OF MAX2 1-LIKE3 (SMXL3), SMXL4, and SMXL5 act as cell-autonomous key regulators of phloem formation. The three genes formed an uncharacterized subclade of the SMXL gene family that mediates SL signaling. SLs depend on the F-box protein MORE AXILLARY GROWTH2 (MAX2). SL 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 SL signaling was unknown. In her study Eva-Sophie demonstrated that, within the SMXL gene family, specifically SMXL3/4/5 deficiency results in strong defects in phloem formation, altered sugar accumulation, and seedling lethality. By comparing protein stabilities, we showed that SMXL3/4/5 proteins function differently to canonical SL signaling mediators, although being functionally interchangeable with those under low SL signaling conditions. These observations revealed a fundamental mechanism of phloem formation and indicated that diversity of SMXL protein functions is essential for a steady fueling of plant stem cell systems.

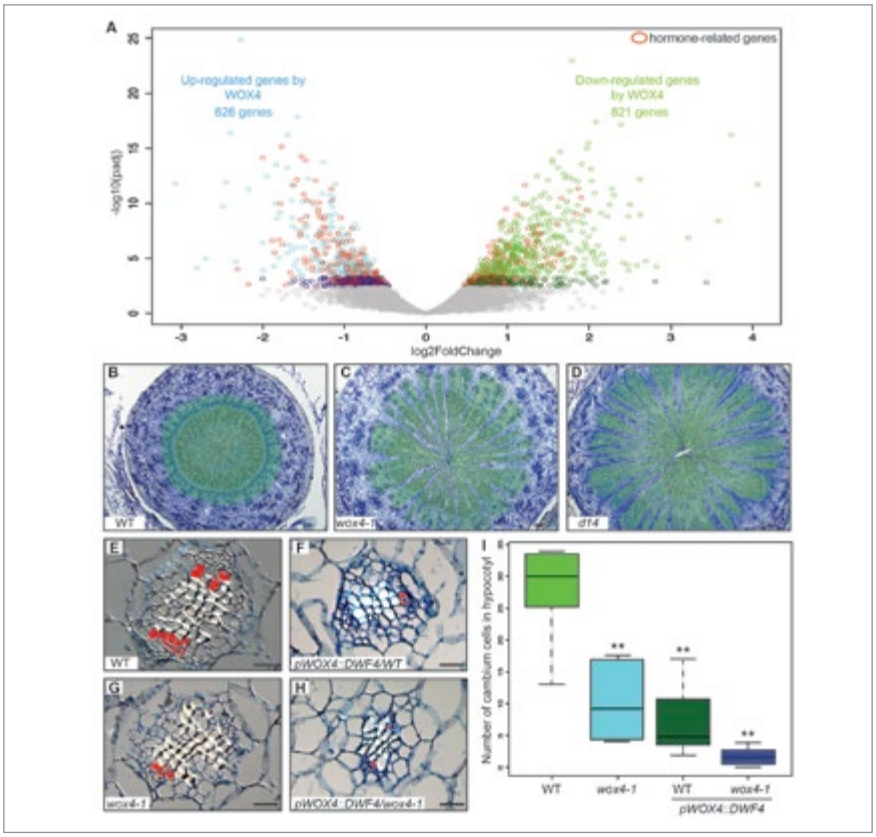

Figure 3
(A) Identification of *WOX4-dependent* genes. Volcano-Plot of RNA sequencing results from RNA extracted from *pWOX4:WOX4-GR/wox4-1* seedlings. The comparison was made between seedlings in which WOX4 was activated by dexamethasone or with a control solution. The blue dots represent genes up-regulated by WOX4, the green dots represent genes down-regulated by WOX4. Red dots highlight hormone-related genes altered by WOX4.
(B-D) The SL-signalling mutant *d14* shows a comparable cambium defect as the *wox4* mutant. Toluidine blue-stained cross-sections of mature hypocotyls. The stele anatomy is highlighted in green.
(E-H) The number of cambium stem cells is reduced by local expression of the BR biosynthesis gene *DWF4*. Toluidine blue-stained cross-sections of young hypocotyls. Cambium cells are highlighted in red.
(I) Quantification of the number of cambium cells in cross-sections. (** for p-value < 0.05).

Planned research and new directions

Two important milestones achieved since 2013 had major impacts on our research and allowed the initiation of several new and exciting directions. First, this was the transfer of our group from Vienna to Heidelberg at the beginning of 2015 enabled through the approval of a Heisenberg fellowship by the DFG. Second, the recruitment of an ERC Consolidator grant in 2015 boosted our research substantially and facilitated our integration into the Heidelberg research community.

Naturally, expansion of differentiated and rigid organs does not only involve the integration of developmental but also mechanical constraints. Because plant cell walls are the major determinants of these properties, we are currently aiming for the characterization and modulation of cell wall properties in the context of radial plant growth. Here, we established Brillouin spectroscopy in our lab in collaboration with the Advanced microscopy facility of the Vienna BioCampus and the Heidelberg Molecular Life Sciences (HMLS) equipment program (Elsayad et al., 2016). Brillouin spectroscopy is an emerging all-optical method for determining mechanical properties of sub-cellular structures and radial plant growth is currently used by us as one case study to determine its applicability on distinct plant growth processes. These activities are also integrated into the DFG research consortium FOR2581 »Morphodynamics«.

The second development which materialized in 2016 is computational modelling of radial plant growth. Ivan Lebovka, a PhD student in the lab, integrated distinct intercellular communication cascades regulating cambium activity and patterning to establish a dynamic 2D-model of an organ growing laterally. Strikingly, the model can simulate tissue conformations found in various genetic backgrounds harboring disturbed cambium regulation suggesting that the predictive power of the model is high. This model will not only be developed further but also be integrated into different projects running in the lab as another tool to understand the non-intuitive behavior of a complex and difficult-to-observe growth process.

Third, by discovering that SMXL proteins are major determinants of phloem formation, we opened a new research direction toward molecular mechanisms of cell fate specification. SMXL proteins are nuclear proteins whose molecular function is obscure. Dissecting this function will not only reveal the mode of action of a class of novel developmental regulators but also allow the integration of development and physiology – an effort in which context we were part of the former EcTop initiative »Metabolism and Development« of the Heidelberg Excellence Cluster.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 11, number of citations 2013-2017: 957, h-index (2013-2017): 5, total h-index: 14 (according to Thomson Reuters).

Brackmann K, Jouannet V, Qi J, Schlamp T, Grünwald K, Sanchez P, Greb T (2017) Spatial specificity of auxin responses coordinates wood formation. bioRxive doi: https://doi.org/10.1101/142885

Wallner, E.S., López-Salmerón, V., Belevich, I., Poschet, G., Jung, I., Grünwald, K., Sevilem, I., Jokitalo, E., Hell, R., Helariutta, Y., Agustí, J., Lebovka, I., and Greb, T. (2017) Strigolactone and karrikin-independent SMXL proteins are central regulators of phloem formation. Curr Biol 27(8):1241–1247.

Elsayad K, Werner S, Gallemi M, Guajardo ERS, Zhang L, Jaillais Y, Greb T, Belkhadir Y (2016) Correlative 3D mapping of high-frequency mechanical properties of the extracellular matrix in live plant cells. Sci Signal 9(435):RS5.

Greb, T., and Lohmann, J.U. (2016) Plant Stem Cells. Curr Biol 26(17):R816-R821.

Gursanscky, N.R., Jouannet, V., Grünwald, K., Sanchez, P., Laaber-Schwarz, M., and Greb, T. (2016) MOL1 is required for cambium homeostasis in Arabidopsis. Plant J 86(3):210-220.

2.7 DR. GUIDO GROSSMANN
INDEPENDENT RESEARCH GROUP MOLECULAR ORGANIZATION OF CELLULAR MEMBRANES

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Fields of Interest
Cell polarity, plasma membrane organization, cytoskeleton, membrane transport, cell-cell communication, environmental sensing, organismal development in complex environments.

Brief summary of work since 2013
The Grossmann group studies root-environment interactions on the cellular and multi-cellular scale. Our primary line of research utilizes root hair development in Arabidopsis thaliana as a model system to understand phenotypic plasticity on the single-cell level. Over the past years, we focused on the initiation and regulation of polar tip growth. We compiled protein localization data into a detailed time-line of the early steps of root hair initiation and identified novel candidates with roles in creating distinct polar domains at the plasma membrane, recruiting the tip growth machinery into these domains, or regulating cell expansion.

A second line of research is the study of calcium signalling and cell-to-cell communication in plants. We identified and quantified spatio-temporal calcium signatures in processes as diverse as fertilization-triggered signalling in gametes or environmental sensing in roots. Our current focus lies on stress-elicited intercellular signal propagation across root tissues to understand how plants communicate information about stress conditions to distal cells to enable systemic responses.

Microfluidic technology development for root imaging with environmental control is the third pillar of our laboratory. We are developing RootChips, integrated microfluidic perfusion and imaging platforms that facilitate microscopic studies of root development and root-environment interactions under precisely controlled conditions. A major challenge in the field of root-environment interactions was to experimentally simulate environmental complexity. Our newly developed organ-on-a-chip devices now provide the possibility to investigate questions of cell-autonomy and systemic control of root development in dynamic and non-uniform microenvironments.

Major contributions since 2013
Plants possess the ability to adapt their overall architecture in response to changing environmental conditions. To understand the mechanisms that enable and determine the plasticity of plant development we need to decipher the signalling networks that help process external stimuli and the molecular machineries that determine plant shape from cells to organs.

Over the past years, we employed genetically encoded biosensors to advance our understanding of intracellular signalling events, in particular regarding transient elevations of the second messenger calcium. Calcium plays a pivotal role in numerous cellular processes, and calcium elevations can occur as single amplitudes or as oscillations and their intracellular dynamics are known to encode specific information about the stimulus (Dodd et al. 2010, Allen et al. 2001). The visualization of calcium signals can also be used to detect signalling between cells. In a collaboration with Thomas Dresselhaus (Regensburg) we recorded calcium signals during fertilization in three cell types of the female gametophyte of Arabidopsis thaliana, namely synergids, central cell and egg cell (Denninger et al., 2014). We observed oscillatory calcium transients in synergids upon contact with the approaching pollen tube, as well as specific calcium signals in the central and egg cell upon sperm cell release from the pollen tube and additional signatures in the moment of plasmogamy.

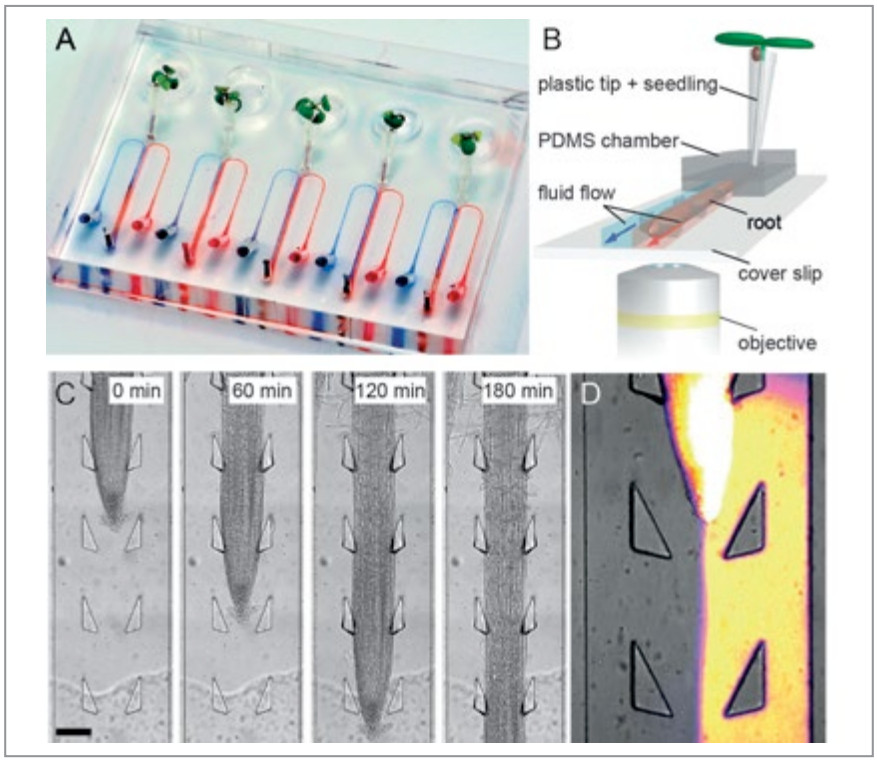

Figure 1
Chips for plant science. The Grossmann lab uses organ-on-chip technology to enable quantitative measurements on roots under dynamic conditions. The depicted dual-flow-RootChip utilizes laminar flow for asymmetric perfusion of growing roots. This technique was key to gain new insights how roots perceive and respond to external stimuli and develop in heterogeneous environments.

There is mounting evidence that calcium signatures exhibit specificity to different stimuli also on the multicellular level (Behera et al., 2016; Choi et al., 2014). In a collaboration with Karin Schumacher (COS), we detected calcium patterns that propagated within roots with stimulus-specific characteristics (Keinath et al. 2015). This intercellular communication is thought to depend on additional responses including, but not limited to, surface-potential changes and the production of reactive oxygen species (ROS) (Dubiella et al., 2013; Steinhorst and Kudla, 2014). We developed methods to trace calcium elevations quantitatively in Arabidopsis roots as they propagate within the organ to determine propagation dynamics upon treatment with different stress elicitors and eventually reveal mechanisms of signal propagation and information encoding (Brugman et al., unpublished). We focused on responses to PAMPs (pathogen-associated molecular patterns) such as bacterial peptide flg22 or the elicitor chitosan. The spatial calcium distribution in response to PAMPs is amenable to tracking of the calcium wave front and lets us extract data on involved root zones, cell types, directionality and velocity. At the same time, pharmacological and genetic approaches are being used to unveil the underlying mechanisms of rapid cell-cell communication.

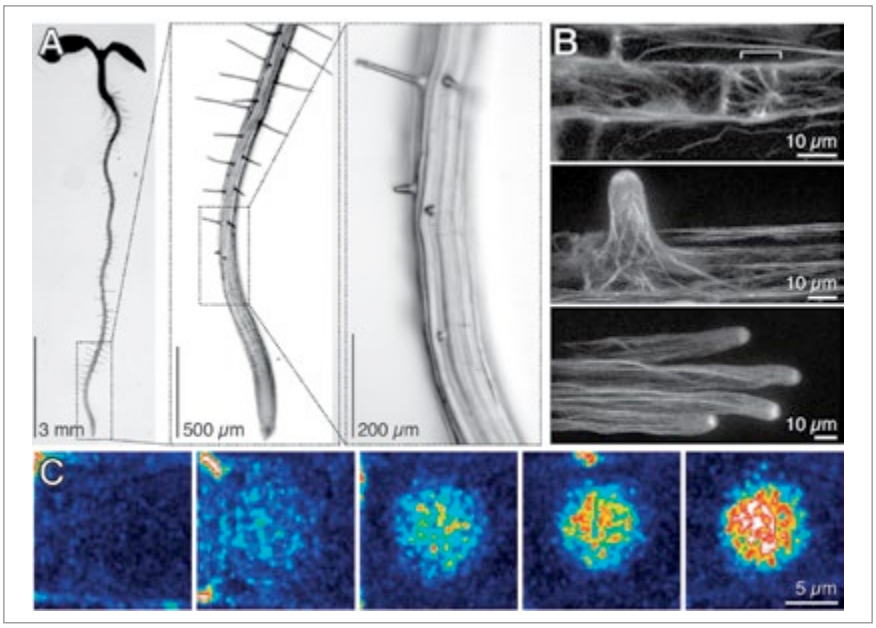

Figure 2
Emergence of cell polarity. Root hairs serves as model for the initiation and manifestation of a new polar axis in cells. The Grossmann lab studies timed interactions within polarity networks that drive symmetry breaking, polar membrane protein recruitment, and the regulation of the tip growth machinery.

To address the regulation of the molecular machinery of cellular growth under changing environmental conditions, we use Arabidopsis root hair development as model system. Despite the conserved nature of polar growth, our understanding of the mechanisms how the plasma membrane is specified locally and how protein recruitment is coordinated is rather limited. Before aiming to understand the regulation of tip growth through environmental conditions, we therefore focussed on resolving the step-wise build-up of the molecular machinery and followed the timed association of 34 proteins involved in root hair initiation (Denninger et al., unpublished). We primarily analysed known and putative interactors of the Rho-like GTPases of plants (ROPs) that play a central regulatory role in tip growth. The identification of putative interactors was partly guided by the Arabidopsis membrane protein interactome network (MIND) (Jones et al. 2014), to which we contributed to. Our time-line analysis of root hair initiation identified candidates that not only associated with the root hair initiation domain concurrently with the GTPase ROP2, but also exhibited, through genetic and microscopic analyses, to play a key role in hair initiation. Even functional ectopic domains could be generated in lines overexpressing selected markers (Denninger et al., in preparation). Our results suggest a mechanism involving recruitment factors that mark the root hair initiation domain and are necessary for the polar accumulation of Rho-GTPases to the plasma membrane.

In addition to the regulatory machinery of polar growth, we recently collaborated with Christopher Grefen (Tübingen) on the characterization of the GET (guided entry of tail-anchored proteins) pathway in plants, identifying a requirement of this pathway for root hair growth (Xing et al. 2017).

Our research on intercellular communication as well as on root development depends on live-imaging of roots under controlled environmental conditions. We had previously developed the RootChip, a microfluidic imaging and perfusion platform for Arabidopsis roots (Grossmann et al., 2011). In collaboration with Claire Stanley (ETH Zürich), we developed a series of new devices, including, among others, a design that allows investigating how roots perceive and respond to local stimuli and develop in asymmetric environments (Stanley, Shrivastava et al. 2017). Here, one side of the root can be exposed to beneficial and the other side to adverse conditions. Using this device, we demonstrated cell-autonomous regulation of root hair growth under local availability of phosphate, a nutrient that often exhibits patchy distribution in soil.

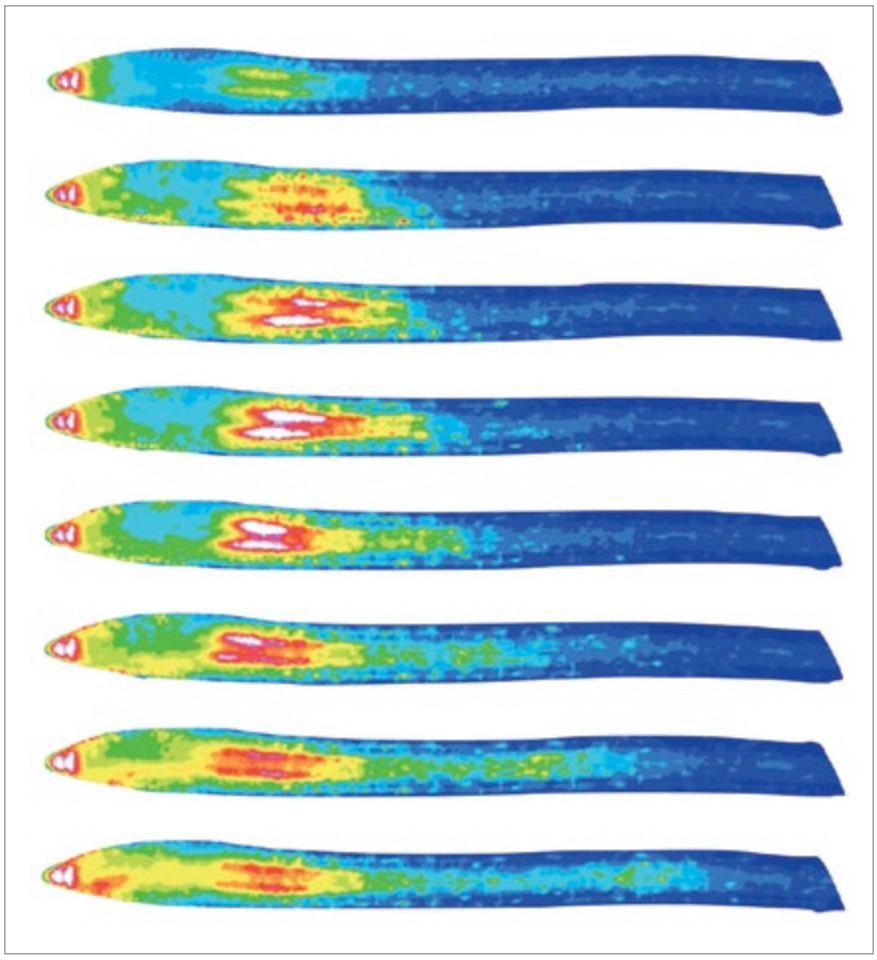

Figure 3
From environmental sensing to cell-cell communication. Using genetically encoded biosensors, the Grossmann lab visualizes stimulus-specific signalling events to understand information transfer in plants. The time series of a root (interval 1 min) upon treatment with a biotic stress elicitor demonstrates the emerging calcium signatures, the responding regions of the root, as well as intercellular signal propagation.

Planned research and new directions
For the coming years our aims are:

1. Decoding cell-to-cell communication upon perception of environmental stress and understanding mechanisms of signal propagation.
To obtain a high resolution map of multi-dimensional calcium-signal propagation, we currently develop tools to locally stimulate roots at the single cell level. These techniques include genetic manipulation for single-cell stimulation, mechanical and microfluidic micromanipulation and an optogenetic approach. Along with stimulus-specific genetic reporters, we will be able to determine information transfer among cells and tissues. Identified mutants and inhibitors from our current work will then allow us to dissect the molecular mechanisms of signal propagation and pave the way for a decoding of inter-cellular communication in plants.

In a collaboration with the group of information theoretician Jürgen Pahle (BioQuant, U. Heidelberg) we are in the process of identifying information-carrying signal components in the stimulus-specific three-dimensional calcium signatures and aim to quantify (in bits/s) information transmission in time and space.

2. Unveiling the mechanisms that control plasma membrane-bound protein dynamics and interaction networks under changing environmental conditions.
Our focus regarding tip growth control in root hairs will lie on the further characterization of the candidate proteins that were identified during the time-line analysis. These include several previously undescribed upstream regulators of ROP activity.

To further understand membrane protein dynamics at the plasma membrane in general and at the root hair initiation domain in particular, we need to investigate the dynamic distribution of membrane constituents and their interaction networks with the highest possible resolution in space and time. We recently secured funding for a variable-angle epifluorescence microscope (Konopka and Bednarek, 2008). First experiments revealed the dynamic formation of transient protein clusters at the emerging tip. Our setup is further equipped with a galvo-scanner module that will enable photomanipulation and protein-diffusion measurements. Furthermore, we recently began to employ FLIM-FRET measurements to reveal protein-protein interaction networks during polar growth initiation.

In addition to these visualization approaches, we will also employ genetic screens on root hair growth regulation. We recently developed a microscope setup for time-lapse imaging of vertically growing roots. Along with an automated root-hair-tracking analysis, this setup will be used to perform genome-wide-association studies (GWAS) to identify novel regulators of phenotypic plasticity.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 13, number of citations 2013-2017: 318, h-index (2013-2017): 10, total h-index: 17 (according to Thomson Reuters, ResearcherID: D-3537-2014).

Stanley, CE*, Shrivastava, J*, Brugman, R, van Swaay, D, and Grossmann, G. (2017) Dual-flow-RootChip reveals local adaptations of roots towards environmental asymmetry at the physiological and genetic level. New Phytol. DOI:10.1111/nph.14887 (* equal contribution)

Xing, S, Mehlhorn, D, Wallmeroth, N, Asseck, LY, Kar, R, Voß, A, Denninger, P, Schmidt, VAF, Schwarzländer, M, Stierhof, YD, Grossmann, G and Grefen, C. (2017) Loss of GET pathway orthologues in Arabidopsis thaliana causes root hair growth defects and affects SNARE abundance. Proc Natl Acad Sci U S A. 114(8):E1544-E1553

Keinath NF, Waadt R, Brugman R, Schroeder JI, Grossmann G, Schumacher K, and Krebs M. (2015) Live Cell Imaging with R-GECO1 Sheds Light on flg22- and Chitin-Induced Transient [Ca^{2}+]cyt Patterns in Arabidopsis. Mol Plant, 8(8), 1188-1200

Denninger, P, Bleckmann, A, Lausser, A, Vogler, F, Ott, T, Ehrhardt, DW, Frommer, WB, Sprunck, S, Dresselhaus, T, and Grossmann, G. (2014) Male-female communication triggers calcium signatures during fertilization in Arabidopsis. Nat Commun, 5, 4645.

Jones, AM, Xuan, Y, Lalonde, S, Xu, M, Wang, RS, Ho CH, You, CH, Sardi, MI, Parsa, SA, Smith-Valle, E, Su, T, Frazer, KA, Pilot, G, Pratelli, R, Grossmann, G, Acharya, BR, Hu, HC, Engineer, C, Villiers, F, Ju, C, Takeda, K, Su, Z, Dong, Q, Assmann, SM, Chen, J, Kwak, JM, Schroeder, JI, Albert, R, Rhee, SY, and Frommer, WB. (2014) Border control – a membrane-linked interactome of Arabidopsis. Science, 344 (6185), 711-716.

2.8 DR. ANNIKA GUSE
INDEPENDENT RESEARCH GROUP MOLECULAR BASIS OF CORAL SYMBIOSIS

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Fields of Interest
Endosymbiosis, host-symbiont interaction, cell-cell communication, cellular organization, cell division, chromatin organization

Brief summary of work since 2013
Mutualistic symbioses are key to evolutionary and ecological novelties. Eukaryotic cells evolved through the acquisition of bacteria, and coral reef ecosystems depend on the endosymbiosis between corals and photosynthetic dinoflagellates that provide critical nutrients to their hosts (Figure 1). Most corals produce non-symbiotic larvae that acquire symbionts anew each generation via phagocytosis into host endodermal cells. We have only a limited understanding of symbiosis establishment, primarily because corals are unsuitable as laboratory systems. My lab uses *Aiptasia*, a marine sea anemone and emerging model for coral symbiosis, as a platform for studying the molecular mechanisms of host-symbiont interaction. We have generated a tool box that allows us to launch a novel research program to uncover mechanisms of symbiont phagocytosis, metabolic exchange and the coordination of cell functions in a hypothesis-driven manner. We have obtained an ERC Consolidator grant »SYMCELLS« starting in 2017 to exploit our powerful model system and developed resources to answer fundamental questions about endosymbiosis such as: Which cells take up symbionts and what are the underlying molecular mechanisms of symbiont phagocytosis? What´s the nature of the symbiosome, the phagolysosome-like organelle in which symbionts reside and how does the symbiont avoid intracellular digestion? How are key nutrients transferred from the symbiont to the host focusing on the molecular mechanisms underlying sterol transfer? By answering these questions, I envision that we will make important contributions in unraveling fundamental aspects of symbiosis, a phenomenon that shaped many evolutionary innovations and continues to allow adaptation to ecological niches.

Major contributions since 2013
Over the last few years, my lab has been a key contributor for establishing the larvae of *Aiptasia* as an experimental platform for studying the molecular mechanisms of host-symbiont interaction (Figure 2). Specifically, we developed a robust protocol to induce *Aiptasia* spawning in the lab by simulating a lunar cycle using artificial blue light which allows access to a basically unlimited number of *Aiptasia* larvae (Grawunder et al., 2015). Taking advantage of the constant availability of fertilized eggs and developing embryos, we have analyzed the timing and main features of embryonic and larval development in *Aiptasia*. Within 48 h, *Aiptasia* embryos develop into fully functional, free-swimming planula larvae with two tissue layers, the endo- and the ectoderm, an oral pore that connects the environment to the prominent gastric cavity and the apical tuft as a sensory organ on the aboral site of the larvae. Larvae phagocytose symbionts most efficiently between 2 days post-fertilization (after mouth formation) and declines ~2 weeks

post-fertilization. Initially, symbionts are predominantly acquired by cells in the aboral endoderm; however, over time the region of uptake expands throughout the endoderm. Thus, symbiont phagocytosis may be accomplished by specific cells within the endoderm (Bucher et al., 2016).

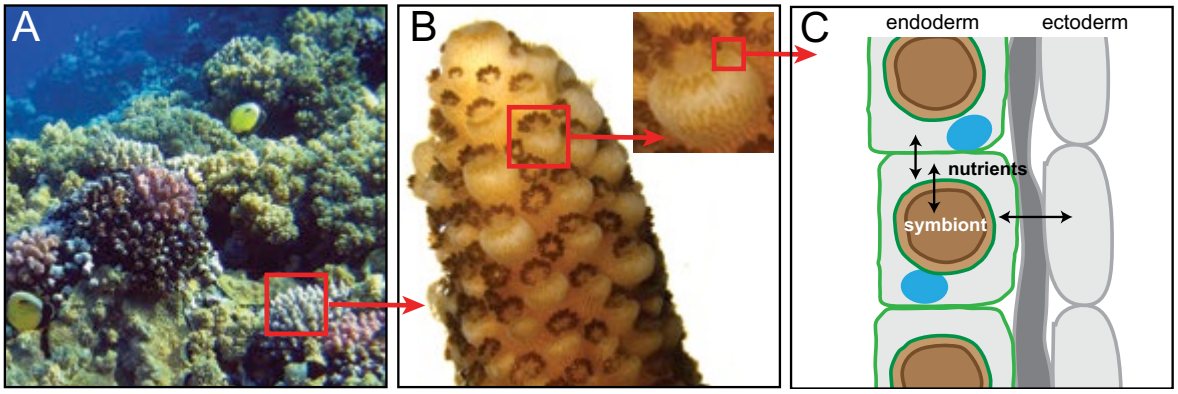

Figure 1
Reef-building corals depend on nutrient transfer by intracellular dinoflagellate symbionts. (A) Coral reefs are formed by coral colonies (B) comprising many individual polyps (inset). (C) Symbionts reside inside endodermal cells and transfer nutrients to support the metabolic needs of the coral host.

We participated in the genome sequencing of *Aiptasia* and used RNA-Seq approaches to identify symbiosis-specific candidate genes (Baumgarten et al., 2015; Wolfowicz et al., 2016) paving the way for a hypothesis-driven analysis of symbiosis establishment. Towards that end, we have developed critical molecular tools such as *in situ* hybridization and qPCR to analyze expression of candidate genes, confocal microscopy and immunofluorescence protocols to analyze protein localization at the cellular level, established first biochemical approaches such as protein extraction, western blotting and metabolomics assays and have recently succeeded in microinjecting of dyes and mRNA into fertilized eggs as the basis for developing functional and genome editing approaches to further extend our experimental tool box.

Building up on these technical advances, we are now addressing the open questions as outlined above. For example, we are investigating the role of the Niemann-Pick Type C (NPC2) proteins in the host for accepting and using symbiont-derived sterols. We and others found that NPC2 gene expression is upregulated during symbiosis and the NPC2 gene family is, in comparison to humans that only have a single gene copy, expanded in corals and anemones indicating a conserved function in symbiosis. NPC2 is a highly conserved lysosomal protein that is essential for cholesterol metabolism in humans and thus to maintain membrane homeostasis in each cell. Because corals themselves cannot synthesize cholesterol and lipid-rich prey is scarce in tropical marine environments, we hypothesize that symbiont-derived sterols may be key for cellular functions in symbiotic animals. In accordance with this idea, symbionts are known to synthesize cholesterol as well as various sterol derivatives such as campesterol, stigmasterol and gorgosterol all of which may be transferred from the symbionts to the host to support its nutrition. To test this, we used quantitative gas chromatography – mass spectrometry (GC-MS) assays to correlate sterol composition of algae in culture, symbiotic and non-symbiotic anemones and corals. We found that sterol composition differs between non-symbiotic and symbiotic animals in that animals devoid of symbionts only contain cholesterol while symbiotic animals also incorporate various unusual sterols presumably derived from the symbionts. Interestingly, the sterol composition varies on symbiont type housed suggesting that the host has the capacity to adapt to distinct sterol profiles provided by its partner. Using qPCR, we show that expression of multiple distinct NPC2 genes correlates with its physiological state such as genes are upregulated in symbiosis in *Aiptasia* and corals when compared to the non-symbiotic conditions. We have generated various NPC2-specific antibodies to determine the subcellular localization and develop various approaches to analyze the function of NPC2 proteins in symbiosis including testing direct binding between NPC2 proteins and various sterols.

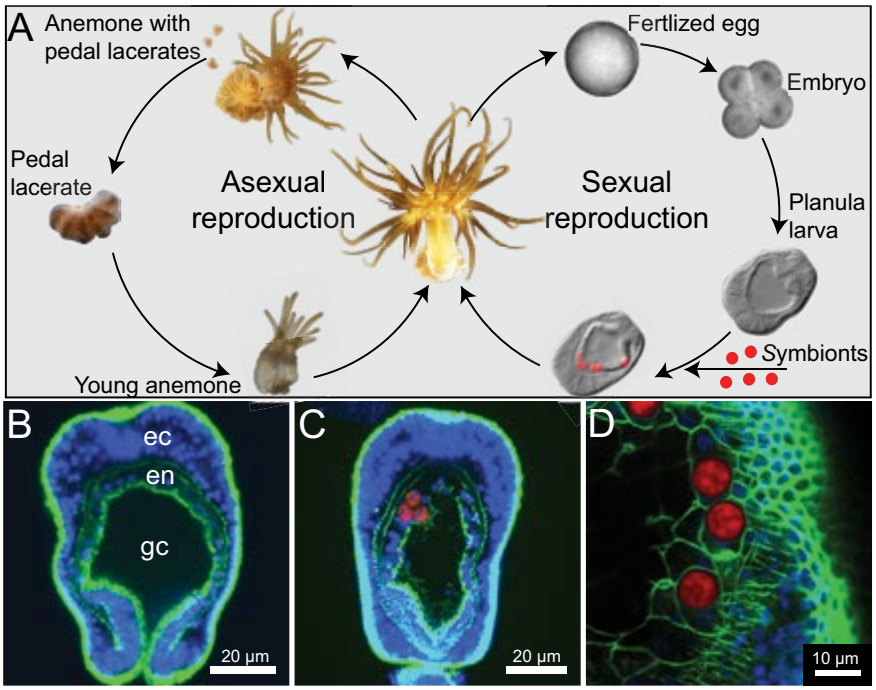

Figure 2
(A) Life cycle of the symbiotic model anemone *Aiptasia*. Clonal lines are maintained through asexual reproduction and non-symbiotic larvae take up symbionts from the environment recapitulating symbiosis establishment in corals. (B)-(D) Confocal microscopy of *Aiptasia* larvae. Channels: phalloidin-stained F-actin (green); Hoechst-stained nuclei (blue); symbiont autofluorescence (red). Larvae have a large gastric cavity (gc), an ectoderm (ec), and an endoderm (en) (B) where algal symbionts reside intracellularly (C). Close-up of endodermal cells shows that symbionts occupy a majority of the host cell cytoplasm (D). *[for display purposes here, red autofluorescence in D was digitally enhanced to overcome auto-fluorescence from symbionts]*

In addition to the work described above, we are also interested in symbiosis specificity, defined as the selection of compatible symbionts among a group of similar algal types. Symbiosis specificity is of great ecological importance, as it has been suggested to affect the host´s physiological properties such as temperature sensitivity (e. g. bleaching susceptibility) and growth rate. We have completed the first comparison of symbiosis specificity between *Aiptasia* and field-collected coral larvae and found that the selection mechanisms are similar for both genera under controlled conditions. We have also identified multiple distinct steps during symbiosis establishment during which compatible symbionts are distinguished from incompatible ones as the basis to elucidate the molecular mechanisms underlying symbiont selection (Wolfowicz et al., 2016).

Planned research and new directions

In the future, we aim to expand our research on the role of NPC2 proteins in symbiosis. We will map the cellular utilization and transport machinery of symbiont-derived sterols in symbiotic anemones and test the importance of transferred sterols to the host. To do so, we will use gain- and loss-of function approaches in combination with chemical inhibition and complementation experiments using (modified) commercially available and custom-made sterols. This analysis will uncover novel mechanisms of the metabolic dependencies between symbiont and host, a key-concept of mutualistic symbioses.

We also aim to molecularly dissect the principles underlying symbiosis establishment including symbiont recognition, mode of phagocytosis and how symbionts avoid digestion by the phagolysosome. To this end, we are using single-cell transcriptomics to characterize distinct cell types within the *Aiptasia* larval endoderm. In the next step, we will use fluorescence microscopy to identify and molecularly characterize symbiosis-specific cells by cellular markers. Specifically, we aim to identify phagocytosis receptors and the phagocytotic machinery involved in symbiont uptake and we have already identified many candidates by RNA-Seq. We plan to test the function of candidate receptors in symbiont uptake by gain- and loss-of-function analysis and monitor their dynamic behavior using live imaging of symbiont phagocytosis.

Together with our transcriptomic and functional analysis, we aim to reveal the evolutionary origin of symbiosis-specific cells, the nature of the organelle hosting the symbionts and whether (and how) symbionts actively manipulate the host´s pathogen clearance machinery to allow long-term residency. Ultimately, to enhance the cellular resolution of our research, we plan to develop a cell culture system for symbiosis. An *Aiptasia* cell culture system will complement our larval system and together, both will allow a holistic analysis of the evolution, ecology, cell biology and biochemistry of symbiosis establishment.

Selected publications since 2013
Number of peer-reviewed articles 2013-2017: 6, number of citations 2013-2017: 78, h-index (2013-2017): 4, total h-index: 8 (according to Thomson Reuters).

*Jones, V.A.S., *Bucher, M., Hambleton, E.A., Guse, A. (2017). Microinjection to deliver protein, mRNA and DNA into zygotes of the cnidarian endosymbiosis model *Aiptasia* sp. bioRxiv, doi:10.1101/187278 (* equal contribution)

Wolfowicz, I., Baumgarten, S., Voss, P.A., Hambleton, E.A., Voolstra, C.R., Hatta, M., Guse, A. (2016). *Aiptasia* sp. larvae as a model to reveal mechanisms of symbiont selection in cnidarians. Sci. Rep. 6, 32366

Bucher, M., Wolfowicz, I., Voss, P.A., Hambleton, E.A. & Guse, A. (2016). Development and symbiosis establishment in the cnidarian endosymbiosis model *Aiptasia* sp. Sci. Rep. 6, 19867

Grawunder, D., Hambleton, E.A., Bucher, M., Wolfowicz, I., Bechtoldt, N. & Guse, A. (2015). Induction of gametogenesis in the cnidarian endosymbiosis model *Aiptasia* sp. Sci. Rep. 5, 15677

Baumgarten, S., Simakov, O., Esherick, L.Y., Liew, Y.J., Lehnert, E.M., Michell, C.T., Li, Y., Hambleton, E.A., Guse, A., Oates, M.E., Gough, J., Weis, V.M., Aranda, M., Pringle, J.R. & Voolstra, C.R. (2015). The genome of *Aiptasia*, a sea anemone model for coral symbiosis. Proc. Natl. Acad. Sci. 112, 11893-8

2.9 PROF. DR. RÜDIGER HELL
PLANT MOLECULAR BIOLOGY

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Fields of Interest
Molecular biology and physiology, regulation of metabolism, metabolite sensing, nutrition, environmental stress, gene expression, protein modification and quality control

Brief summary of work since 2013
The Department of Molecular Biology of Plants investigates functional interactions between the continuously changing entities of metabolism, development and environment. The selection of sulfur metabolism as a platform to study these interactions has been very rewarding. This comprises questions of plant nutrition, growth control and stress defenses. The variety of approaches opens the way to discovery of processes of general relevance for plant biology: it deepened our understanding of the regulatory interaction of nutrients (i. e. sulfur and iron metabolism), the role of redox in generative development (i. e. glutathione requirement), of drought stress acclimation (i. e. redox homeostasis), the relevance of redox control processes in photosynthesis (i. e. provision of cysteine), and contributed to numerous other collaborative projects (see publications 2013–2017). In addition, new approaches have been initiated that aim at the integration of general metabolism and growth, again using sulfur metabolism as trigger, and the epigenetic regulation of gene expression during sulfate deficiency.

This open strategy allows to link and support several projects within the department. The research group of Markus Wirtz is fully integrated and connected by our strong shared interests in sulfur-based drought responses and protein turnover during stress. The department hosts the Metabolomics Core Technology Platform since summer 2013 that is funded by the University's Future Concept of the Excellence Initiative. This goes along with the support of the independent research group »Plant defense metabolism« of Emmanuel Gaquerel that is funded by the same source. All units within the department are engaged in teaching at Bachelor, Master and PhD levels.

Major contributions since 2013
Towards the understanding of the interaction of different metabolic pathways the consequences of starvation of sulfate, iron and potassium were studied using global gene expression and metabolomics in *Arabidopsis thaliana* (Forieri et al., 2017; Fig. 1). The reactions to different nutrient deficiencies have hardly been compared but led to the hypothesis of the existence of a core response to nutrient deficiencies. In a systems approach we could show that in fact only 130 genes are jointly regulated by all three nutrients, but that nutrient-specific patterns of gene expression and metabolite levels arise. The latter include fingerprints of jasmonic acid, salicylic acid and absisic acid that were found for the first time in starved roots. A specific co-regulation had been predicted between sulfur and iron metabolism (Forieri et al., 2013), and indeed we found that iron deficiency caused regulation of a different set of genes of the sulfur assimilation pathway compared with sulfur deficiency itself, which demonstrates the presence of specific signal-transduction systems for the cross-regulation of the pathways.

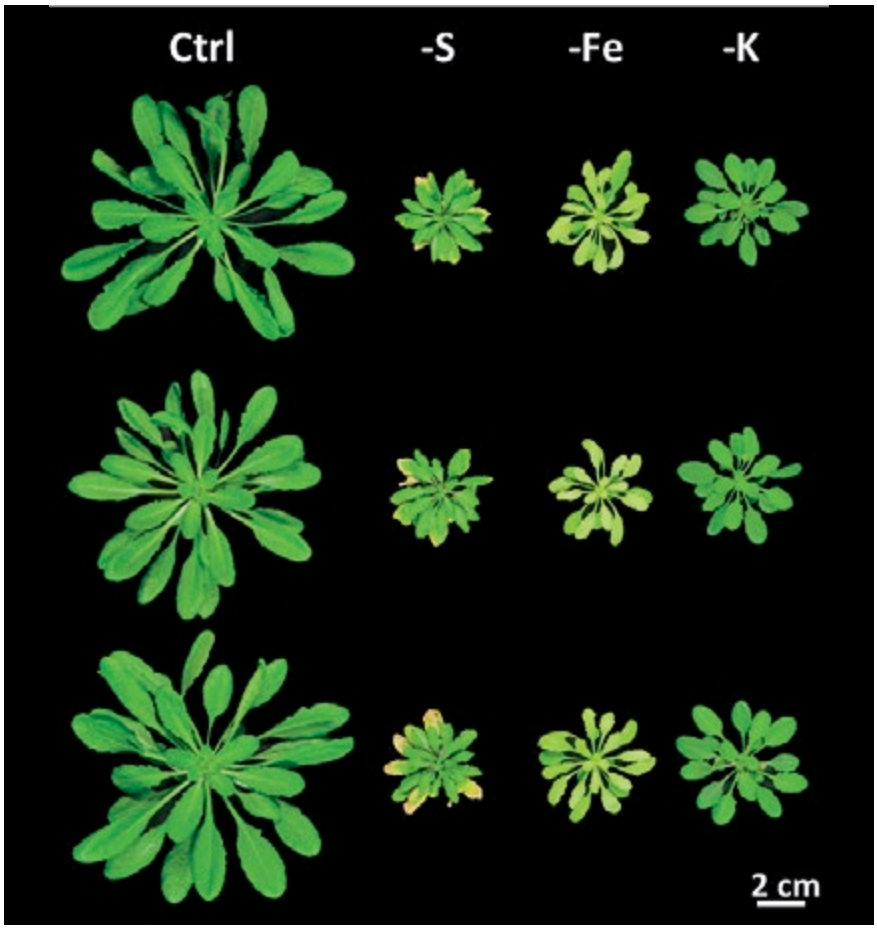

Figure 1
Phenotype of seven week-old *Arabidopsis thaliana* plants hydroponically grown under different nutritional regimes (Ctrl, full nutrient supply, -S, sulfur deficiency, -Fe, iron deficiency, -K, potassium deficiency)

Our previous analyses had revealed that reduction of sulfate to sulfide exclusively takes place in plastids, but that incorporation of sulfide into cysteine occurs in plastids, mitochondria and the cytosol. However, the subunits of the cysteine synthase complex, serine acetyltransferase (SERAT) and *O*-acetylserine (thiol) lyase (OAS-TL), are differently abundant and regulated in the three compartments. Towards understanding of compartmental functionalization of cysteine synthesis we found that mitochondria surprisingly have a special role in tuning the overall cysteine synthesis rate, while chloroplasts seemed to be of little importance under non-stressed conditions. Sulfide has a dual role as intermediate of the assimilatory reduction pathway and as toxin of cytochrome c oxidase. By combination of reverse genetics and H_2S fumigation we showed that mitochondria are particularly important for detoxification of sulfide, while formation of thiosulfate and glutathione in the cytosol provided major sinks for excess sulfide (Krüssel et al., 2014; Birke et al., 2015a, b). Indeed, we could show that mitochondria carry a complex inner and outer membrane system for uptake and exchange of sulfur compounds (Lee et al., 2014). Generation of all major *oastl* double loss-of-function mutants in combination with radiolabeled tracer studies showed that subcellular localization of OAS-TL proteins is more important for efficient cysteine synthesis than total cellular OAS-TL activity in leaves. The absence of *oastl* triple embryos ruled out any relevant alternative route of sulfur fixation. Interestingly, analyses of *oastlABC* pollen demonstrated that the presence of at least one functional OAS-TL isoform is essential for the function of the male gametophyte (Birke et al., 2013). With respect to the seemingly less important role of chloroplast cysteine synthesis we found that this becomes indeed relevant under high light stress conditions (Speiser et al., 2014).
This sparked our interest in redox control processes in chloroplasts under stress (Dietz and Hell, 2015; Kovacs et al., 2016) and led to the investigation of the plastid cysteine synthase complex (Fig. 2). The current data indicate that, despite highly similar primary sequences, the chloroplast OAS-TL A protein differs considerably from the other OAS-TLs in mitochondria and cytosol, giving rise to weaker binding properties with SERAT towards an active cysteine synthase complex that are likely used to modulate the rate of cysteine synthesis.

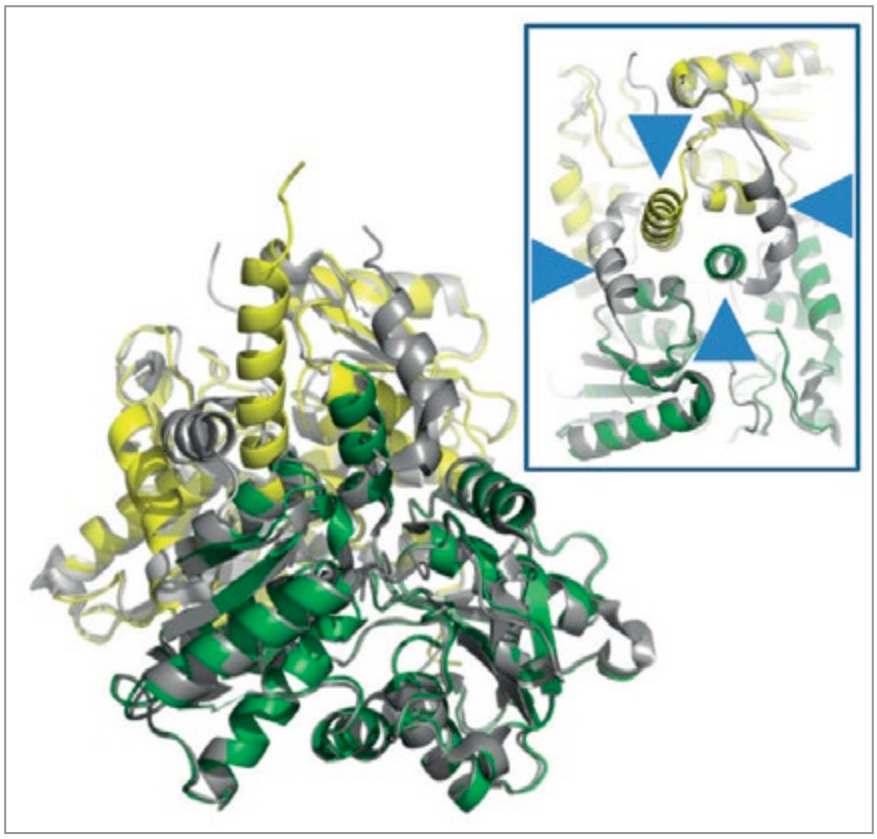

Figure 2
Overlay of the crystal structures of cytosolic OAS-TL A (grey) and plastid OAS-TL B (green) from Arabidopsis. Arrows in the insert indicate the folding of helix 9 in OAS-TL A (black) and OAS-TL B (red) in both monomers of the OAS-TL dimer (with J. Knoop, I. Sinning, Biochemistry Centre Heidelberg)

These findings pointed to a special relationship between redox control and sulfur metabolism. Abiotic stress conditions in many cases result in the formation of reactive oxygen species, either derived from the plastid and mitochondrial electron chains or NADPH oxidases. Since drought will be the major limiting factor for plant production in this century, we established a controlled drought stress system for *Zea mays* that allowed the parallel analysis not only of leaves, as is usually done, but also of roots (Ahmad et al., 2016). We found that during drought-stress maize leaves suffer from sulfate supply and accordingly are unable to synthesize sufficient cysteine to maintain their glutathione levels that are required to scavenge ROS. In contrast, roots were unable to export sulfate but reduced it to maintain their glutathione redox balance. This finding parallels the growth arrest of the shoots and continued elongation of roots, contributing the characteristic root-to-shoot shift under drought stress and providing a potential goal for breeding od improved varieties of maize.

Planned research and new directions

The fundamental reactions and pathways of primary metabolism have been elucidated, mostly based on genetic and biochemical approaches. The next challenges are to understand the interactions between different pathways and how the changes that are imposed onto the organism by the environment are translated via metabolism into optimization of growth and development. The study of sulfur metabolism proved to be highly suitable tool and will therefore also be used in the future to assess these processes at the genetic, cellular and organismal level using *Arabidopsis thaliana* as model.

In the previous report period the search for the sensing mechanism that triggers the canonical sulfate deficiency response identified the cytosolic cysteine synthase complex as one element of the early transduction chain (Speiser et al., in preparation). This work will be continued to complete all components of the sensor system. In the future the link between the long term sulfate deficiency response (i.e. >24 hours) and the general growth response will be the target (Fig. 3).

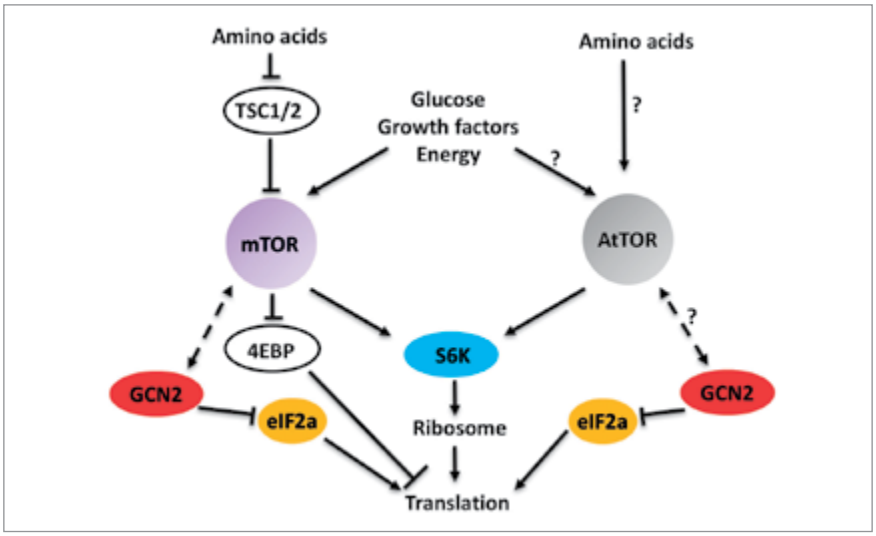

Figure 3
Target of rapamycin (TOR) signaling in animals (left side) and plants (right side)

Ongoing experiments use an alternative approach to classical sulfate deficiency experiments. It consists in knock-down mutants in the reduction branch (sulfite reductase) and the amino acid branch (serine acetyltransferase triple KO) towards cysteine synthesis. Both genetic systems generate strongly lowered flux through cysteine synthesis. The immediate consequences of internal limitation of cysteine show that translation is not just slowed down by lack of cysteine, but that active downregulation of the translation machinery takes place. Remarkably, the limitation by the amino acid branch is sensed by the GCN2 kinase system, while the sulfur branch is sensed via target of rapamycin (TOR) regulation. Indeed, when we tested external sulfur deficiency this caused downregulation of TOR activity leading to decreased translation, lowered root meristem activity and elevated autophagy for remobilization of internal resources (Fig. 4). Thus, plants differentially sense the two precursors of the amino acid and not cysteine levels themselves. This adds to several differences between plant and yeast/mammalian TOR sensing. It is tempting to speculate that this is an adaptation to (photo)autotrophic growth, opening the possibility to unravel these differences with respect to the triangle of primary sulfur metabolism, sugar metabolism and general growth control. In collaboration with A. Pfeiffer and J. Lohman we contributed to the dissection of the TOR kinase as a central integrator of light and metabolic signals in the activation of stem cells in the shoot apex (Pfeiffer et al., 2016).

The second major future research area will be the epigenetic control of nutrient metabolism. S-adenosylmethionine is the methyl donor for DNA methylation, suggesting a direct connection between sulfur availability and the capacity for DNA methylation. Current work indicates specific changes in the methylome of sulfate-deprived plants including a cluster of methylation sites in the promoter of a sulfate transporter gene, and a global hypomethylation at extended starvation. We have already contributed to the characterization of mutants in carbon-1 metabolism that is known to be fueled by S-adenosylmethionine. Together with the groups of Steven Jacobsen, UCLA, and David Salt, Nottingham, we found indications that a nucleus-localized sensing or synthesis system operates towards silencing of transposable elements but also sulfate deficiency induced methylome modifications (Groth et al., 2016, Huang et al., 2016). Our preliminary data in addition point to a memory effect of repeated sulfate deprivation phases that is based on DNA methylation. Future work will address the mechanisms involved in these changes, identify the target genes and extend to other mineral nutrients such as nitrate.

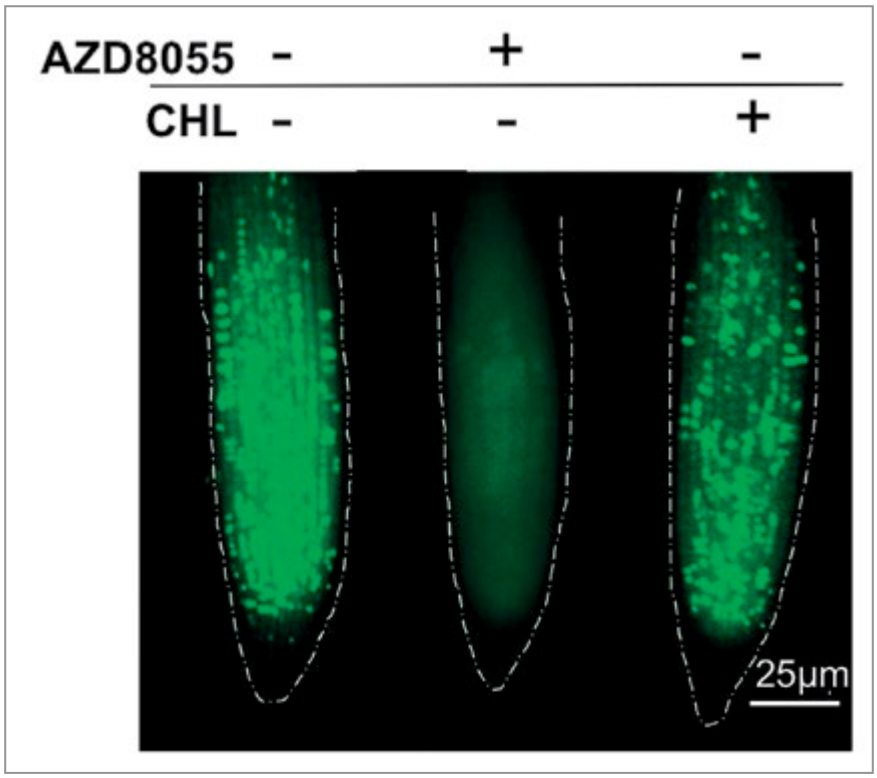

Figure 4
Root meristem activity (Edu staining) in 7-day old wildtype Arabidopsis seedlings treated with TOR inhibitor (AZD8055) or GCN2 activator (Chlorosulfuron, CHL) for 2 hours. Scale bar, 25 µm

Selected publications since 2013
Number of peer-reviewed articles 2013-2017: 47, number of citations 2013-2017: 387, h-index (2013-2017): 12, total h-index: 47 (according to Thomson Reuters).

Forieri, I., Sticht, C., Reichelt, M., Gretz, N., Hawkesford, M.J., Malagoli, M., Wirtz, M., Hell, R. (2017) Systems analysis of metabolism and the transcriptome in *Arabidopsis thaliana* roots reveals differential co-regulation upon iron, sulfur and potassium deficiency. Plant Cell Environm. 40, 95–107.

Pfeiffer, A., Janocha, D., Dong, Y., Medzihradszky, A., Schöne,S., Daum, G., Suzaki, T., Forner, J., Langenecker, T., Schmid, M., Wirtz, M., Hell, R., Lohmann, J. U. (2016) Integration of light and metabolic signals for stem cell activation at the shoot apical meristem. eLife, doi: 10.7554/eLife.17023

Groth, M., Moissiard, G., Wirtz, M., Wang, H., Garcia-Salinas, C., Ramos-Parra, P. A., Bischof, S., Feng, S., Cokus, S.J., John, A., Smith, D. C., Zhai, J., Hale, C. J., Long, J. A., Hell, R., Díaz de la Garza, R. I., Jacobsen, S. E. (2016) MTHFD1 controls DNA methylation in Arabidopsis. Nature Comm. 7, 11640. doi: 10.1038/ncomms11640

Linster, E., Stephan, I., Bienvenut, W.V., Maple-Grødem, J., Myklebust, L.M., Huber, M., Reichelt, M., Sticht, C., Møller, S.G., Meinnel, T., Arnesen, T., Giglione, C., Hell, R., Wirtz, M. (2015) Proteome imprinting by N-terminal acetylation is a vital hormone-regulated switch during drought stress. Nat. Comm. 6:7640.doi: 10.1038/ncomms8640

Birke, H., Heeg, C., Wirtz, M., Hell, R. (2013) Successful fertilization requires the presence of at least one major *O*-acetylserine(thiol)lyase for cysteine synthesis in pollen of Arabidopsis. Plant Physiol. 63: 959 – 972

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Fields of Interest
Stress-induced surveillance mechanisms, protein-quality control, protein-modifications, hormone biosynthesis, metabolite signaling, transcriptional networks

Brief summary of work since 2013
Plants face a multitude of biotic and abiotic stresses during development as a result of their sessile life-style. In the last four years our research focused on the characterization of stress-induced surveillance mechanisms that enable plants to adapt to these environmental challenges. We made used of the previously established stress-models, drought and sulfate deficiency, to uncover the importance of co-translational proteome imprinting by N-terminal acetylation (NTA) for cellular surveillance. In particular, we studied the contribution of NTA to control the stability of cytosolic proteins. However, we recently widened the focus and began to analyze also the role of NTA in plastids. Furthermore we identified a direct link between endogenous sulfate allocation from the root to the shoot and biosynthesis of the stress-related hormone abscisic acid (ABA).

Major contributions since 2013
At the end of the previous report term, we showed in close collaboration with Prof. Chengbin Xiang (USTC, China) that sulfate availability impacts ABA steady state levels in seedlings of *Arabidopsis thaliana* (Cao et al., 2014). We elaborated on this finding in collaboration with Prof. Cornelia Herschbach, who identified sulfate as an early xylem-borne signal upon drought, and demonstrated that sulfate rapidly closes stomata in a QUAC1-dependent manner (Malcheska et al., 2017). Currently, we are connecting both findings by establishing the molecular mechanism by which sulfate induces stomata closure. We were able to show that sulfate must be incorporated into cysteine to induce stomata closure. Determination of cytosolic ABA concentration by life-cell imaging with the ABAleon2;1 probe unambiguously demonstrates that application of sulfate or cysteine increases ABA levels in guard cells. In agreement with these findings sulfate-induced stomata closure requires ABA biosynthesis and ABA signaling that activates ROS production by plasmalemma localized NADPH oxidases (Batool et al., submitted).

NTA is a prevalent protein modification affecting 70–80 % of cytosolic proteins and is catalyzed by six mainly ribosome associated N-terminal acetyltransferases (named NatA to NatF) in plants. In the previous report term, we identified the NatA complex as the major contributor to imprinting of cytosolic proteins by NTA. In this report term characterization of NatA depletion mutants revealed the importance of NTA for establishing the drought stress response. The dynamic regulation of NatA activity upon ABA application and during drought stress was the basis for the observed rapid decrease of NTA frequency under both conditions and is sufficient to induce stomata closure (Linster et al., 2015).

In order to provide a molecular explanation for the significant decrease of stomatal aperture in the NatA depleted plants, we are currently analyzing the function of NTA to act

as a specific degradation signal that regulates protein-turnover of NatA and NatB substrate proteins. In agreement with the antagonistic action of NatA and NatB on the stability of the plant immune receptor SNC1 (Xu et al., 2015), the feeding of radioactively labelled amino acids uncovered enhanced global protein turnover in NatA depleted plants, while depletion of the NatB decreased turnover of proteins. In accordance with these results proteasome activity was antagonistically regulated in mutants of the NatA and NatB complex. Our results strongly suggest an important contribution of NTA to control the stability of the cytosolic proteome (Forero et al., in preparation). Interestingly, NTA is not restricted to cytosolic proteins in plants (Dinh et al., 2015).

Planned research and new directions

Research of the next four years will focus on two aspects: How does sulfate trigger the biosynthesis of the plant hormone ABA? How does N-terminal protein acetylation regulate stability of cytosolic proteins and is NTA in plants integrated within the N-end rule pathway? To this end we established an innovative fluorescence-based tool to determine protein-turnover of selected candidates *in vivo* by non-invasive life cell imaging (Fig. 1). The importance of the N-end rule pathway for degradation of acetylated proteins will be analyzed in collaboration with Dr. Daniel Gibbs (University of Birmingham, UK), who identified loss-of-function mutants for Not4 type E3-ligases. These E3 ligases have been shown in yeast to selectively ubiquitinylate proteins in response to their N-terminal acetylation status.

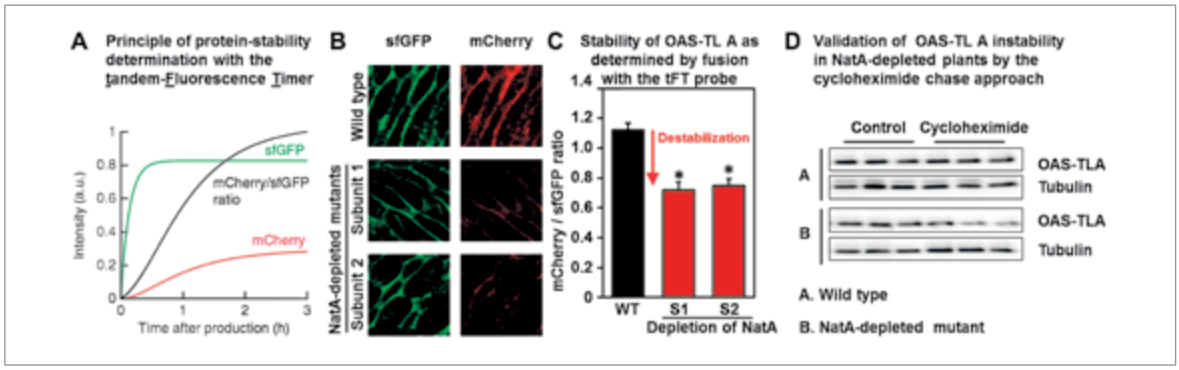

Figure 1
N-terminal acetylation by the NatA complex stabilizes a cytosolic OAS-TL protein
A) Schematic overview on the tandem Fluoresecence Timer-based (tFT) approach to quantify protein dynamics in living cells. B) Fluorescence signal for sfGFP (green) and mCherry (red) after expression of a bona fide NatA substrate (cytosolic OAS-TL A) in the wild type and two NatA depleted plants. C) Quantification of the mCherry to sfGFP signal ratio from signals shown in B. D) The significant destabilization of cytosolic OAS-TL A due to absent acetylation of the N-terminus was confirmed by application of the cycloheximide chase approach. Tubulin served as a loading control.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 31, number of citations 2013-2017: 384, h-index (2013-2017): 12, total h-index: 32 (according to Thomson Reuters).

Groth, M, Moissiard, G, Wirtz, M, Wang, H, Garcia-Salinas, C, Ramos-Parra, PA, Bischof, S, Feng, S, Cokus, SJ, John, A, Smith, DC, Zhai, J, Hale, CJ, Long, JA, Hell, R, Diaz de la Garza, RI & Jacobsen, SE *(2016)* MTHFD1 controls DNA methylation in Arabidopsis. Nat. Commun. 7, 11640

Xu, F, Huang, Y, Li, L, Gannon, P, Linster, E, Huber, M, Kapos, P, Bienvenut, W, Polevoda, B, Meinnel, T, Hell, R, Giglione, C, Zhang, Y, Wirtz, M, Chen, S & Li, X (2015) Two N-terminal acetyltransferases antagonistically regulate the stability of a nod-like receptor in Arabidopsis. Plant Cell 27, 1547-1562.

Linster, E, Stephan, I, Bienvenut, WV, Maple-Grodem, J, Myklebust, LM, Huber, M, Reichelt, M, Sticht, C, Geir Moller, S, Meinnel, T, Arnesen, T, Giglione, C, Hell, R & Wirtz, M *(2015)* Downregulation of N-terminal acetylation triggers ABA-mediated drought responses in Arabidopsis. Nat. Commun. 6, 7640

Dinh, TV, Bienvenut, WV, Linster, E, Feldman-Salit, A, Jung, VA, Meinnel, T, Hell, R, Giglione, C & Wirtz, M *(2015)* Molecular identification and functional characterization of the first N-acetyltransferase in plastids by global acetylome profiling. Proteomics 15, 2426-2435.

Cao, MJ, Wang, Z, Zhao, Q, Mao, JL, Speiser, A, Wirtz, M, Hell, R, Zhu, JK & Xiang, CB (2014) Sulfate availability affects ABA levels and germination response to ABA and salt stress in *Arabidopsis thaliana*. Plant J. 77, 604-615.

2.10 PROF. DR. THOMAS W. HOLSTEIN
MOLECULAR EVOLUTION AND GENOMICS

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Fields of Interest
Molecular evolution of development, Wnt and TGF-β signaling, transcriptional networks; biology of regeneration and stem cells; cell type evolution (nerve cells, nematocytes), axis formation

Brief summary of work since 2013
To understand the origin and evolution of key regulators in animal development, our lab is analyzing cnidarians, simple diploblastic animals with a gastrula-like body plan and an ancient nervous and stem cell system. We are working with embryos of the sea anemone *Nematostella* and with the freshwater *Hydra*, famous for its almost unlimited life span and regeneration capacity. We discovered cnidarian Wnt signaling and we were engaged in sequencing the *Nematostella* and *Hydra* genomes. Our work revealed an unanticipated genomic complexity of these ancient organisms with intriguing similarities to vertebrates. It also indicated that the genetic repertoire required for setting up the bilaterian body plan was already present in the common cnidarian/bilaterian ancestor. Recently, we also discovered that Nodal signaling is essential for setting up an axial asymmetry along the main body axis during *Hydra* budding (branching). In bilaterians, Nodal signaling is essential for setting up the left-right axis. This supports an evolutionary scenario according to which another major core signaling cassette pre-dated the cnidarian-bilaterian split and was co-opted for various modes of axial patterning in cnidarians and bilaterians. Our work on *Nematostella* neurogenesis revealed that β-catenin signalling is crucial for the early induction of the embryonic nervous system. It also induces Bmp signalling, which is indispensable for maintenance and asymmetric neuronal patterning along the secondary (directive) axes. Our data provide a new view on both, the evolution of the origin of the bilaterian central nervous system (CNS) and the origin of animal body axes.

Major contributions since 2013
Molecular evolution and genomics. A limitation of the first *Hydra* genome assembly (including Sanger sequencing at the J. Craig Venter Institute) was a scaffold N50 of only 92.5 kb (Chapman et al., 2010). In 2015, DNA samples from the original *Hydra* strain (Rob Steele, Irvine) and Chicago libraries were generated by Dovetail Genomics. The new assembly generated by Dovetail Genomics (*Hydra* 2.0) has a scaffold N50 of ~1 MB. We contributed to the new *Hydra* Genome Project by providing one of the two transcriptomes that are currently available (https://research.nhgri.nih.gov/*Hydra*/).

Proteomic and transcriptomic analysis of *Hydra* regeneration. In order to understand the regeneration capacity of *Hydra*, we performed the first integrative transcriptome and SILAC proteome study on *Hydra* head regeneration (Petersen et al., 2015). Using a combined transcriptomic and stable isotope labelling by amino acids in cell culture proteomic/phosphoproteomic approach, we studied stem cell-based regeneration in *Hydra* polyps. As major contributors to head regeneration, we identified diverse signalling pathways adopted for the regeneration response as well as enriched novel genes. Our global analysis reveals

two distinct molecular cascades: an early injury response and a subsequent, signalling driven patterning of the regenerating tissue. A key factor of the initial injury response is a general stabilization of proteins and a net upregulation of transcripts, which is followed by a subsequent activation cascade of signalling molecules including Wnts and transforming growth factor (TGF) beta-related factors. We observed moderate overlap between the factors contributing to proteomic and transcriptomic responses suggesting a decoupled regulation between the transcriptional and translational levels. Our data also indicate that interstitial stem cells and their derivatives (e. g., neurons) have no major role in *Hydra* head regeneration. Remarkably, we found an enrichment of evolutionarily more recent genes in the early regeneration response, whereas conserved genes are more enriched in the late phase. In addition, genes specific to the early injury response were enriched in transposon insertions. Genetic dynamicity and taxon-specific factors might therefore play a hitherto underestimated role in Hydra regeneration.

Figure 1
Hydra magnipapillata

Nodal-signaling is braking radial symmetry. Our data demonstrate that Wnt signaling has a major role in the setting up the primary, anterior-posterior body axis. Since the dorsal-ventral and left-right asymmetries of bilaterians are driven by TGF-β signaling, we also analysed the role of Wnt and TGF-β signaling during bud formation, *Hydra's* asexual mode of reproduction. Here, we identified a Nodal-related gene (Ndr) in *Hydra*, which is essential for setting up a new lateral signalling centre, inducing a new body axis of a budding polyp that is orthogonal to the mother polyp's axis. Ndr is expressed exclusively in the lateral bud anlage and induces Pitx, which encodes an evolutionarily conserved transcription factor that functions downstream of Nodal. Reminiscent of its function in vertebrates, Nodal acts downstream of β-Catenin signalling. Our data support an evolutionary scenario in which a »core-signalling cassette« consisting of β-Catenin, Nodal and Pitx pre-dated the cnidarian-bilaterian split. We presume that this cassette was co-opted for various modes of axial patterning: for example, for lateral branching in cnidarians and left-right patterning in bilaterians.

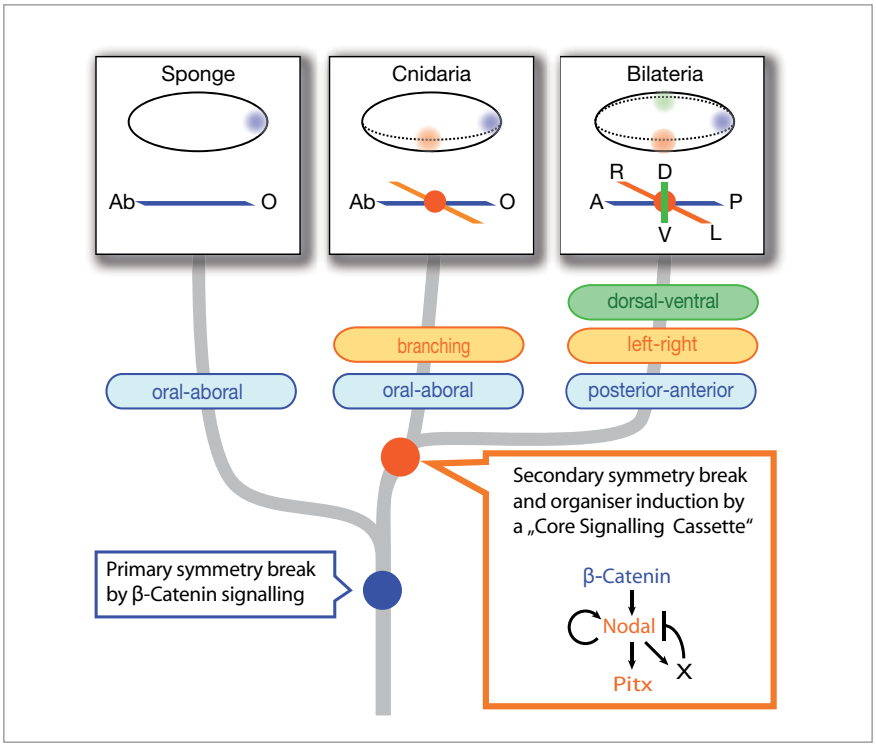

Figure 2
Nodal signalling and secondary body axes. The primary Wnt/β-Catenin axis (blue) was established by a primary symmetry break in the last common metazoan ancestor to pattern anterior (aboral) posterior (oral) axis. In the *Ur-Eumetazoa*, the »Core Signalling Cassette« comprising β-Catenin/Nodal/Pitx signalling components evolved to induce a symmetry break and further body axes.

Origin of a centralized nervous system. Closely linked to the patterning of an animal body plan is the formation of the nervous system. The emergence of nerve cells is one of the key novelties in animal evolution. The general view holds that the bilaterian CNS can be traced back through evolution to a nerve net in a cnidarian-like ancestor. Although cnidarian neurogenic TFs and neuropeptide-positive neurons exhibit a clear position dependency along the oral-aboral body axis, it was unknown how the basic mechanisms of neural induction and formation of central nervous systems have evolved in cnidarians. We found in *N. vectensis* embryos that β-catenin signalling is crucial for the early induction of the embryonic nervous system that starts at the blastula/gastrula transition with the development of the oral nervous system. β-catenin in activity at the blastopore induces specific neurogenic genes required for development of the oral nervous system. β-catenin signalling induces also Bmp signalling, which, at later larval stages, becomes indispensable for the maintenance and asymmetric patterning of the oral nervous system along the primary and secondary (directive) axes. We hypothesize that the consecutive and functionally linked involvement of b-catenin and Bmp signalling in the formation of the cnidarian oral nervous system reflects an ancestral mechanism that evolved before the cnidarian/bilaterian split.

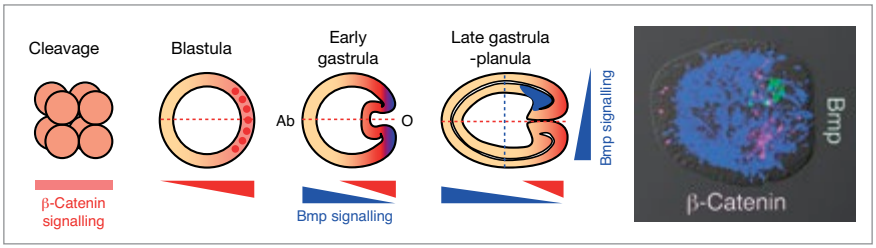

Figure 3
Development of the oral nervous system by β-Catenin and Bmp signalling in N. vectensis. Gradients of β-Catenin and Bmp activation in the development of the embryonic oral nervous system (left) are shown. The induction of the oral nervous system is not depending on Bmp signaling at the blastula stage. At the planula stage, asymmetric suppression of Bmp activity along the secondary axis becomes indispensable for the expression Arp6 transcription factors and the development of GLWa+ neurons at one side (green). Other neuronal populations (magenta and blue) exhibit a radial symmetrical pattern (see Figure 9, Watanabe et al 2014, Nat. Commun. 5:5536 doi: 10.1038/ncomms6536).

Planned research and new directions
We will address two main questions:
(i) Stem cell plasticity in *Hydra*
(ii) The Wnt code: Evolutionary origin of Wnt ligand-receptor interactions

Stem cell plasticity in *Hydra*. Similar to plants, *Hydra* polyps exhibit an almost unlimited regeneration capacity and immortality. Based on new functional studies using siRNA, CRISPR/Cas9, and ChIP-Seq studies we are currently working on the control mechanisms of *Hydra*'s stem cell homeostasis including epigenetic signatures (histone deacetylases). We focus our work on somatic interstitial stem cells that give not only rise to somatic cells but also to germline precursors. To unravel this process, we started our analyses with Dr. Nishimiya-Fujisawa by using cell-type-specific RNA-Seq profiling revealing specifically expressed genes in (somatic) multipotent stem cell lines that are giving rise to the egg or sperm lineage. This approach will be helpful for identifying the underlying the common mechanism for the longevity of somatic stem cells and the germline. We presume that our experiments will yield important insights into the evolution of the germline and stem cells. The project is embedded in the DFG CRC 873 (A1) on stem cells.

The Wnt code: Evolutionary origin of Wnt ligand-receptor interactions. The pattern of Wnt activation in cnidarians is highly reminiscent to the morphogen gradient of Wnt/β-catenin signaling that regulates anteroposterior neural patterning during vertebrate brain development and was characterized as the »Wnt code«. The interplay of different Wnt ligands, receptors and co-receptors is an unsolved fundamental question for Wnt research. We interfere with the function of specific Wnts *in vivo* using either siRNA knock-down or CRISPR/Cas9 knock-out approaches. We will also analyze the specificity of different Fzd-Lrp5/6-combinations for the different Wnt-ligands in cell-based TOPFLASH Wnt reporter assays and cell-free biochemical ligand-receptor assays. Since the spatio-temporal expression pattern of different Wnt proteins as well as their diffusion range may specifically affect the functional relevance of the Wnt code, we will also use transgenic animals expressing tagged Wnts to monitor their diffusion range. By this combined approach, we will shed light on the evolution of the Wnt pathway. This project is a part of the newly established DFG CRC 1324 (A5) on Wnt signaling.

Selected publications since 2013
Number of peer-reviewed articles 2013-2017: 10, number of citations 2013-2017: 67, h-index (2013-2017): 6, total h-index: 35 (according to Thomson Reuters).

Gavelis, G.S., Wakeman, K.C., Tillmann, U., Ripken, C., Mitarai, S., Herranz, M., Özbek, S., Holstein, T.W., Keeling, P.J., Leander, B.S. (2017). Microbial arms race: Ballistic »nematocysts« in dinoflagellates represent a new extreme in organelle complexity. Sci Adv. 3:e1602552.

Petersen, H.O., Höger, S.K., Looso, M., Lengfeld, T., Kuhn, A., Warnken, U., Nishimiya-Fujisawa C., Schnölzer, M., Krüger, M., Özbek, S., Simakov, O., and Holstein T.W. (2015). A Comprehensive Transcriptomic and Proteomic Analysis of *Hydra* Head Regeneration. Mol Biol Evol. 32:1928-47. PMID: 25841488

Watanabe, H., Schmidt, H.A., Kuhn, A., Höger, S.K., Kocagöz, Y., Laumann-Lipp, N., Ozbek, S. and T. W. Holstein. (2014). Nodal signaling determines biradial asymmetry in *Hydra*. Nature: 515:112-510. PMID: 25156256

Watanabe, H., Kuhn, A., Fushiki, M., Agata, K., Özbek, S., Fujisawa, T., Holstein, T.W. (2014). Sequential actions of β-catenin and Bmp pattern the oral nerve net in *Nematostella* vectensis. Nat Commun. 5:5536. PMID: 25534229

Holstein, T.W., and V. Laudet. (2014). Life-history evolution: at the origins of metamorphosis. Curr Biol. 24: R159-61. PMID: 24556439.

PROJECT LEADER
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Fields of Interest
Microtubule dynamics, actin dynamics, adhesion, cytoskeleton, axon guidance, nervous system, live imaging, super-resolution

Brief summary of work since 2013
My lab wants to understand how microtubule architecture is maintained in advancing neurons and how the network can be regulated to respond to guidance cues. A highly dynamic population of microtubules probes the peripheral domain or the growth cone and interacts with actin. Previously, we identified a microtubule plus-tip interacting protein (MT+TIP) called CLASP to be required for axon guidance downstream of the repulsive guidance molecule Slit in Drosophila. Our biochemical studies demonstrated CLASP's interaction with both microtubules and actin (Engel et al. 2014). Depletion of XCLASP1 (Xenopus CLASP1) in embryos revealed that XCLASP1 promoted axon extension and microtubule advance into the growth cone periphery (Marx et al., 2013). Currently we use in silico simulation of microtubule dynamics based on experimental data obtained from spinal cord growth cones. We use the software Cytosim (collaboration with F. Nedelec, EMBL, Heidelberg) to model individual dynamic microtubules and microtubule-actin interactions based on parameters extracted from neuronal growth cones.

Major contributions since 2013
The role of the microtubules in neuronal outgrowth: We found that CLASP1 in Xenopus is expessed during axonal outgrowth (Fig. 1A). CLASPs are microtubule end binding proteins (Fig. 1C), which regulate microtubule dynamics in mitosis and interphase cells. We established quantitative analysis of microtubule dynamics to study the role of CLASP in primary neurons. Using spinal cord neurons of *Xenopus laevis* expressing movement of EB3 comets wer recorded with single microtubule resolution and the data processed by automated particle tracking established in a collaboration with Karl Rohr at the Bioquant (Marx et al., 2013). Reducing *Xenopus* CLASP1 by morpholino injection demonstrated that XCLASP1 promotes axon outgrowth (Fig. 1C) and microtubule advance. Interestingly, the effect of XCLASP1 depletion was very similar to treatment with very low doses of Taxol: in growth cones with reduced CLASP levels, MTs reached the periphery at lower speeds, similar to growth cones treated with very low doses of taxol (5nM). In both cases axons grew slower and showed defects in keeping direction (Marx et al., 2013). In line with the finding that CLASP can bind to actin (Engel et al. 2014), XCLASP1 knockdown also had an effect on the organization of the actin cytoskeleton (Marx et al., 2013).

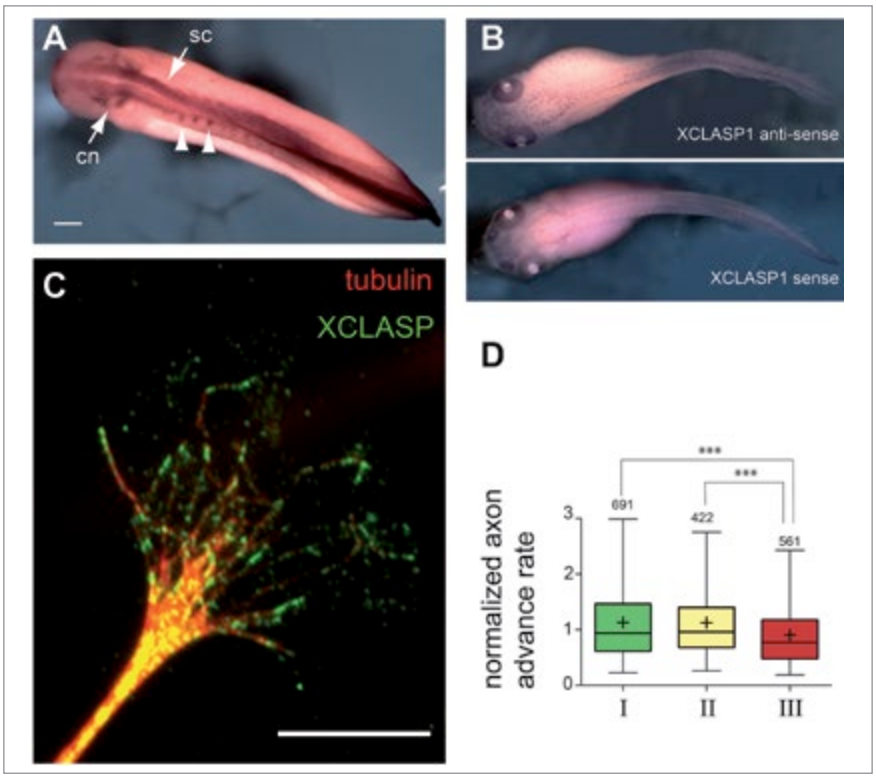

Figure 1
XCLASP1 depletion in spinal cord neurons results in reduced axonal outgrowth. (A) In situ hybridization of XCLASP1 riboprobe reveals localization of XCLASP1 mRNA expression in longitudinal fascicles of the spinal cord (sc), axons of motor neurons (arrowheads), and cranial nerves (cn) in stage 28 embryos. Scale bar, 200 µm. (B) In situ hybridization of XCLASP1 in stage 41 embryos with antisense probe detects no signal above sense riboprobe. (C) XCLASP1 localizes to MT plus ends in neuronal growth cones as detected by Xorbit antibody. Scale bar, 10 µm. (D) Axon advance rates of isolated spinal cord neurons in culture are lower for XCLASP-depleted neurons (III) than for control (I) or control-morpholino injected neurons.

In-vivo 2-photon imaging of brain development: To study the formation of brain nuclei from precursor cells and their projection in brain development requires to document large areas of the brain over extended time periods. In a collaboration with the group of Dr. Matthias Carl, we established time lapse imaging over 4 days on living zebrafish larvae using 2-photon microscopy (Beretta et al. 2017). This allowed us to identify precursor cells of the habenulae and document their contra- and ipsilateral projections to their target regions located 300µm caudally over several days. The importance of an intermediate targets for projects was shown by photoablation (Beretta et al. 2017). The origin of the cells was demonstrated by photoconversion of a reporter fluorescent protein (Beretta et al. 2016).

Planned research and new directions

Modeling of microtubule dynamics: based on experimental data obtained from spinal cord growth cones we are modeling microtubule behavior on the level of individual microtubules. In collaboration with François Nédélec (EMBL, Heidelberg) we use the software Cytosim to model dynamic microtubules and microtubule-actin interactions in realistic growth cone morphologies. The model uses parameters extracted from imaging experiments, such as growth/shrinkage rates and microtubule dynamicity (catastrophe, rescue). We have established a steady-state model of microtubule dynamics and observed good accordance with distribution of MTs in imaging data. In the model, actin bundles are necessary to allow microtubule ends to explore the entire growth cone periphery. Interactions of microtubules with actin bundles play a decisive role: Obstacles result in a slow down of microtubule polymerization and therefore more frequent catastrophe. This change in catastrophe contributes to localization of plus ends in the periphery, which in turn might be important for delivery of signal molecules. To complement our results from simulation, we are designing experiments, where we enhance actin-microtubule interactions, e. g. by expressing proteins that act as transient crosslinkers.

Superresolution microscopy: Structured illumination microscopy has provided a good tool to study cytoskeletal organization in growth cones (Marx et al. 2013) and yeast (Rüthnick et al. in press). We have now extended our superresoltuion imaging to 4-channels, by optimizing labeling and post-acquisition alignment of channels. This provides us with super-resolution imaging to study colocalizations in a rich-context such as adhesion, clustered receptors etc.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 8, number of citations 2013-2017: 32, h-index (2013-2017): 4, total h-index: 17 (according to Thomson Reuters).

Rüthnick, D., Neuner, A., Dietrich, F., Kirrmaier, D., Engel, U., Knop, M., and E. Schiebel, Characterization of spindle pole duplication reveals a regulatory role for NPCs. J. Cell Biol. in press

Beretta, C.A., Dross, N., Guglielmi, L., Bankhead, P., Soulika, M., Gutierrez-Triana, J.A., Paolini, A., Poggi, L., Falk, J., Ryu, S., et al. (2017). Early Commissural Diencephalic Neurons Control Habenular Axon Extension and Targeting. Curr. Biol, 27, 270-278.

Engel, U., Zhan, Y., Long, J.B., Boyle, S.N., Ballif, B.A., Dorey, K., Gygi, S.P., Koleske, A.J., and Vanvactor, D. (2014). Abelson phosphorylation of CLASP2 modulates its association with microtubules and actin. Cytoskeleton 71, 195-209.

Heinrich, D., Ecke, M., Jasnin, M., Engel, U., and Gerisch, G. (2014). Reversible Membrane Pearling in Live Cells upon Destruction of the Actin Cortex. Biophys. J. 106, 1079-1091.

Marx, A., Godinez, W.J., Tsimashchuk, V., Bankhead, P., Rohr, K., and Engel, U. (2013). Xenopus cytoplasmic linker-associated protein 1 (XCLASP1) promotes axon elongation and advance of pioneer microtubules. Mol. Biol. Cell, 24, 1544-1558.

PROJECT LEADER
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Fields of Interest
Molecular evolution in cnidarians, organelle morphogenesis, nematocyst biology, proteomics, structure-function studies in Wnt signaling and ECM molecules

Brief summary of work since 2013
Our research is focused on evolutionary and structure/function aspects of extracellular matrix and Wnt signaling molecules. Our model organism is the cnidarian *Hydra*, which represents one of the most basal metazoan animals and a sister group to the bilateria. A unique feature of cnidarians is the possession of a stinging organelle called »nematocyst«, which has been one of the main subjects of our studies. In these two research areas, we have contributed novel evolutionary and molecular findings using functional proteomics and genomics, as well as focused structure/function studies on selected proteins.

Major contributions since 2013
In the nematocyst field, we have made significant contributions by the characterization of a new protein elastomer (Cnidoin) responsible for the ultrafast nematocyst discharge (Fig. 1) and by elucidating the functional and global evolutionary consequences of a single mutation in short cysteine-rich domains, which are responsible for the macromolecular assembly of nematocysts. In addition, in cooperation with a Canadian group, we recently discovered that protozoan ballistic organelles are a product of convergent evolution, contradicting the textbook knowledge that they share a common evolutionary origin with cnidarian nematocysts. In a project funded by HEIKA, we have been able to screen for novel small molecule inhibitors of *Hydra* TRPN channels responsible for nematocyst discharge. Another project funded by the BW-Stiftung has led to a novel application of nematocyst proteins in synthetic fibers used as stem cell substrates. In the field of Wnt signaling, we have published the first comprehensive mutational study on mouse Wnt3 based on the crystal structure of the Wnt3/Fzd8 complex. In addition, we have defined a novel role for *Hydra* Thrombospondin, a prominent ECM protein and factor of the *Hydra* mesoglea, as negative regulator of Wnt signaling in the hypostomal organizer. A proteomic analysis of the *Hydra* mesoglea for the first time allowed identifying ECM factors regulated during organizer patterning (Fig. 2).

Planned research and new directions
Future research on nematocysts will focus on the subproteome of the tubule structure and novel molecular factors essential for tubule morphogenesis. Furthermore, we will initiate an siRNA screen to identify additional ECM factors involved in pattern formation and morphogenesis in *Hydra*. An initial DFG-funded project in this context is focused on the role of a metalloprotease in Wnt signaling and pattern formation, which started in 2016.

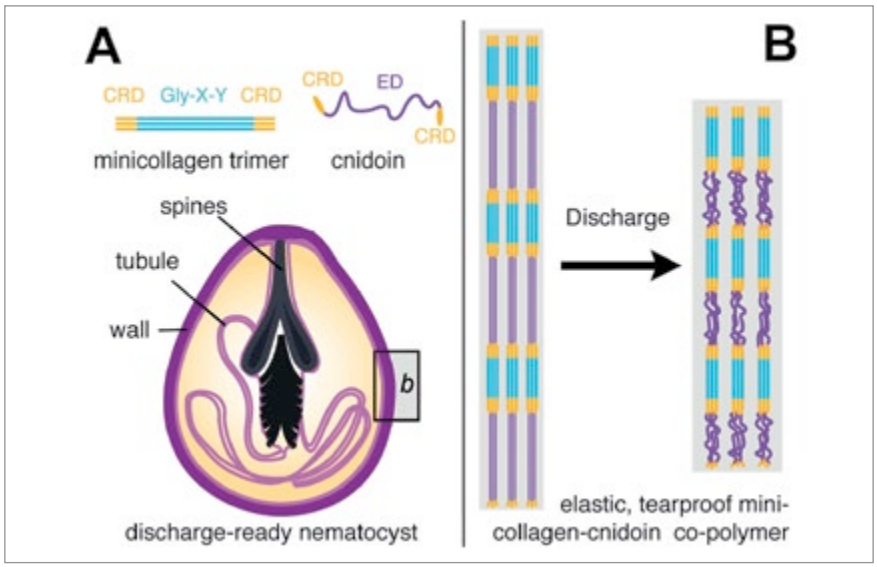

Figure 1
Model of nematocyst capsule polymer from minicollagens and the elastic, silk-like Cnidoin.

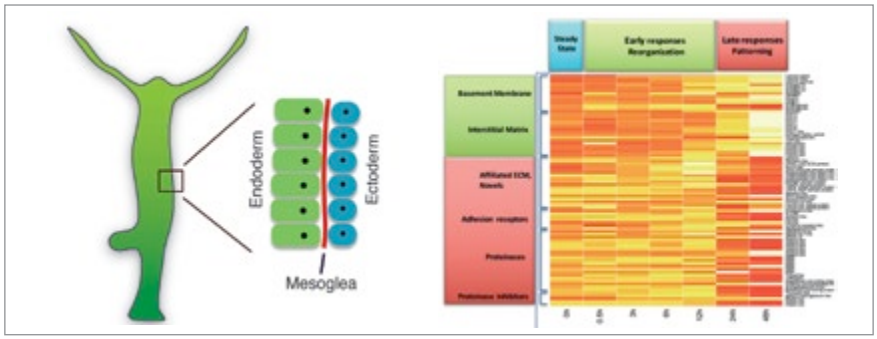

Figure 2
The *Hydra* mesoglea is an ECM connecting the epithelial bilayer. The heat map shows the regulation of the matrisome during head regeneration.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 9, number of citations 2013-2017: 55, h-index (2013-2017): 4, total h-index: 23 (according to Thomson Reuters).

Gavelis G.S., Wakeman K.C., Tillmann U., Ripken C., Mitarai S., Herranz M., Özbek S., Holstein T., Keeling P.J. and Leander B.S. (2017). Microbial arms race: Ballistic »nematocysts« in dinoflagellates represent a new extreme in organelle complexity. Sci. Adv. 2017;3: e1602552

Tursch, A., Mercadante, D., Tennigkeit, J., Grater, F., and Ozbek, S. (2016). Minicollagen cysteine-rich domains encode distinct modes of polymerization to form stable nematocyst capsules. Sci Rep 6, 25709.

Beckmann, A., Xiao, S., Muller, J.P., Mercadante, D., Nuchter, T., Kroger, N., Langhojer, F., Petrich, W., Holstein, T.W., Benoit, M., et al. (2015). A fast recoiling silk-like elastomer facilitates nanosecond nematocyst discharge. BMC biology 13, 3.

Kumar, S., Zigman, M., Patel, T.R., Trageser, B., Gross, J.C., Rahm, K., Boutros, M., Gradl, D., Steinbeisser, H., Holstein, T., et al. (2014). Molecular dissection of Wnt3a-Frizzled8 interaction reveals essential and modulatory determinants of Wnt signaling activity. BMC biology 12, 44.

Watanabe, H., Schmidt, H.A., Kuhn, A., Hoger, S.K., Kocagoz, Y., Laumann-Lipp, N., Ozbek, S., and Holstein, T.W. (2014). Nodal signalling determines biradial asymmetry in *Hydra*. Nature.

2.11 DR. AMAL J. JOHNSTON
INDEPENDENT RESEARCH GROUP GERMLINE BIOLOGY

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Fields of Interest
Female and male germlines and gametes; convergent evolution of reproductive systems; higher order regulatory network; transcription factors; stress and epigenetics; sexuality and apomixis

Brief summary of work since 2013
Our long-standing thrust is to unravel sex-specific developmental mechanisms across evolutionarily extant biological systems in order to dissect molecular signatures of adaptive evolution. Evolution of convergent and divergent reproductive mechanisms that regulate gamete (eg. protected egg) and spore development can be traced throughout eukaryotes (Fig. 1). Transcription factors and underlying regulatory networks are of our primary focus, and we use a transcriptional repressor protein Retinoblastoma (pRb), otherwise known as RETINOBLASTOMA RELATED in plants, as a main hub to tease apart the connecting nodes. We and other participating labs have established that RBR controls gametic differentiation, apomixis-like behaviour during (mega)spore development, stem cell homeostasis and stress. We have identified key transcription factors tethered by RBR to control egg cell development in *Arabidopsis*. In addition, we have also focused how RBR controls male germline development and stress in stark contrast to the somatic development in *Arabidopsis*. In order to understand how RBR network operated along evolution, we have initiated studies in moss, a lower plant lineage believed to have thrived prior land plant evolution following limiting water and abundant light conditions (Fig. 1).

Whereas in animals germ cells represent stem cell populations that are set aside early in development during embryogenesis, plant germlines differentiate late in development prior the onset of meiosis, developing into haploid female and male gametophytes (Fig. 1). Genetic modifications in female germline development (eg. avoidance of meiosis, parthenogenesis) may lead to clonal offspring in asexual organisms via a natural cloning process known as apomixis. We have made use of *Boechera*, an apomictic relative of *Arabidopsis*, to understand how stress and meiosis are inter-related, and also how parthenogenesis and violation of imprinting are mechanistically correlated. A detailed RNA-sequencing identified several transcription factors connected to stress and reproduction eg. NAC factors, and also key genes involved in DNA repair and meiosis. Genome-wide DNA methylation changes seem to allow for transcriptional shifts prior and at apomeiotic and parthenogenetic development.

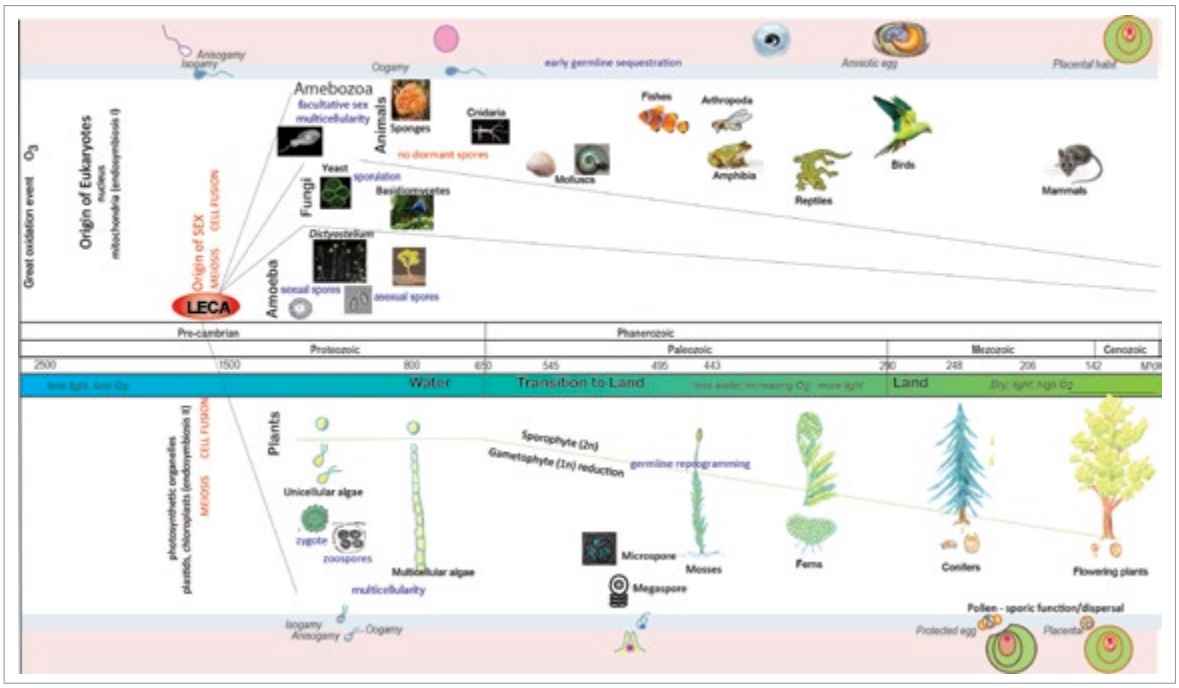

Figure 1
Eukaryotic evolution of reproductive systems

Major contributions since 2013

In addition to DNA methylation and stress being predominant denominators controlling apomixis development, we noted that a maternalized MADS transcription factor which is otherwise known for its role in imprinting is regulated by DNA methylation during parthenogenesis. Further, we noted that ovular heterochrony and perturbation of stem cell homeostasis accompany apomictic initiation, such that meiosis is modified. Unfortunately, we know almost nothing about how RBR participates in the apomictic process in the wild. In line with a finding from another lab that established the role of RBR in *Arabidopsis* during apomixis-like development and stem cell homeostasis, we noted that down-regulation of RBR could be relevant for initiation of apomixis. Further, we observed that RBR regulates male germline development particularly transition from meiosis to mitosis, and stress amelioration. There is a stark context on to how RBR controls these events across source and sink tissues, and might indicate the mechanistic connection between RBR, cell cycle and metabolism in general. Depletion of RBR also leads to aberrant starch and lipid accumulation in pollen indicating how RBR is involved in the control of storage reserves there.

As part of technology development drive, we generated a suite of modules termed as *Byepass* that allow for gene stacking by homology-based cloning in yeast and/or bacteria, or by using rare endonucleases. Among others, a palette of GFP-derivatives for reporter fusions have been incorporated, and also elements that are necessary for bimolecular fluorescence complementation, dominant-negative forms, loss and gain of function of genes are incorporated. Assembled modules can cargo to plant systems for functional analyses. We demonstrate the versatile application of *Byepass* in non-plant systems as well. These tools are currently being utilized in our work and will be also available for the community upon publication.

We have identified a core transcriptional network tethered by RBR that regulates egg cell development, stress homeostasis and zygotic transition. By chromatin immuno-precipitation we could demonstrate that some of these specific transcription factors are also regulated by RBR-mediated protein-DNA interaction, and the Polycomb complexes target similar genes at the same location in the DNA. Therefore, there are potential cross-talks mediated by an RBR associated higher-order network in operation during egg development.

We have completed three major RNA-sequencing projects and a bisulfite-seq (for DNA methylation) project during this period, and an additional bisulfite-seq project is ongoing. Similarly a large-scale ChIP-seq project is ongoing to identify potential RBR targets during development. One PhD project and four Master projects have been completed to date, and our results are disseminated in three international meetings via invited talks and by additional local lectures.

Planned research and new directions

Whereas our work on gamete development and apomixis using higher plant systems progressed well, we have faced a significant lag phase in the evo-devo project that aimed to compare developmental pathways centered on RBR in moss. Knock-down of two out of three copies of RBR did not reveal meaningful phenotypes, and further work needs to be done in this area. Besides a PhD project centered on this aspect was discontinued in the second year and training of a new personnel incurred further delays amidst prolonged sick-leave of two senior scientists in the lab. The evo-devo project was, therefore, further shaped in a distinct angle to understand zygote versus spore evolution pathways in collaboration with external labs, branching out from amoeba to plants. rblA, the homolog of RBR in *Dictyostelium* is known for controlling spore and stalk pathway and we identified transcription factors down stream of RBR/rblA, and established mutants that perturb spore development. In *Arabidopsis*, the corresponding paralog OESTEREI, which is expressed in the spores in addition to the egg cells, and loss-of-function mutants aberrantly develop twin embryos (Fig. 2). Ongoing work aims to connect the RBR-OESTEREI network across distinct evolutionary distance using *Arabidopsis*-moss-amoeba.

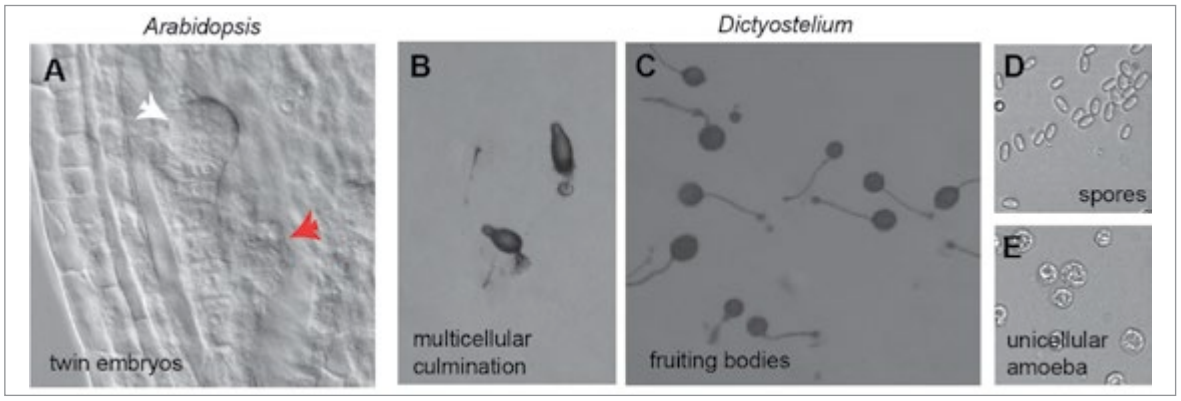

Figure 2
OSTEREI TF controls reproductive development
(A) A mutation in *Arabidopsis* OSTEREI (OSI) causes developmental transition to form twin embryos. (B-E) Asexual/apomictic spore germination in *Dictyostelium*

A very recent finding that connects RBR, histone/DNA methylation and stress are currently being followed up in *Arabidopsis*, and we aim to connect these nodes to metabolism too. One possible node connecting microtubules and RBR during stress and development is readily discernible, and is also being continued. Distinct RBR-specific regulatory elements are currently mapped and are expected to reveal germline-specific repression and activation. Genome-wide RBR ChIP-seq is at present ongoing, and is expected to provide a clear picture of this intriguing regulon.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 3, total h-index: 6 (according to Thomson Reuters).

Tayyab, M. (2017). Molecular genetic framework of vegetative and male Germline Development, and Abiotic Stress, Mediated by *RETINOBLASTOMA RELATED* in *Arabidopsis thaliana*. PhD thesis, Faculty of Biosciences, Heidelberg University.

Shah, J.N., Kirioukhova, O., Pawar, P., Tayyab, M., Mateo, J.L. and Johnston, A.J. (2016). Depletion of Key Meiotic Genes and Transcriptome-Wide Abiotic Stress Reprogramming Mark Early Preparatory Events Ahead of Apomeiotic Transition. Front Plant Sci 7, 1539.

Zinta, G., Khan A., AbdElgawad, H., Verma V and Srivastava AK. (2016). Unveiling the Redox Control of Plant Reproductive Development during Abiotic Stress. Front Plant Sci 7, 700.

Khan, A. and Zinta, G. (2016) Drought Stress and Chromatin: An Epigenetic Perspective: 571-586. In. Drought Stress Tolerance in Plants 2, 571.

2.12 PROF. DR. MARCUS A. KOCH
BIODIVERSITY AND PLANT SYSTEMATICS

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Fields of Interest
Plant systematics and evolutionary biology, adaptation and trait evolution, biodiversity assessment and protection, botanical garden and herbarium collections, knowledge databases, Brassicaeae

Brief summary of work since 2013
A major achievement since 2013 was the successful contribution with two projects within second funding period of the DFG priority programme »Adaptomics« (DFG-SPP 1529), which was launched together with Ute Krämer (Heidelberg, now Bochum) in 2009. Central scientific objective of this Programme is to obtain fundamentally novel, comprehensive and increasingly predictive insights into the molecular solutions that plant species develop to match local environmental demands. By concentrating on the *Brassicaceae* family that exhibits remarkable ecological diversity, this Programme takes advantage of progress in the molecular understanding of the *Brassicaceae* model plant *Arabidopsis thaliana* and capitalizes on recent seminal advances in sequencing technology and bioinformatics. Our department is central to the SPP with the *BrassiBase* knowledge database project, but unravelled the evolutionary history and trait evolution of various important groups and hundreds of species. A species check-list for the entire family with nearly 4000 taxa has been finalized serving actually even as a blueprint for herbaria in Europe to restructure their collections with 100,000s of vouchers.

Since 2014 genomic approaches and respective informatics pipelines have been established, which allow us to characterize evolutionary processes in space and time with the highest resolution and also focus more easily on non-model systems (e. g. Cheddar Pink, *Dianthus gratianopolitanus* as an example for high priority species in international conservation strategies; St. John´s wort, *Hypericum perforatum*, as an example of the evolution of cryptic gene pools and associated non-sexual reproduction, the Gingko-tree as a »living fossil« or invasive Turkish Warty-Cabbage, *Bunias orientalis*. Of particular interest is a better understanding of adaptation to cold environment in evolutionary terms. Here we develop the arctic-alpine genus *Cochlearia* towards a system to study genome-environment interactions to better understand how phenotypic plasticity and genetic variation contribute to cold adaptation and study the effect of rising temperatures in northern hemisphere arctic-alpine biota.

Figure 1
Cochlearia pyrenaica (Brassicaceae) in its alpine environment (here Scotland).

Major contributions and research highlights since 2013

Our research focus on cruciferous plants is substantially contributing to establishing various important model groups in plant evolutionary biology such as *Arabis alpina, Noccaea caerulescens*, both relatives of *Arabidopsis*. However, not only are we providing a solid scientific basis for single species, but our department is establishing *Brassicaceae* as one of the most important model systems in dicotyledonous angiosperms at the familial level. Our impact in this regard is documented by a series of publications at different taxonomic levels addressing a broad spectrum of evolutionary processes and topics. We developed a spatio-temporal evolutionary framework for the family and were able to relate significant biological transitions such as species radiation, genome duplications, diploidization with major environmental transitions during the last 25 million years (Hohmann et al. 2015).

We continue to focus on the evolution of wild relatives of *Arabidopsis*. The most important achievement was the presentation of the first comprehensive phylogenetic and evolutionary perspective on this important genus based on whole genome sequence data (Novikova et al. 2016). For this genus we further worked on the development of a hypothesis in a natural hybrid zone in Austria which may explain how a narrowly adapted species (*A. lyrata*) has colonized a broad ecological niche. The idea involves interploidal and interspecies gene flow where the transfer of genetic material has potentially provided the source of new allelic variation for local adaptation (Muir et al. 2014). Using whole genome sampling of individuals from an aölpine introgression zone and a phylogenomics approach to correctly identify single copy homologous genes, we could confirm (genomically) the results apparent at the population level. Limestone populations of *A. lyrata* have been introgressed by *A. arenosa*, and there is evidence that *A. lyrata* has moved and colonised onto a new bedrock type (from a cline apparent in introgression from south to north). We are currently using genomic tools to identify signatures of genomic interferences.

Major efforts were undertaken to compile and archive research on *Brassicaceae* into a comprehensive framework. The *BrassiBase* knowledge database system, as it is now known, is a developing online-accessible knowledge and database system of cross-referenced information and resources on *Brassicaceae* (Cruciferae). Information on taxonomy, systematics and evolution, chromosome numbers, character traits, germplasm resources, and accurate enumeration of all species, genera and tribes is documented. Biological, molecular and evolutionary knowledge is exponentially increasing in the mustard family but because of the complex and overwhelming biological diversity of the family, it is difficult to assess research results within a larger evolutionary framework. This was the rationale for the second release, which builds on the taxonomic content and synergizes

taxonomic information into a »Taxonomy Tool« with several new features such as a »Phylogenetics Tool« and a »Cytogenetics Tool« (Kiefer et al. 2014). With 2017 we have completed the species checklist of the entire family and incorporated an interactive key to the family and provide a tool to display any important morphological trait and character over the entire family. These tools will largely facilitate research on trait evolution and some emphasis on the evolution of flower traits is demonstrated by the research profile of Claudia Erbar (see separate report).

Evolutionary breeding systems have been studied on large spatial continental scales in detail and revealed not only fundamental insights into the dynamics of breeding systems and cryptic gene pools (ranging from apomixis to full sexual reproduction) but also highlighted the influence of introgression and hybridization during speciation and evolution. Our studies on the crucifer *Boechera* highlighted Pleistocene differentiation patterns at a North American and continental scale (Mau et al. 2015), and this work elucidated the dynamics of the evolution of apomixis in the widest sense, indicating multiple independent realizations of this trait. Focus on molecular and cellular aspects of apomixes in *Boechera* is given with the research group of Anja Schmidt (see separate report).

Revisions and detailed taxonomic-systematics treatments have been completed for difficult genera (*Arabis* and *Aubrieta*) from biodiversity hotspots such as the Irano-Turanian region and Greece (Karl & Koch 2014, Koch et al. 2017) and led also to the recognition and description of new taxa.

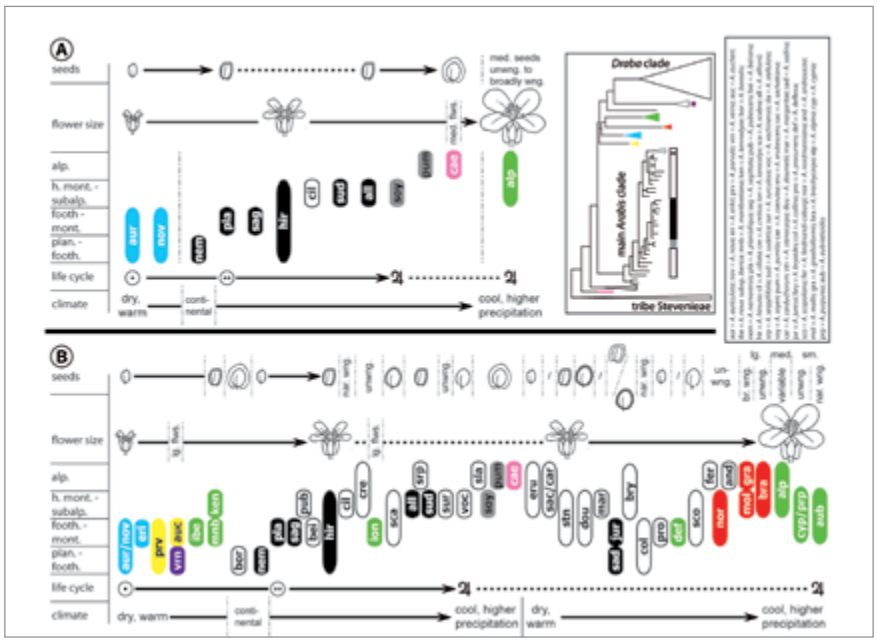

Figure 2
Adaptive flower and seed trait evolution in *Arabis* (Karl & Koch 2014).

As one example of scientific work with important collections and associated information here »The Werner Rauh Heritage Project« serves as one example. The project is based on the life-time work of Prof. Rauh, director of the Botanical Garden, since during his time (1960–1982) and until his retirement until 1994, he made more than 36 expeditions, mainly to South- and Central-America, as well as to the south of Africa and particularly to Madagascar. From these journeys, he brought back innumerable plants to the Botanical Garden in Heidelberg, which are a valuable part of today's living collection and of the Herbarium (HEID). During his expeditions he scribed hundreds of booklets (~90) with detailed information not only about the plants collected, but recording vegetation and geology and cultural heritage of the regions he visited. As part of this record, all these information were made available and are being processed within »The Werner Rauh Heritage Project« knowledge database system. Further research with strong curatorial collection management is highlighted with the profile of Andreas Franzke and Dmitry German (see separate short report).

Planned research and new directions

A major task will be to further develop genomic and transcriptomic tools and approaches in our department to address evolutionary questions with high resolution in space and time. We selected the genus *Cochlearia* is an evolutionary cold-adapted group of young species of Pleistocene origin. It is an excellent evolutionary study system that allows us to address questions in relation to parallel trait evolution, reticulate speciation networks and polyploid evolution. The system is also an ideal means of contrasting local adaptation with phylogenetic inertia and in resolving classical questions in plant biogeography such as species radiations in cold temperate climates. This work is embedded into an ambitious project to resolve the evolutionary history and phylogenetics of the entire *Brassicaceae* family utilizing whole genome sequence data. Family-wide genomic data are analysed within the framework of the entire Superrosid clade, thereby allowing elaboration on a comprehensive time-calibrated completely phylogenetically resolving framework with important divergence time estimates from within the entire family. These data will allow testing for the hypothesis that overall species richness of the family is well explained by high frequencies of polyploidization with more than 43 % neopolyploid taxa, but is not directly associated with an increase in genome size, e. g. via ancient polyploidization events, nor exhibits obvious general lineage-specific constraints. Instead, we aim to elaborate on the hypothesis that after mesopolyploidization events a severe lag-phase was needed for diploidization until (and often under new environmental conditions) diversification started. Thereby, our aim is to test the idea that these spatio-temporal diversification patterns may correlate among the various evolutionary lineages, thereby allowing us to draw conclusions about major past-climate/environmental transitions contributing to the radiation of the entire family in concert with extensive polyploidization from within the last 35 million years.

A second focus will be on conservation genetics and biodiversity research of selected species and biota. A particular focus will be on the calcicole-calcifuge phenomenon shown by a major proportion of plant species. This describes the observation on strict preferences of plant species to the respective and very different bedrock types, often displayed by sister species. A deeper understanding of this phenomenon will provide some further insights into the understanding of current day plant distribution and evolution.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 41, number of citations 2013-2017: 1968, h-index (2013-2017): 10, total h-index: 38 (according to Thomson Reuters).

Koch, M.A., Karl, R., and German, G.A. (2017) Underexplored biodiversity of Eastern Mediterranean biota: Systematics and evolutionary history of the genus *Aubrieta* (*Brassicaceae*). Ann. Bot. 119, 39-57.

Novikova, P., Hohmann, N., Nizhynska, V., Lanz, C., Tsuchimatsu, T., Muir, G., Guggisberg, A., Paape, T., Schmid, K., Fedorenko, O., Holm, S., Säll, T., Schlötterer, C., Marhold, K., Widmer, A., Sese, J., Shimizu, K.K., Weigel, D., Krämer, U., Koch, M.A., and Nordborg, M. (2016) Sequencing of the genus *Arabidopsis* reveals a complex history of non-bifurcating speciation and abundant trans-specific polymorphisms. Nat. Genet. 48, 1077-1082.

Hohmann, N., Wolf, E., Lysak, M., and Koch, M.A. (2015) A Time-Calibrated Road Map of *Brassicaceae* Species Radiation and Evolutionary History. Pl. Cell 27, 2770-2784.

Mau, M., Lovell, J.T., Corral, J.M., Kiefer, C., Koch, M.A., Aliyu, O.M., McKay, J., and Sharbel, T.F. (2015) Hybrid apomicts trapped in the ecological niches of their sexual ancestors. Proc. Nat. Acad. Sci. (PNAS) US 112, 2357-2365.

Karl, R., and Koch, M.A. (2014) Phylogenetic signatures of adaptation: The *Arabis hirsuta* species aggregate (*Brassicaceae*) revisited. Perspectives Pl. Ecol. Evol. Syst. (PPEES) 16, 247-264.

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Fields of Interest
Flower ontogeny, flower morphology, flower ecology, angiosperm systematics, evolution of the angiosperms, biodiversity, evolutionary biology

Brief summary of work since 2013
Our research is focused on floral developmental and morphological features for understanding relationships between structure, function, systematics, and phylogeny as well as on plant-flower-interactions. Projects that have been pursued in detail are the diversity of nectaries in angiosperms and style diversity in Asteraceae. Since nectar-secreting tissues show some variation in the location and histological structure, these characters can be used in systematics. Nectar secretion already early in angiosperm evolution raises the question about the driving force behind the development of nectaries. The in-depth research on style morphology and mode of secondary pollen presentation in the family Asteraceae (with regard to function and phylogeny) is in progress for the whole family. Five papers on basal groups of this largest angiosperm family are published within the reporting period. Additionally, the sex- and breeding-behaviour of the Sicilian snail-shell bee (*Rhodanthidium siculum*) and their flower visits were studied during two field trips.

Major contributions since 2013
In basal angiosperms, in a number of families and genera nectar is offered, though not in great amounts and in addition to pollen as a reward. The diverse structures (nectary sites and histology), the rare occurrence, and scattered distribution of nectaries in the basal groups indicate convergent evolution. What is the selective advantage of nectar secretion in pollen flowers? Since the nectar production is limited mainly to the female phase of the proterogynous flowers, it may be concluded that the nectar assures the attractiveness of the flower in the non-pollen presenting phase. In addition, during searching for the sparse nectar, the insect may come into contact with each stigma thus compensating the economic disadvantage of a choricarpous compared to a coenocarpous gynoecium.
Based on in-depth studies on style morphology of basal subfamilies and tribes of Asteraceae, we establish 15 style types in view of shape, bifurcation and distribution of stylar hairs and stigmatic tissue. The high diversity of stylar shapes in the basal groups of Asteraceae is remarkable. Our studies support many of the DNA-based clades by style morphology. It is generally assumed that the basal groups of Asteraceae exhibit a continuous stigmatic area on the inner surface of the stylar. However, we found that in these groups an inversely U-shaped stigma on the inside of the stylar branches occurs, realized either by the loss of receptivity of the papillate median tissue or by adhesion of the stylar branches to each other mediated by their median ventral tissues.

The sexual and breeding behaviour of *Rhodanthidium siculum* was almost unknown. We could demonstrate for the first time the high frequency of copulation (with different partners), the composition of larval supply in the snail shells used for breeding, and the sealing, transporting and burying of the filled snail shells. *R. siculum* differs from most other bee species because males are mostly significantly larger than females. In addition, males and females vary in size. A surprising observation was that not only the large males, the territory owners, mate with the female provisioning the nest but also smaller males. Because the small males mostly cannot win the struggle for the nest, they pursue a »sneaking strategy«. Whenever possible, they copulate with a female either when the large male is absent from the nest during a foraging flight or if he tolerates the mating (after several matings perhaps he is exhausted). If we assume that sperm of sequential matings is somewhat mixed, this would result in an advantage for the population. Perhaps, there is a genetic disposition of the different body sizes of the females, which would be advantageous for the breeding success in correlation with the different snail-shell sizes.

Figure 1
Rhodanthidium siculum. a, Large male guarding the snail shell. b, Copulation. c, Females closes the snail shell after loading the snail shell with nectar and pollen and laying 1–2 eggs. d, Female visiting a flower of *Lotus creticus*, a main source for pollen and nectar.

Planned research and new directions

The research on flower development will continuously go on. One focus will be on the mode of corolla tube formation in the euasterids, especially in scarcely investigated families. Our future research on nectaries will concentrate on Brassicaceae. The insect-pollinated flowers of Brassicaceae exhibit a receptacular nectary of the mesophyllary type. In Brassicaceae, the receptacular nectaries show a high diversity as regards number and position as well as shape and size. We want to characterize the tribes by their nectaries (SEM). On the other hand, we want to trace some possible trends, which occurred several times in parallel and for the underlying evolutionary constraint.

The work in progress on the detailed comparative study (SEM, histology) concerning style morphology of Asteraceae (including about 400 species of about 260 genera covering all 44 tribes) and type of secondary pollen presentation will be continued ending up in the atlas-like compilation.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 9, number of citations 2013-2017: 112, h-index (2013-2017): 3, total h-index: 12 (according to Thomson Reuters)

Erbar, C. & Leins, P. (2017). Sex- and breeding-behaviour of the Sicilian snail-shell bee (*Rhodanthidium siculum* Spinola, 1838; Apoidea-Megachilidae- preliminary results. APIS 11: 317-328 DOI: 10.1007/s11829-016-9489-x

Erbar, C. (2016). Unique style morphology in the monotypic Famatinanthoideae-Famatinantheae, a recently established subfamily and tribe of Asteraceae. Syst. Bot. 41: 796–806.

Erbar, C. & Leins, P. (2016). Styles and new stigma characters in Mutisieae s.str. (Asteraceae-Mutisioideae) in comparison with genera of traditionally circumscribed Mutisieae. Plant Div. Evol. 131, 363–393.

Erbar, C. & Leins, P. (2015). Diversity of styles and mechanisms of secondary pollen presentation in basal Compositae – new insights in phylogeny and function. Flora 217, 109-130.

Erbar, C. (2014). Nectar secretion and nectaries in basal angiosperms, magnoliids and non-core eudicots and a comparison with core eudicots. Plant Div. Evol. 131, 63-143.

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Fields of Interest
Developmental genetics, epigenetics, evolution, sexual and asexual plant reproduction (apomixis), plant reproductive lineage (germline), RNA helicases, transcriptional profiling

Brief summary of work since 2013
During the recent years, my research was focussed on the investigation of the genetic basis underlying sexual and asexual plant reproduction through seeds (apomixis). In longer terms, a detailed understanding of these fascinating developmental processes is a precondition for targeted manipulation of plant reproduction for crop improvement. Until 2015 (as Postdoc in the laboratory of Ueli Grossniklaus, University of Zürich) my work covered two major aspects of plant developmental genetics: the dissection of epigenetic regulatory pathways controlling seed and endosperm development (Schmidt et al., 2013; Florez-Rueda et al., 2016), and the cell-type specific transcriptome analysis to study reproductive development in apomictic *Boechera ssp*. (Schmidt et al., 2014). Since moving to Heidelberg in 2016, I mainly focus on the role of RNA helicases for plant reproduction and aspects of their evolution. An additional aim is to gain more insights into the gene regulatory networks controlling the formation of sexual and apomictic reproductive lineages using *Boechera* as model system.

Major contributions since 2013
Apomixis has long been recognized for its outstanding potential for crop improvement, as it leads to the formation of offspring that is genetically identical to the mother plants. In apomicts, endosperm can either be formed dependent on central cell fertilization (pseudogamy) or autonomously. Using *Arabidopsis thaliana*, we identified that MEDEA, a core component of the FERTILIZATION-INDEPENDENT SEED Polycomb Repressive Complex 2 (FIS-PRC2), interacts with the DNA METHYLTRANSFERASE MET1 to repress autonomous endosperm development (Schmidt et al., 2013).

Additional core components of apomixis are omission or abortion of meiosis by the first cell of the apomictic germline, the apomictic initial cell (AIC), and parthenogenesis, meaning development of the egg cell into an embryo without preceding fertilization. In order to study the molecular mechanisms underlying core components of apomixis, the genus *Boechera* is an ideal model system, as it harbors a large variety of sexual and apomictic accessions at low ploidy levels (2n, 3n). In Zurich, we have been using the triploid apomict *B. gunnisoniana* to undertake a comprehensive cell-type specific transcriptome profiling of cells of the reproductive lineage isolated by laser assisted microdissection (LAM) (Schmidt et al., 2014). As additional tool for genomic and transcriptomic studies, we established an annotated reference transcriptome for *B. gunnisoniana*, including the identification of *A. thaliana* homologues (Schmidt et al., 2014). This work allowed the analysis of genes and pathways active in the apomictic germline and serves as excellent starting point for further investigations of apomixis in *Boechera*.

Since my start in Heidelberg in spring 2016, the method of LAM for transcriptional profiling of reproductive tissues, overcoming the challenge to sample small and rare tissue types developing enclosed by maternal flower tissues, has successfully been set up in Heidelberg (Figure 1). This provides the basis to extend the transcriptional analyses to deepen the understanding of the gene regulatory pathways underlying apomixis and sexual reproduction in *Boechera*.

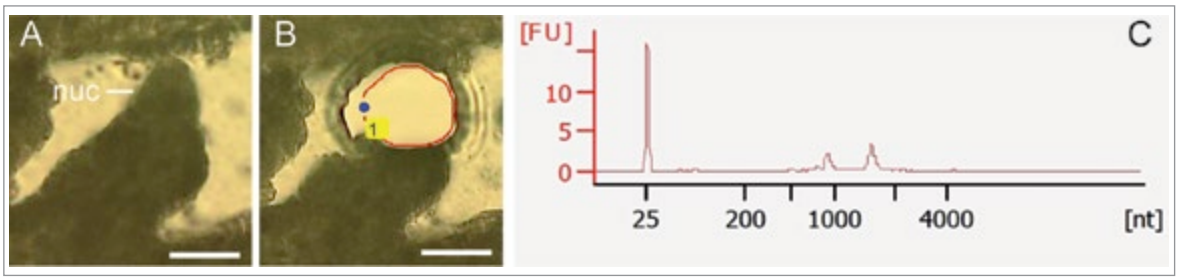

Figure 1
LAM of reproductive tissues in *Boechera*. A, B) 8 µm thin section of an ovule from *Boechera divaricarpa* (A) before and (B) after LAM of the nucellus (nuc; part of the ovule harboring the AIC). B) The red line indicates the laser path. scale = 20 µm. C) Bioanalyzer profile from controls after LAM indicates good RNA quality for downstream transcriptome analysis.

Planned research and new directions

The importance of RNA helicases for the formation of the plant reproductive lineage has previously been uncovered (Schmidt et al., PLoS BIOL 2011). Importantly, plants carrying a mutant allele of the RNA helicase *MNEME (MEM)* show features of apomixis (Schmidt et al., PLoS BIOL 2011). However, to date knowledge is limiting about the roles of RNA helicases for plant sexual and apomictic reproduction. A central goal of my current research is the investigation of the roles of selected RNA helicases for plant reproduction and aspects of their evolution. To this aim, functional analysis in *Arabidopsis thaliana* is combined with genomic and transcriptomic work in *Boechera*. Mutant analysis is ongoing in order to identify RNA helicases important for reproductive development (Figure 2). Moreover, the analysis of allelic variants from a number of different sexual and apomictic *Boechera* accessions has been initiated in order to identify allelic variants correlated with apomixis, and further transcriptional analyses from sexual and apomictic *Boechera* accession are planned to deepen the understanding of the gene regulatory networks governing plant germline formation.

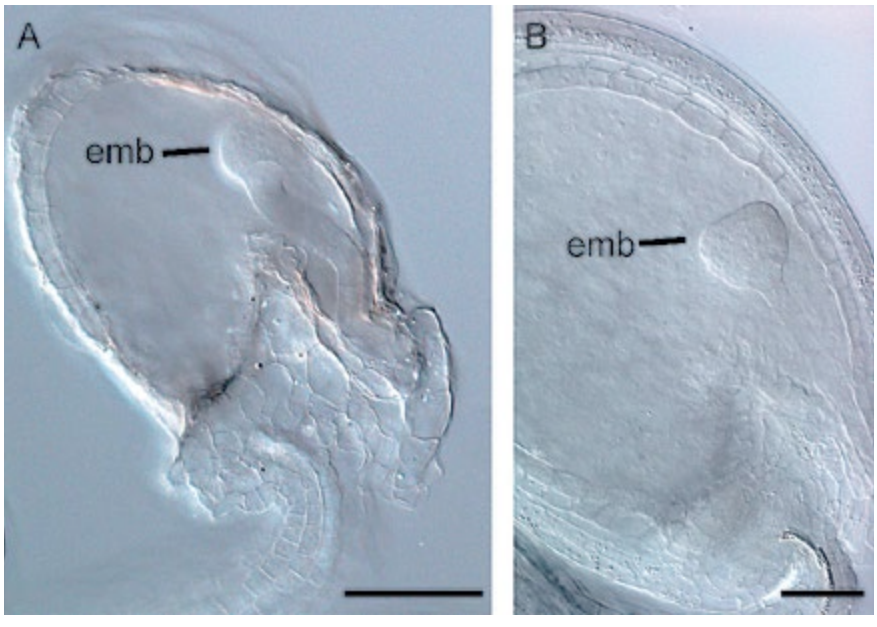

Figure 2
Mutant analysis. A) Based on preliminary results, plants carrying a mutation in a novel candidate RNA helicase frequently lead to abortion of embryogenesis. B) Wild-type like embryo from the same plant. scale = 50 µm.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 8, number of citations 2013-2017: 72, h-index (2013-2017): 4, total h-index: 11 (according to Thomson Reuters).

Florez Rueda, A.M., Paris, M., Schmidt, A., Widmer, A., Grossniklaus, U., and Städler, T. (2016). Genomic imprinting in the endosperm is systematically perturbed in abortive hybrid tomato seeds. Mol Biol Evol. 33(11):2935-2946. doi:10.1093/molbev/msw175.

Florez Rueda, A.M., Grossniklaus, U., and Schmidt, A. (2016). Laser-assisted microdissection as tool for transcriptional profiling of individual cell types. J Vis Exp. 10(111). doi: 10.3791/53916.

Schmidt, A., Schmid, M.W., and Grossniklaus, U. (2015). Plant germline formation: common concepts and developmental flexibility in sexual and asexual reproduction. Development 142(2):229-41. doi: 10.1242/dev.102103.

Schmidt, A., Schmid, M.W., Klostermeier, U.C., Qi, W., Guthörl, D., Sailer, C., Waller, M., Rosenstiel, P., and Grossniklaus, U. (2014). Apomictic and sexual germline development differ with respect to cell cycle, transcriptional, hormonal and epigenetic regulation. PLoS Genet. 10(7):e1004476. doi: 10.1371/journal.pgen.1004476.

Schmidt, A., Wöhrmann, H.P., Raissig, M.T., Arand, J., Gheyselinck, J., Gagliardini, V., Heichinger, C., Walter, J., and Grossniklaus, U. (2013). The Polycomb group protein MEDEA and the DNA methyltransferase MET1 interact to repress autonomous endosperm development in Arabidopsis. Plant J. 2013 73(5):776-87. doi: 10.1111/tpj.12070.

2.13 PROF. DR. URSULA KUMMER
MODELLING OF BIOLOGICAL PROCESSES

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Fields of Interest
Systems biology, computational methods for modeling and analysis, information processing in signalling and metabolic networks, functional dynamics

Brief summary of work since 2013
Research in the group focuses on one side on the development of computational methods of methods for the modeling, simulation and analysis of biochemical networks. On the other hand, application projects on relevant biological systems are of major importance for the group. On the methodological side, during the last years we focused a lot on the development of methods for the stochastic, discrete description of biological processes. Thus, we developed a new method for parameter estimation in stochastic, dynamic systems and another one to efficiently estimate parameters for stochastic systems using distribution data from high-throughput experiments like FACS. As always, we already indlcuded or are in the process to include the respective algorithms in our software COPASI, that we develop together with my colleague Pedro Mendes (Connecticut, USA) and his group. The software COPASI has just been selected as a national resource for modeling and receives funding from the BMBF as such. By now, our download numbers per release exceed 10,000. On the application side we continued projects on information processing in neutrophils and hepatocytes and set-up multi-scale models for TNFa-signalling, as well as INFa signalling, in the latter case spanning scales from whole body to intracellular signalling. We successfully established methodology to integrate quantitative proteomics data with whole-genome scale metabolic models and aquired a very thorough understanding of metabolic rearrangements during pH-shifts in Enterococcus faecalis. Finally, we started several collaborative projects with COS members. In an EcTop project we are trying to understand the distribution of control in sulfur assimilation in Arabidopsis during changing environmental conditions (with Rüdiger Hell). Together with Karin Schumacher we investigate pH maintenance in plant cell compartments (SFB 1101) and calcium signalling in plant cells (FOR 964).

Major contributions since 2013
Method developement:
Compared to conventional ODE modeling, modeling based on stochastic formalisms dealing with discrete particle numbers has poor access to sophisticated analysis methods which hampers its application. Nevertheless, it is necessary to apply this formalism when dealing with subsystems e. g. with low particle numbers as often seen in signal transduction or in genetic networks. Therefore, we have developed a parameter estimation technique that allows parameter fitting to experimental data in stochastic systems (e. g. Bergmann et al., 2016). Furthermore to account for modern experimental high-throughput techniques, we also developed a method for parameter estimation from distribution data (Fig. 1) (Aguilera et al., 2017).

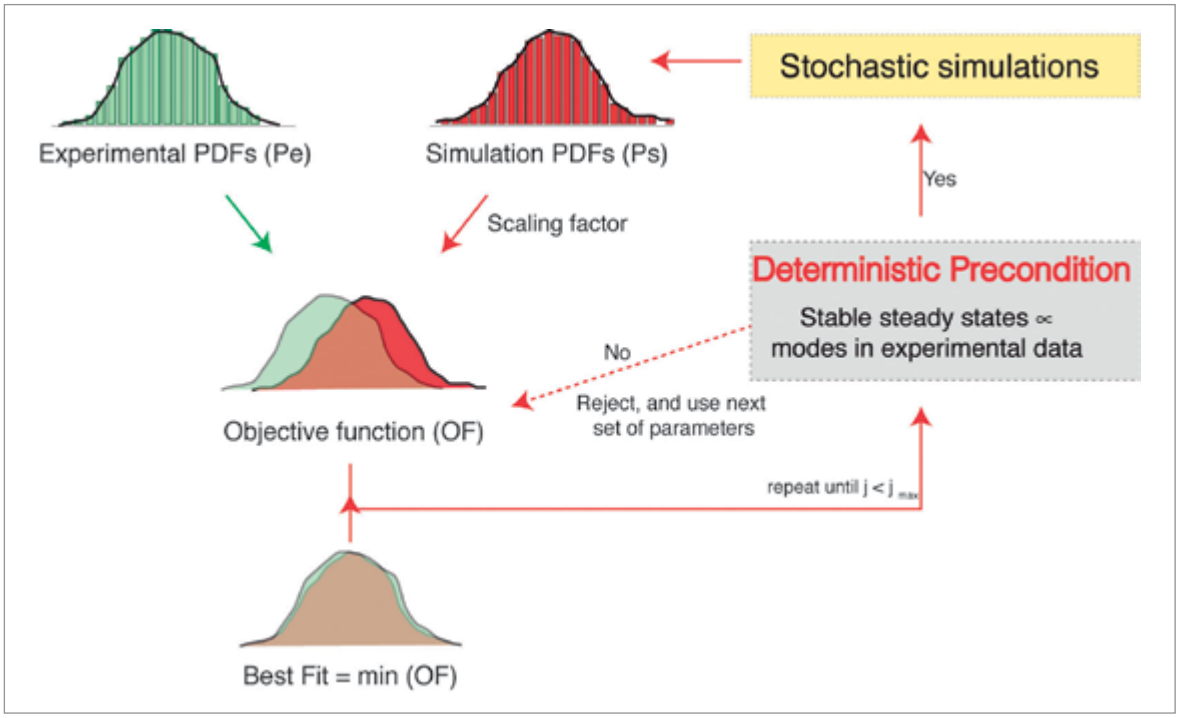

Figure 1
Algorithm to efficiently fit stochastic models to experimental data: The algorithm solves a model in the deterministic and stochastic regimes. A condition observed in the experimental data is defined (e. g. a certain mean steady state). A set of parameter values is evaluated in the deterministic regime to test if the model reproduces this condition. If the condition is met the stochastic simulation is performed. Otherwise, the parameter values are rejected. The parameter set that best reproduces the experimental data is given by the minimum value interatively obtained after the iterative evaluation of the objective function.

Information processing in signalling networks:
We extended the previously reported and established model on intracellular NfkB sginalling in hepatocytes to decipher the respective contributions of other non-parenchymal liver cells to LPS induced TNFa signalling. Thus, based on experimental data on cytokine production we were able to quantitatively model the respective contribution of endothelial cells, macrophages and stellate cells which produce TNFa in response to LPS stimulation to the intracellular NfkB response in hepatocytes.

Also the previously established hepatocytic IFNa model has been integrated with a whole body PBPK (physiologically based pharmacokinetic) model which computes IFN distribution in the body after injection. With this model, we can estimate a) how much IFN arrives at the liver after the injection of a certain dose and b) how strong will the intracellular response be after the administration of a specific dose. This project is pursued in collaboration with Bayer, Leverkusen.

Understanding metabolic networks:
As mentioned above we have successfully established (for the first time) a method to integrate time-dependent quantitative whole-cell proteome data (e. g. SWATH data) with a full-fletched whole-genome-scale metabolic model. In collaboration with Ruedi Aebersold (ETH Zürich) and the group of Bernd Kreikemeyer (University Hospital Rostock) we measured metabolic (Fig. 2) and proteome changes during pH shift experiments in Enterococcus faecalis, a facultative pathogen and cause of many multi-resistant infection. We then integrated the data in a genome-scale computational model that allows us to analyse metabolic rearrangements during the environmental change. We could show that almost all observed changes are due to the higher energy demand at lower pH and are well predicted by the model (Großeholz et al., 2016).

Planned research and new directions
Method development
On the methodological side we certainly will further continue developing and maintaining our software package COPASI. We are still planning for the possibility to do 2D-simulations, mimicking live-cell imaging data (in collaboration with Peter Bastians, IWR).

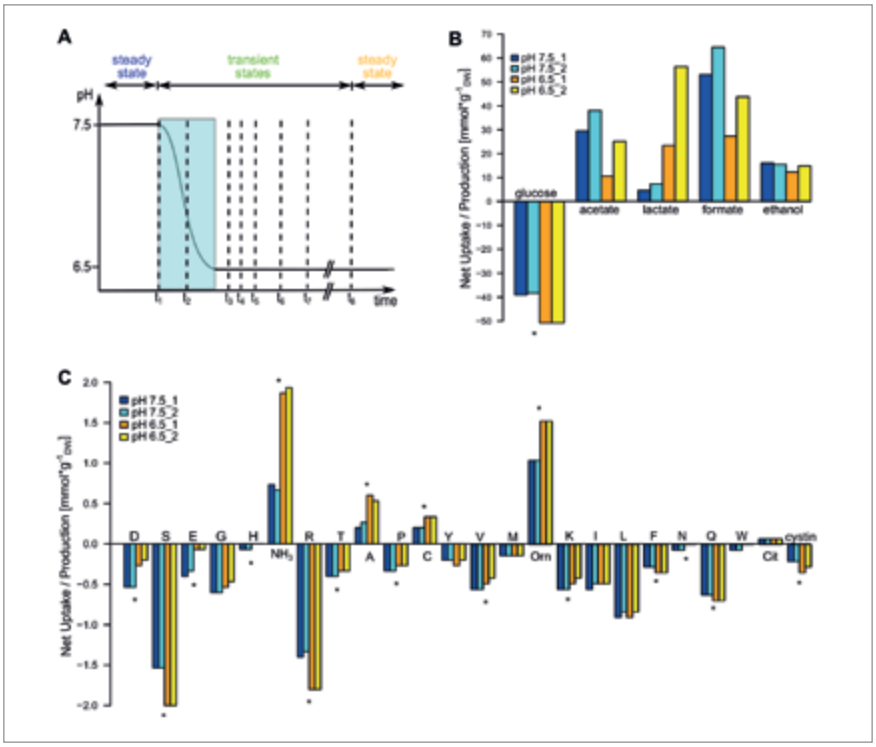

Figure 2
Experimental setup and metabolite measurements during pH-shift in E. faecalis. Data were used to constrain a genome-scale model reproducing metabolic behaviour at different pHs (Großeholz et al., 2016).

Information processing in signalling networks
Within the Researcher Group FOR 964 we are investigating how different calcium dependent proteins decipher specific and experimentally measured calcium signals. In addition, we want to understand the advantages and disadvantages of frequency vs amplitude coding in calcium signalling.

Understanding metabolic networks
Based on our first studies we are interested in how environmental changes or stresses shape the metabolism in an organism. So, we are planning to extend this research and analyse different species and environmental clues.

Selected publications since 2013
Number of peer-reviewed articles 2013-2017: 13, number of citations 2013-2017: 1192, total h-index: 25 (according to Google Scholar).

Beuke K, Schildberg FA, Pinna F, Albrecht U, Liebe R, Bissinger M, SchirmacherP, Dooley S, Bode JG, Knolle PA, Kummer U, Breuhahn K, Sahle S. (2017) Quantitative and integrative analysis of paracrine hepatocyte activation by nonparenchymal cells upon lipopolysaccharide induction. FEBS J. Mar;284(5): 796-813.

Großeholz, Ruth, et al. (2016) Integrating highly quantitative proteomics and genome-scale metabolic modeling to study pH adaptation in the human pathogen Enterococcus faecalis. *npj Systems Biology and Applications* 2: 16017.

Levering J, Fiedler T, Sieg A, van Grinsven KW, Hering S, Veith N, Olivier BG, Klett L, Hugenholtz J, Teusink B, Kreikemeyer B, Kummer U. (2016) Genome-scale reconstruction of the Streptococcus pyogenes M49 metabolic network reveals growth requirements and indicates potential drug targets. J Biotechnol. Aug; 20; 232:25-37.

Veith N, Solheim M, van Grinsven KW, Olivier BG, Levering J, Grosseholz R,Hugenholtz J, Holo H, Nes I, Teusink B, Kummer U. (2015) Using a genome-scale metabolic model of Enterococcus faecalis V583 to assess amino acid uptake and its impact on central metabolism. Appl Environ Microbiol. Mar; 81(5):1622-33.

Hübner K, Surovtsova I, Yserentant K, Hänsch M, Kummer U. (2013) Ca2+ dynamics correlates with phenotype and function in primary human neutrophils. BiophysChem.; 184:116-25.

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Fields of Interest
Modeling of biochemical reaction networks, computational methods for systems biology, complex dynamical systems, software for life science research

Brief summary of work since 2013
The general area of research of the group is computational modeling of biochemical reaction networks. The activities of the group span a range from applied modeling, methodological work, to tools development and other services for the scientific community.
– Applied modeling: Within the framework of the Virtual Liver network the TNF stimulated NfκB signaling network has been studied (in collaboration with the group of Dr. Kai Breuhahn, University Hospital Heidelberg). The group was also involved in the study of metabolic networks in different organisms.
– Methodological research: The group has developed computational methods for parameter estimation in stochastic models. It has also worked on parameter identifyability, time scale analysis of dynamical models, and numerical methods for simulating spatial (partial differential equation based) models.
– Tools development and services for the scientific community: The group is a long time main contributor to the software project COPASI (www.copasi.org) that provides a tool for setting up, simulating and analysing kinetic models of reaction networks. The group is also intensively involved in international efforts for software interoperability and data exchange standardisation (e. g. www.sbml.org)

Major contributions since 2013
A key feature of systems biology is the tight interaction of computational and experimental research. From the modeling/computational side this requires a solid methodological basis for creating and simulating mathematical models, and for comparing the simulation results to experimental data, both for parametrization and validation of the model. While these methodological basis is well established for classical deterministic modeling approaches, this is not the case for stochastic modeling. The group has over the last years developed techniques for parameter estimation in stochastic systems that allow the parametrization of models with compuatinal efficiency that is comparable to parameter estimation in deterministic models (Main contributor of this work was postdoctoral researcher Dr. Christoph Zimmer).

Another important aspect is that advanced computational methods are made available to the scientific community, preferably including scientists who are not necessarily experts in numerical methods or computer programming. Therefore, the group develops software tools that implement sophisticated methods with an easy user interface. The main project is COPASI (www.copasi.org), a tool for creating, simulating and analysing models of biochemical reaction networks. Is is developped in a long established international cooperation with the groups of Prof. Pedro Mendes (Manchester University), Dr. Stefan Hoops (Virginia Tech) and Prof. Ursula Kummer (BIOQUANT). On the one hand, COPASI

is used as a platform for methodological research. On the other hand, it is provided as a service for the scientific community. By now it is well established as a standard tool for kinetic modeling in systems biology. The main publication about COPASI (Hoops et al. 2006) has received more than 850 citations. The user forum on www.copasi.org has about 1000 registered users and is used very actively. New releases of the software typically get several thousand downloads. COPASI is provided as free software. Features of COPASI include the standard methods for quantitative modeling of metabolic, signalling, or gene regulation networks, i.e. stochastic and deterministic simulation, steady state stability analysis, metabolic control analysis, generalized sensitivity analysis, stoichiometric network analysis, parameter estimation, etc. It also includes more advanced model analysis features, e.g. time scale analysis or elaborate optimization facilities.

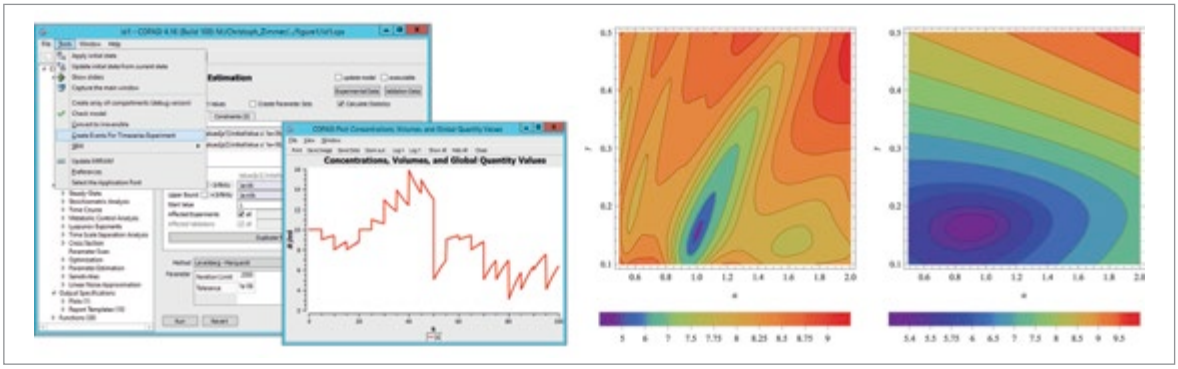

Figure 1
Illustration of methodological improvements for parameter estimation and its software implementation. Left: The COPASI user interface, showing the setup of a parameter estimation, and the simulation of a time series with discrete events based on measured data. Right: The fitness landscape (two different objective functions) of a parameter estimation for the Lotka-Volterra model. The rightmost panel illustrates that the improved objective function contains no local minima and is therefore easier to minimise. Figure taken from Bergmann et al. 2016.

Planned research and new directions

The group will continue to work on modeling of signaling networks in collaboration with its experimental collaborators. The development of COPASI will also be continued. A new area of research will be the modeling of processes where the experimental data consists of live cell images. This will require the investigation of spatial modeling techniques, especially simulation algorithms for spatial models, and the development of tools to handle such models, especially for integrating the modeling results with the microscopy images obtained from our experimental partners.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 10, number of citations 2013-2017: 21, h-index (2013-2017): 3, total h-index: 13 (according to Thomson Reuters).

Beuke, K., Schildberg, F. Pinna, U. Albrecht, R. Liebe, M. Bissinger, P. Schirmacher, S. Dooley, J.G. Bode, P. A. Knolle, U. Kummer, K. Breuhahn, S. Sahle (2017): Quantitative and integrative analysis of paracrine hepatocyte activation by nonparenchymal cells upon lipopolysaccharide induction. FEBS Journal 284(5), 796–813, doi:10.1111/febs.14022

Bergmann, F., S. Sahle, C. Zimmer (2016): Piecewise parameter estimation for stochastic models in COPASI. Bioinformatics 32(10), 1586–1588, doi: 10.1093/bioinformatics/btv759

Zimmer, C. and S. Sahle (2015): Deterministic inference for stochastic systems using multiple shooting and a linear noise approximation for the transition probabilities. IET Systems Biology 9(5), 181–192, doi: 10.1049/iet-syb.2014.0020

Levering, J., U. Kummer, K. Becker, and S. Sahle (2013): Glycolytic oscillations in a model of lactic acid bacteria metabolism. Biophysical Chemistry 172, 53–60

2.14 JUNIOR PROF. DR. STEFFEN LEMKE
INDEPENDENT RESEARCH GROUP EVOLUTION OF MORPHOGENESIS

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Fields of Interest
Evo-Devo, Gastrulation, Morphogenesis,
Gene regulatory networks

Brief summary of work since 2013
The animal kingdom exhibits fundamental differences in early development that seem to have arisen very abruptly in evolution, as if a new mode of development were a matter of throwing a switch. An example is gastrulation, the first shape-giving process of animal development, which establishes the basis of the entire body plan. Gastrulation appears to have undergone repeated and reversible changes over the course of evolution, including a long-recognized transition from single-cell ingression to whole-tissue invagination and vice versa. In the past five years we have established early fly development as a unique system to study the genetic, cell biological, and molecular mechanisms underlying the evolution of gastrulation, with unprecedented power to functionally test hypotheses through experiments that recapture genetic events of the past. In a pioneering study that serves as a proof-of-principle for future work, we could show how a single »switch gene« was sufficient to transform mesoderm ingression in the midge *Chironomus riparius* to Drosophila-like tissue invagination, and vice versa. Our results have offered a new and surprising explanation for the sudden appearance of morphogenetic novelty: a few genetic changes effect major changes in phenotypes. It is still unclear how a single gene can switch a morphogenetic state without disrupting parallel processes, and whether or not our results reflect a general principle in the evolution of gastrulation. We continue to address these questions by leveraging our technological innovations and fly models to dissect the evolution of gastrulation from a cell-biological perspective.

Major contributions since 2013
Work in our lab has established fly gastrulation as genetic system to study principles of morphogenetic evolution. We and others have generated genome and transcriptome sequence assemblies over the last years and for multiple fly species at key positions in the phylogenetic tree (Klomp et al., 2015; Vicoso and Bachtrog, 2015). In parallel we have developed protocols for genetic loss- and gain-of-function analyses (Klomp et al., 2015), as well as protocols and vector sets for transgenesis (Caroti et al., 2015). Our toolkit allows us to visualize cell membranes and nuclei in vivo during gastrulation and to record cell shapes, positions, and the cytoskeleton of the entire embryo with high spatiotemporal resolution by using light sheet microscopy. Using this newly established experimental system, we demonstrated gastrulation among flies is sufficiently complex to study major morphogenetic transitions between selected fly species and the reference Drosophila melanogaster. We have specifically focused on questions that addressed how coordinated invagination evolved from stochastic ingression, how epithelial continuity originated by a putative loss of tissue separation, how an epithelial fold emerged by changes in genetic pre-patterning, and how convergent extension was affected by the evolution of tissue folding.

Ingression versus invagination. To understand how coordinated tissue invagination evolved, we compared mesoderm internalization between the midge Chironomus riparius and D.melanogaster. We could show that mesoderm invagination in Drosophila is a novel feature; in C.riparius and other insects the mesoderm ingresses as single cells. We then narrowed the differences in the developmental programs of the two flies to a few critical genes. Finally, we could demonstrate that single »switch genes« were sufficient to transform mesoderm ingression in Chironomus to Drosophila-like tissue invagination, and vice versa (Urbansky et al., 2016). Our results offer a new and surprising alternative explanation for the evolution of gastrulation: a few genetic changes effect major changes in phenotypes. It is still completely unclear how a single gene can switch a morphogenetic state without disrupting parallel processes, and whether or not our results reflect a general principle in the evolution of gastrulation. We will address these questions by leveraging our technological innovations and fly models to dissect the evolution of gastrulation from a cell-biological perspective.

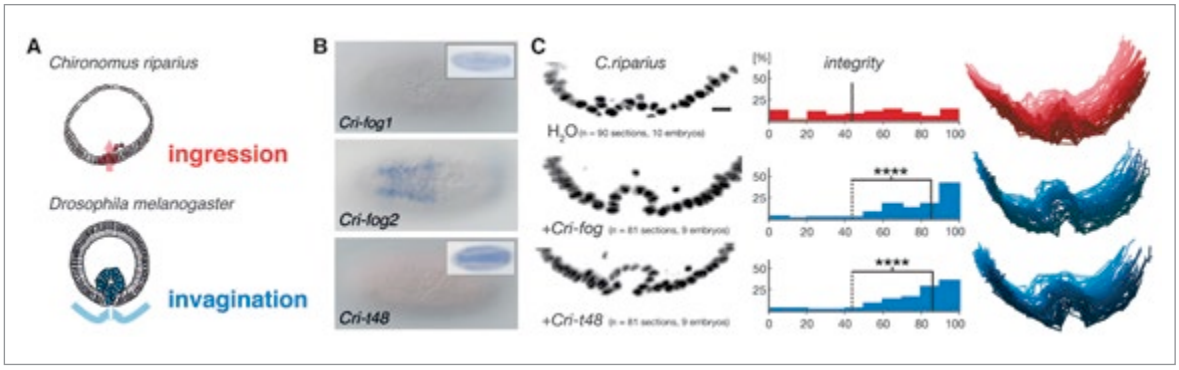

Figure 1
From cell ingression to tissue invagination. (A) Gastrulation in Chironomus and Drosophila represent two distinct modes of cell internalization, i.e. stochastic ingression of individual cells in C.riparius and coordinated invagination of an epithelium in D.melanogaster. (B) The developmental program orchestrating gastrulation is overall conserved in both species; the expression of folded gastrulation (fog) and t48 are an exception. Both genes are expressed along the ventral midline in D.melanogaster (inset), but not completely or not at all in C.riparius. (C) The experimental expression of C.riparius fog or C.riparius t48 are sufficient to invoke epithelial integrity and switch gastrulation in C.riparius from ingression to Drosophila-like invagination (modified from Urbansky et al., 2016).

Epithelial disjunction versus continuity. To elucidate how a program of tissue morphogenesis switched from disjunction and expansion to continuity and moderate stretching, we compared extraembryonic development between the scuttle fly Megaselia abdita and D.melanogaster. In both species, an extraembryonic anlage is pre-patterned in blastoderm embryos by dorsal expression of the homeobox transcription factor Zerknüllt (Zen). The initially columnar zen-expressing cells change their shape and form a thin sheet of non-dividing squamous cells, which gets folded and extends over the neighboring embryo proper. In M.abdita, this extraembryonic epithelium continues to expand, and the fold between embryo and extraembryonic tissue tears apart to generate a separate epithelium, the serosa. The cell-biological basis for this epithelial separation is unknown and could not be solved based on the analysis of fixed specimen (Rafiqi et al., 2008). Based on time lapse recordings, as well as cell and tissue tracking, we were able to identify previously unknown transitions during serosa development. Specifically, we found that the disjunction of serosa and amnion was preceded by a previously unknown tissue pulsation. Genetic analyses suggest that this tissue pulsation is dependent on the activity of metalloproteases and is less pronounced during extraembryonic development in *D.melanogaster*.

A novel head fold. Using a newly generated atlas of comparable gastrulation stages, the side-by-side comparison of different fly species identified a head fold in the Drosophila embryo as an evolutionary novelty of »higher« (i.e. cyclorrhaphan) flies. In *D.melanogaster*, timing and positioning of head fold (cephalic furrow) formation is determined by the input of two overlapping expressed transcription factors, *buttonhead* and *even-skipped*. We found that *buttonhead* and *even-skipped* also overlapped in *M.abdita*, and that, like in *D.melanogaster*, this overlap was required for cephalic furrow formation. This suggests that *buttonhead* and *even-skipped* constitute the conserved regulatory circuit that provides spatio-temporal information for cephalic furrow formation. Remarkably, however, we found that this spatio-temporal circuit was not conserved in *C.riparius*, where the lack of cephalic furrow formation coincided with absence of *buttonhead* and *even-skipped* co-expression. The recent identification of the first bicoid-independent anterior-posterior patterning system in insects (Klomp et al., 2015) provides the required molecular foundation to address how changes in spatio-temporal patterning in *C.riparius* and higher flies are linked

to morphogenetic innovation. In the context of a granted HFSP project the combination of gain-of-function experiments in *C.riparius* and loss-of-function analyses in *M.abdita* and *D.melanogaster* will allow us to address the cell-biological basis, function, and evolution of cephalic furrow formation.

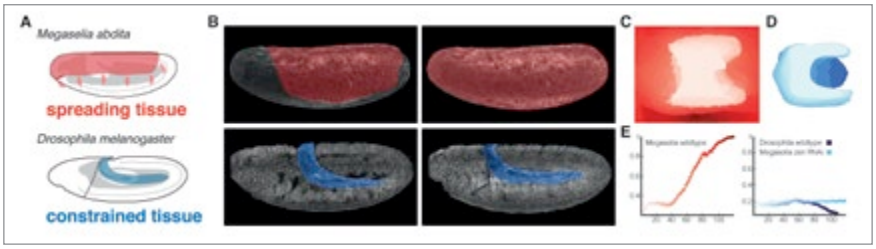

Figure 2
From epithelial spreading to constrained epithelia. (A) Extraembryonic development in flies represents a genetically tractable invertebrate system to study universal principles of epithelial spreading in animals and to address the reason and molecular logic for its loss in a subset of flies like Drosophila. (B-C) Cell- and tissue-dynamics previously inaccessible in non-model species like Megaselia can be visualized (B) and quantified (C-E) at high spatiotemporal resolution using *in toto* imaging on a selective plane illumination microscope (SPIM).

Variation versus robustness in tissue folding and extension. To address whether gastrulation is affected in species thought to be adapted to different climate zones, we generated transgenic reporter lines for different Drosophila species and analyzed gastrulation based on *in toto* time lapse recordings. Building on experience in quantitative image analysis at tissue and cell level (Heermann et al., 2015; Leitte et al., 2012; Paolini et al., 2015), we analyzed convergent extension of the embryo by particle image velocimetry and compared them with the position and depth of various ectodermal tissue folds (Kappe et al., 2016). In these quantitative comparisons, we identified a previously unknown degree of variation, which we confirmed for additional Drosophila species and in higher numbers by additionally analyzing fixed embryos. To our surprise, the observed morphogenetic differences in position and depth of dorsal transverse folds appear to correlate with a tolerance of high temperature stress during embryonic development. Our data suggests that transient transverse folds in the Drosophila embryo can provide a physical scaffold to stabilize embryonic fly development under environmental stress conditions.

Planned research and new directions

Macro-evolutionary change is traditionally seen as the sum of a large number of small, micro-evolutionary changes that are presumably irreversible. Our results suggest that major changes in phenotypes may alternatively stem from a few genetic changes with major effects. We are in a unique position to test whether our results reflect a general principle, and we have identified additional experimental paradigms to test the putative role of morphogenetic switches.

To systematically identify »switch genes« associated with differences in fly gastrulation, we will combine candidate gene and unbiased genome-wide profiling approaches. Our candidate gene approach will be based on the genetic networks controlling the three morphogenetic paradigms. Our unbiased approach will focus on our non-Drosophila fly species and the identification of genes that either differ substantially in their expression level or that appear in the genome of only a subset of species. Genome sequences are available as preliminary data (Chironomus, Episyrphus) or through public databases (NCBI), gene expression will be sampled by RNAseq for selected stages of early development. To functionally test »switch genes« in the context of major morphogenetic transitions, we will investigate identified candidates in gain- and loss-of-function experiments. Specifically, we will ask whether the gain or loss of gene function results in »forward-evolution« or »reverse-evolution« of morphogenesis. Both types of experiments will be performed using established protocols for injection of mRNA and dsRNA. Next, we aim to establish molecular reporters that will allow us to address how »switch genes« are integrated into existing cytoskeletal machineries. We will use in vivo reporters for actin, myosin, junctions, and scaffolding proteins to reveal how »switch genes« instruct cell- and tissue-behavior and thus tune or remodel entire morphogenetic units. To investigate whether these modulations of morphogenesis affect gastrulation only locally or also globally, we will take advantage of our ability to record changes in cell shape, position, and behavior in the context of gastrulation as a whole.

Selected publications since 2013
Number of peer-reviewed articles 2013-2017: 7, number of citations 2013-2017: 187, total h-index: 10 (according to Thomson Reuters).

Urbansky, S., González Avalos, P., Wosch, M. & Lemke, S. (2016). Folded gastrulation and T48 drive the evolution of coordinated mesoderm internalization in flies. eLife 5, e18318.
Caroti, F., Urbansky, S., Wosch, M. & Lemke, S. (2015). Germ line transformation and in vivo labeling of nuclei in Diptera: report on Megaselia abdita (Phoridae) and Chironomus riparius (Chironomidae). Development Genes and Evolution 225, 179–186.

Paolini, A., Duchemin, A.-L., Albadri, S., Patzel, E., Bornhorst, D., González Avalos, P., Lemke, S., Machate, A., Brand, M., Sel, S., et al. (2015). Asymmetric inheritance of the apical domain and self-renewal of retinal ganglion cell progenitors depend on Anillin function. Development 142, 832–839.

Heermann, S., Schütz, L., Lemke, S., Krieglstein, K. & Wittbrodt, J. (2015). Eye morphogenesis driven by epithelial flow into the optic cup facilitated by modulation of bone morphogenetic protein. eLife 4, e05216.

Klomp, J., Athy, D., Kwan, C. W., Bloch, N. I., Sandmann, T., Lemke, S. & Schmidt-Ott, U. (2015). Embryo development. A cysteine-clamp gene drives embryo polarity in the midge Chironomus. Science 348, 1040–1042.

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2.15 PROF. DR. INGRID LOHMANN
DEVELOPMENTAL BIOLOGY

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Fields of Interest
Deciphering the specificity of Hox transcription factors and the role of Hox transcriptional networks in animal development with a focus on the establishment, maintenance and plasticity of neural networks, stem cell homeostasis and the interplay of metabolism and growth control

Brief summary of work since 2013
Cell- and tissue-specific changes in gene expression play a prominent role in development as well as in pathologies, and are regulated by coordinated transcription factor-DNA interactions at defined regulatory elements in the genome. One crucial class of evolutionary conserved transcription factors (TFs) is the Hox proteins, which are used throughout an animal's life to control development and maintenance of all tissues and cell types. In contrast to their important role, the mechanistic cues underlying cell- and tissue-specific Hox transcriptional activities and functions remain poorly understood. In the last years, my group has on the one hand undertaken huge efforts to elucidate mechanisms allowing Hox proteins to regulate their target genes in a highly context-dependent manner. To this end, we apply genomic, proteomics and bioinformatics approaches to elucidate cis- and trans-requirement of Hox proteins crucial for the realization of cell type-specific programs. In addition, my lab studies how Hox proteins translate their regulatory activity into functional output. To this end, we have chosen two processes under Hox control, stem cell maintenance and differentiation in the *Drosophila* testis and control of motor behaviors, in particular movements associated with feeding, to elucidate how Hox proteins regulate downstream processes in a stage- and context-dependent manner thereby achieving a highly specific architectural/morphological output.

Major contributions since 2013
The importance of *Hox* genes in various stem cell systems is well recognized, however so far their function in these systems is less understood. We have chosen the *Drosophila* male stem cell system, the testis, to study Hox dependent control of stem cell maintenance and differentiation. This system provides an excellent system to study these processes, since it is morphologically and molecularly well described. The stem cell niche of the testis consists of the hub or organizing center, a cluster of non-dividing mesodermal cells, and germline stem cells (GSCs), which are enclosed each by a pair of somatic cyst stem cells (CySCs). Both types of stem cells in the testis divide asymmetrically to renew their stem cell identity and to give rise to gonialblasts and somatic cyst cells (SCCs), which will differentiate. Our work in the last years demonstrated that the Hox transcription factor Abdominal-B (Abd-B), active in pre-meiotic spermatocytes of *Drosophila* testes, is essential for positioning the niche to the testis anterior by regulating integrin in differentiated somatic cyst cells. Abd-B also cell non-autonomously controls critical features within the niche including centrosome orientation and division rates of GSCs. By genome-wide binding studies, we found that Abd-B mediates its effects on integrin localization by

directly controlling at multiple levels the signaling activity of the Sev ligand Boss via its direct targets *src42A* and *sec63*, two genes involved in protein trafficking and recycling. Thus, our data showed that Abd-B through local signalling between differentiated cell types provides positional cues for integrin localization, which is critical for placement of the distant stem cell niche and stem cell activity (Papagiannouli et al., 2014; Papagiannouli et al., 2015). Recently, we aimed at studying the regulatory regime controlling stem cells and their progenies as they progress through their developmental program. To this end, we recorded gene activities and genome-wide binding profiles of two transcription factors, Zfh-1 and Abd-A, which are expressed in somatic support cells of the *Drosophila* testis and crucial for fate acquisition within the soma and in the closely adjoining germline. To facilitate data mining and analysis, we generated an interactive online tool. Using this tool, we comprehensively uncovered the differential activity of functional gene classes in the somatic lineage and demonstrate the importance of selected processes for proper germline-soma interaction and differentiation of both lineages. Our data identified key roles for TOR signalling, signal processing V-ATPase proton pumps and the nuclear transport engaged nucleoporins in controlling germline maintenance, proliferation and differentiation from the support side. In summary, our dataset and interactive platform represents a versatile tool for identifying novel gene networks active in stem cell support cells and provides a valuable resource for elucidating the multifaceted regulatory inputs required to guide proper stem cell behaviour (Papagiannouli, Tamirisa et al., Dev Cell, under review).

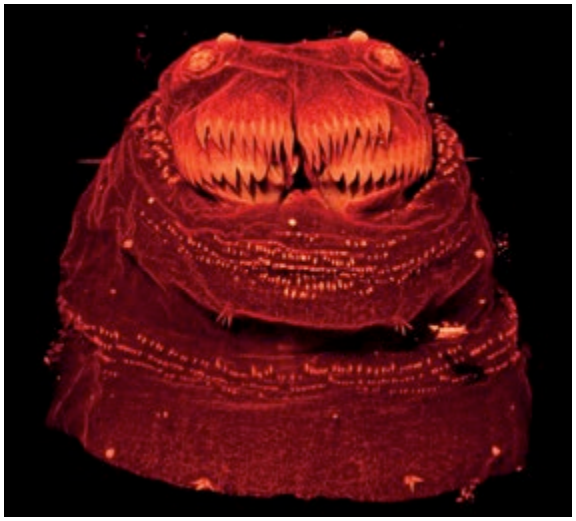

Figure 1
Confocal imaging of a wild-type first instar larva cuticle. First larval cuticles were imaged in frontal view showing the characteristic head structures.

In both vertebrates and invertebrates, the Hox family of transcription factors has emerged as a group of key players in controlling the establishment of motor behaviours, like feeding. Food uptake crucial is for all animals and depends on the rhythmic activity of feeding muscles stimulated by specific brain neurons. Despite its vital importance, critical determinants regulating the development, wiring and connectivity of the neuromuscular feeding unit were largely unknown. We identified the Hox transcription factor Dfd to be expressed and functional in specific neurons and muscles, which are essential for feeding in *Drosophila*. Using genetic, molecular, genomic and behavioural approaches we demonstrated that Dfd is required at subsequent phases in the formation of the feeding unit by directly controlling neuronal specification, axon outgrowth, synapse formation and neurotransmission genes. The synchronous regulation of cell adhesion molecules, like Connectin, in these feeding neurons and muscles furthermore uncovered Dfd as an important factor that might guide the recognition of the interacting synaptic partners. Finally, we were able to show that the *Dfd* homolog *hoxb4* is expressed in neurons projecting towards head muscles in the vertebrate model medaka, indicating a general and conserved role of homology group 4 *Hox* genes in establishing and maintaining neuro-muscular units required for feeding (Friedrich et al., 2016).

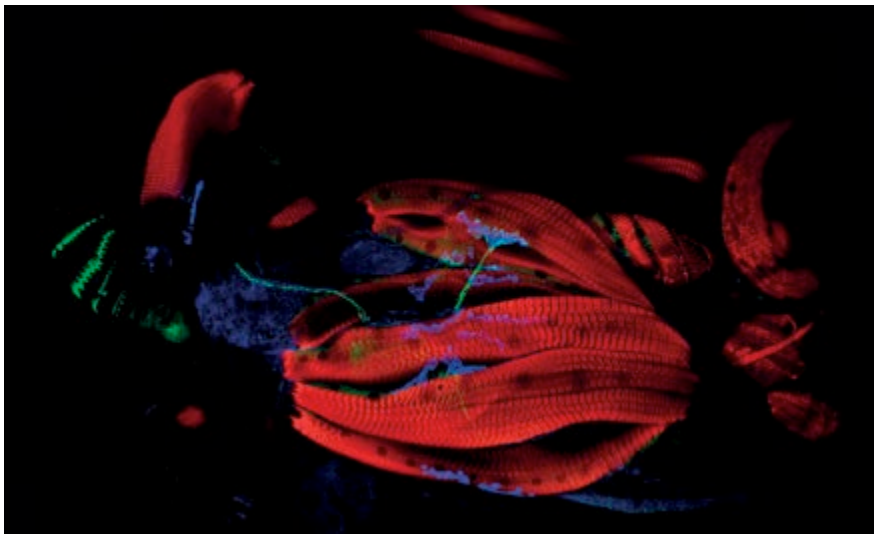

Figure 2
Muscle bundles (red) associated with different parts of the feeding apparatus are innervated by Dfd positive motoneurons (green) to allow synaptic transmission and feeding movements. Functional synapses are labelled with DVGlut (blue).

Besides elucidating the mechanistic basis of Hox proteins, my lab is also interested in understanding tumour formation and progression, in particular how changes in cell-intrinsic properties result in mis-regulation of cell proliferation. Through an *in vivo* RNAi-screen in the *Drosophila* eye-imaginal disc we identified a mitochondrial electron transport chain subunit to enhance Notch-mediated over-proliferation of the developing eye. By transcriptome analysis we found an induction of glycolytic enzymes and nutrient transporters, implying a cell-autonomous adaptation of metabolism in response to mitochondrial stress. Cooperation between mitochondrial stress and the Notch pathway depends on a larval diet with low protein content, suggesting that the proliferative advantage could be based on an increased capacity to take up amino acids. This hypothesis is additionally supported by the observation that knockdown of sugar transporters or lactate dehydrogenase further enhances over-proliferation. In sum, we suggest that the proliferation capacity of eye progenitors is limited by cellular metabolism and that alterations in relative nutrient uptake can alter the behaviour in response to pro-proliferative signals such as the Notch pathway (Sorge et al., in preparation).

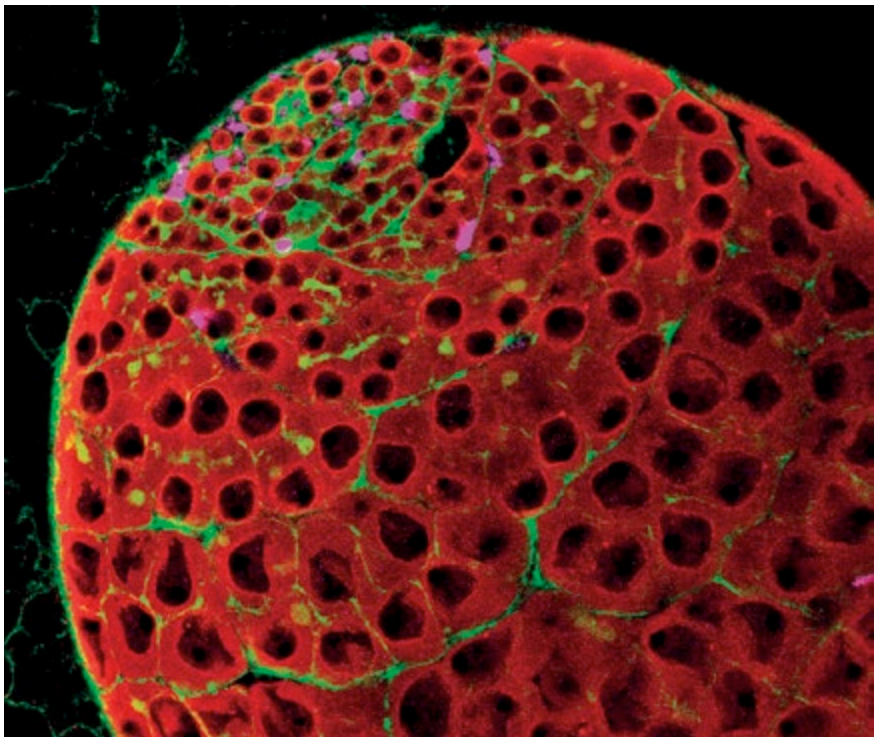

Figure 3
Image of a Drosophila wild-type larval The germline lineage is shown in red (Vasa), the somatic population in green (actin), the somatic stem cells and their progenies in pink (TJ).

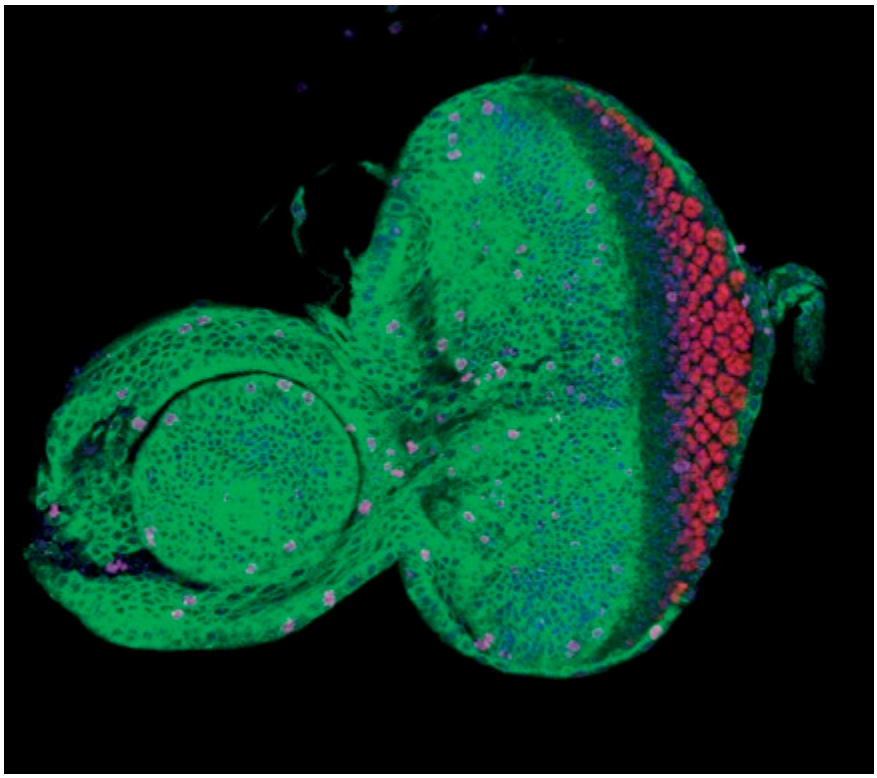

Figure 4
Image of a Drosophila eye-antennal disc, where cells are labelled by eyeless-FLP induced GFP expression (green). Proliferating cells are highlighted by phospho-H3 staining (magenta) and differentiating photoreceptors located posterior to the morphogenetic furrow are labelled with ELAV (red).

Planned research and new directions

In the next years, we will on the one hand continue our efforts to elucidate tissue-specific activities of the Hox transcription factors. To this end, we will focus on three themes emerging from our genomic and proteomic data: 1) control of cell identities by Hox TFs via the repression of alternative fates, 2) Hox control of transcriptional programs by changing the chromatin architecture, and 3) studying a novel function of Hox TFs in alternative splicing.

On the other hand, we will continue to elucidate the mechanistic basis of Hox function in selected biological processes, in particular in stem cell maintenance/differentiation and motor behaviour. Our preliminary data suggest that Hox TFs regulate the expression, the activation as well as repression, of synaptic target selection molecules on interacting motoneurons and muscles, thereby instructing the proper wiring of the neuromuscular system critical for region-specific motor patterns. In order to provide vigorous proof for this hypothesis, we will identify in a tissue-specific manner the full complement of synaptic target specificity factors in Dfd positive motoneurons and muscles, will then interfere large-scale with the function of Dfd and its downstream »specificity« factors in a tissue- and time-specific manner and study the effects on motoneuronal targeting, synaptic wiring and regional motor output. We expect to identify a »synaptic target specificity code« controlled by Hox TFs in the interacting motoneurons and muscles, a hypothesis that will be tested by changing the Hox code in the corresponding cell types. In the *Drosophila* male stem cell system, we will focus on selected processes identified by our profiling strategy. In particular, we will study the function of immune-related processes, an emerging but completely unsolved signature common to many invertebrate and vertebrate systems, and will study its relevance in stress adaptation.

In sum, our research aims at solving the question how sets of highly related and widely expressed top level regulators like the Hox TFs have shaped animal diversity during evolution by simultaneously acting on large fields of cells and small cell populations with extreme spatio-temporal precision.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 8, number of citations 2013-2017: 21, h-index (2013-2017): 3, total h-index: 12 (according to Thomson Reuters).

Friedrich, J., Sorge, S., Bujupi, F., Eichenlaub, M., Schulz, N. G., Wittbrodt, J. and Lohmann I. (2016) Hox function is required for the development and maintenance of the *Drosophila* feeding motor unit. Cell Rep *14*, 850-860.

Domsch, K., Papagiannouli, F. and Lohmann, I. (2015) The Hox-apoptosis interplay in development and disease. Curr Top Dev Biol *114*, 121-158.

Schardt, L., Ander, J.J., Lohmann, I., Papagiannouli F. (2015) Stage-specific control of niche positioning and integrity in the *Drosophila* testis. Mech Dev *114*, 121-158.

Merabet, S. and Lohmann, I. (2015) Towards a new twist in Hox and TALE DNA-binding specificity. Dev Cell *32*, 259-261.

Papagiannouli, F., Schardt, L., Grajcarek, J., Ha, N., Kaspar, P. and Lohmann, I. (2014). The *Hox* gene *Abd-B* controls stem cell niche function in the *Drosophila* testis. Dev Cell 27, 189-202.

2.16 PROF. DR. JAN LOHMANN
STEM CELL BIOLOGY

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Fields of Interest
I am interested in various aspects of plant stem cell regulation and its plasticity: Integration of systemic and local signals; Modulation of regulatory programs by the environment, Epigenetic mechanism of cell fate specification; Mechanisms of stem cell induction by WUSCHEL; Cell behavior in the shoot apical meristem.

Brief summary of work since 2013
Plant stem cells reside at the root tip and the shoot apex and are embedded in specialized structures called meristems, which provide a local environment that regulates the homeostasis between proliferation and differentiation. The homeodomain protein WUSCHEL (WUS) induces stem cell fate in the shoot meristem and its activity is controlled by a negative feedback system involving the *CLAVATA (CLV)* genes. Plant hormones have long been known to play a major role in meristem regulation, but how their activity is integrated with the *WUS/CLV* system had remained elusive. My lab has made important contributions to understanding WUS function, how it is connected to hormone signaling systems, most notably cytokinin and auxin and how the entire system is modulated by the environment. In recent years, my lab has focused on elucidating the mechanisms underlying WUS activity on the one hand by studying the unusual cell-to-cell mobility of WUS protein (Daum et al.; PNAS), deciphering the role of the WUS dependent transcriptional network (Schuster et al.; Developmental Cell, Schuster et al.; Development, Gaillochet et al.; eLife in revision) and elucidating its connection with auxin signaling on the other hand (Miotk et al.; Science in revision). Importantly, I have initiated a new line of research with ERC funding aimed at elucidating the role of the environment in controlling the apical stem cell system and we have made significant inroads in this field, for example by identifying TOR kinase as the central integrator for light and metabolic signals (Pfeiffer et al.; eLife).

Major contributions since 2013
Plant meristems are home to continuously active stem cells, which are controlled by developmental and environmental signals and only daughter cells that exit the stem cell domain acquire the competence to differentiate. In contrast to the situation in most animals, plant stem cells do not contribute to embryogenesis, but remain quiescent until the postembryonic phase of development. In the past years, work in my lab has shed light on the regulatory processes involved in the activation of apical stem cells, their mechanisms to communicate with their niche, and how their differentiation rate is controlled.

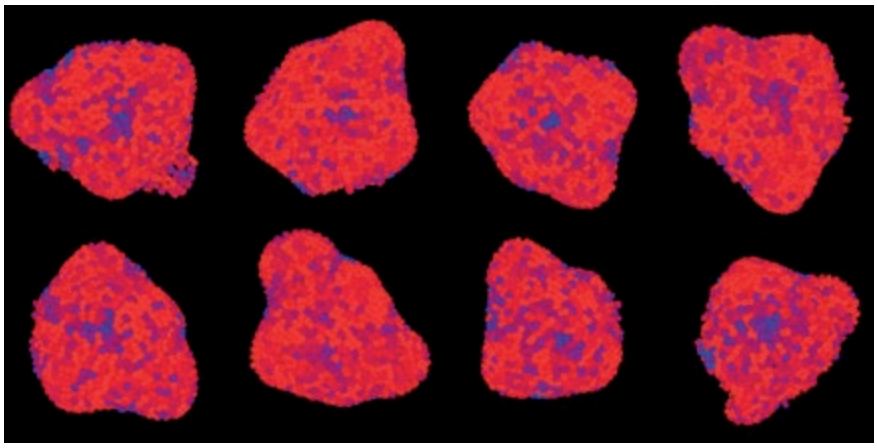

Figure 1
A novel tool to visualize cell proliferation. A monomeric fluorescent timer protein is driven from a cell-cycle regulated promoter. The protein matures from a red to a blue light emitting form and the spectral ratio serves as an excellent proxy for the time passed since the last division. Live cell imaging of plant cohorts reveals striking regional differences in cell proliferation in the SAM.

Using a combination of genetic and physiological perturbations, we have dissected how light and metabolic signals are integrated to overcome stem cell dormancy during germination. Surprisingly, we found that stem cells exist in the absence of stimulating inputs but that their activity is dependent on the presence of the canonical niche signal, the WUS transcription factor. Light is able to activate expression of WUS independently of photosynthesis and this likely involves inter-regional cytokinin signaling. Metabolic signals are transduced to the meristem through activation of the TARGET OF RAPAMYCIN (TOR) kinase. Surprisingly, we found that TOR is also required for light signal dependent stem cell activation, suggesting that TOR kinase acts as a central integrator of light and metabolic signals and a key regulator of stem cell activation at the shoot apex (Pfeiffer et al. eLife 2016).

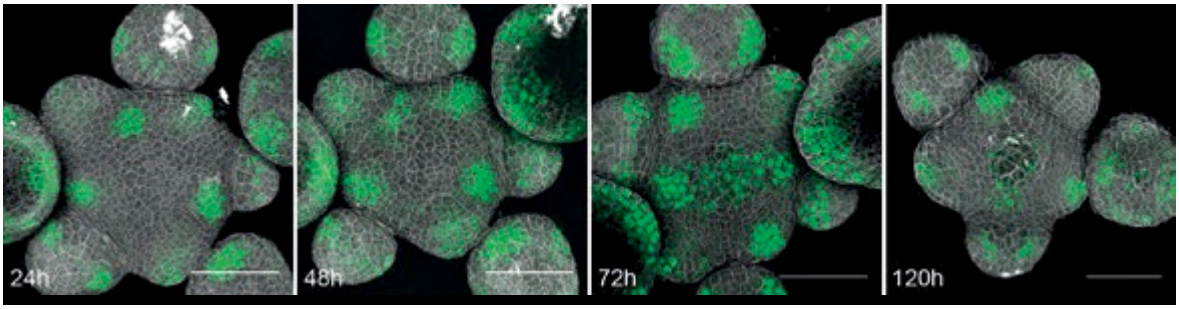

Figure 2
Auxin signalling is triggered in response to loss of stem cell fate. 72 hours after experimental shut down of stem cell maintenance, the auxin signalling reporter DR5v2 is activated in the central stem cell domain. Around 120 hours post induction, cells differentiate and expand.

A long-standing interest of my lab relates to the mechanistic foundation of stem cell induction and maintenance in the shoot apical meristem. While many basic cellular processes are well conserved between animals and plants, cell-to-cell signaling is one function where substantial diversity has arisen between the two kingdoms of life. One of the most striking differences is the presence of cytoplasmic bridges, called plasmodesmata, which facilitate the exchange of molecules between neighboring plant cells and provide a unique route for cell-cell communication in the plant lineage. We were able to show that WUS protein, expressed in the niche, moves to the stem cells via plasmodesmata in a highly-regulated fashion and that this movement is required for its function and thus stem cell activity. Mobility is encoded in the WUS protein sequence and mediated by multiple domains: The homeodomain promotes protein movement, while a non-conserved and potentially unstructured region C-terminal to the homeodomain restricts mobility. Interestingly, this region of the protein is also required for WUS homo-dimerization, suggesting that formation of WUS dimers might contribute to the regulation apical stem cell activity (Daum et al., PNAS 2014).

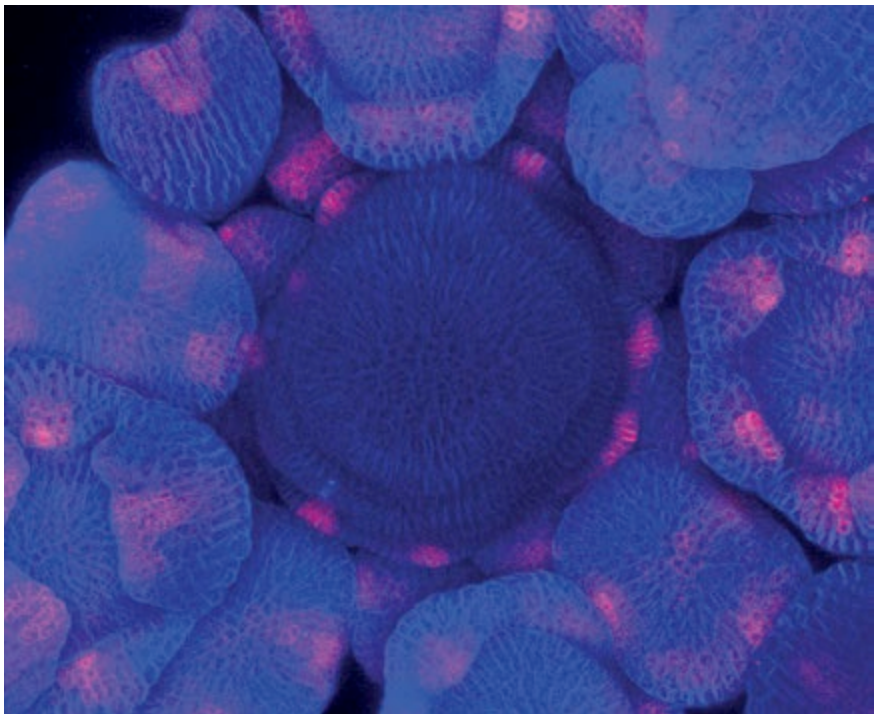

Figure 3
Auxin signalling is supressed in the presence of WUSCHEL. Plants carrying the *clv3* mutations develop massively enlarged apical stem cell systems, due to expanded expression of the stem cell inducing transcription factor WUSCHEL. In contrast to the situation in wild type, where auxin signalling extends from the periphery to the centrally located stem cells, the DR5v2 reported is fully supressed in the SAM of *clv3* mutants.

Another long-term goal of my lab is to elucidate the integration of transcriptional and hormonal signals for controlling stem cell fate in the shoot meristem. The phytohormone auxin is a prime example for a small, highly mobile molecule with instructive roles in a wide range of plant developmental processes. In the context of the shoot apical meristem, local accumulation of auxin is required and sufficient for organ initiation at the periphery. At the same time, stem cells are continuously maintained in the centre of the meristem despite abundant auxin signaling input. We were able to demonstrate that spatial specificity in the auxin response is achieved by direct, pathway-wide transcriptional control by the WUS transcription factor in stem cells. While auxin signaling input is fairly uniform across the shoot meristem, transcriptional output from the auxin pathway is highly localized. Importantly, WUS represses a large number of auxin output and response genes, resulting in an auxin blind spot in the centre of the meristem, which coincides with long term stem cells. This repression cannot be overcome by over-activation of potent upstream signaling components or even downstream response transcription factors, demonstrating that WUS renders stem cells insensitive to auxin. Consequently, interfering with WUS activity allows stem cells to respond to auxin input and will cause their premature differentiation. Our results reveal the mechanisms of a complex regulatory system that confers robustness against fluctuations of a mobile developmental signal and thus prevents termination of an essential stem cell pool (Ma et al., under revision for Science).

In another 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 revealed 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 and demonstrated for the first time that the temporal progression of cell fates is regulated independently from positional identity (Gaillochet et al., under revision for eLife).

Planned research and new directions
Current research in my lab is based on a multi-scale approach using advanced genetic, genomic, molecular, biochemical, and structural methods together with computational analysis and simulation to determine how plant stem cell behavior is regulated by local transcriptional programs, global hormonal signals, as well as the environment.

1. Mechanisms of stem cell induction and maintenance: We are working towards elucidating the crystal structure of WUS and related stem cell regulators to gain an understanding of how these homeodomain transcription factors can bind at least three distinct DNA target motifs, how they are able to form dimers or multimers and how these complexes might affect cell-to-cell mobility. Furthermore, we are investigating the mechanisms underlying the tight regulation of WUS movement from organizing into stem cells by interaction screens and in vivo approaches, including multi-spectral live-cell imaging, and proteomics. To complement these strategies at the genome-wide level, we have developed novel tools for experimentally controlling the activity of WUS in space and time and are using them to delineate the genomic response to this stem cell inducing signal.

2. Integration of hormonal and transcriptional signals in stem cell control: We aim to understand how plant hormones control meristem activity at the mechanistic level. We have carried out extensive protein-protein interaction screens in yeast to identify potential co-regulators of A-type ARR proteins, which we have shown to act as central integrator hubs for WUS, as well as auxin and cytokinin (Leibfried et al. Nature, Zhao et al. Nature). Interestingly, we have identified a topoisomerase as candidate and genetic, as well as molecular evidence suggest that this interaction is relevant in vivo. We are now using this interaction to study how dynamic developmental signals are translated into stable cell behavior. In addition, we have carried out extensive genetic enhancer/suppressor screens in *Arabidopsis* lines that have a modified response to cytokinin and significantly altered transcriptional program without showing overt phenotypes.

3. Environmental control of plant stem cell activity and meristem morphogenesis: In our latest line of research, we focus on the role of the environment in modulating meristem function. To this end we have developed a robust pipeline to record a large number of cellular parameters in vivo, including cell number, cell identity, cell proliferation, cell size etc., which allows us to trace cell behavior in the shoot apex over a wide variety of developmental stages and environmental conditions in diverse accessions of *Arabidopsis thaliana*. We employ this pipeline not only to decipher the environmental response as an adaptive trait, but also when using environmental perturbations as a stimulus to disentangle feedback systems of stem cell control and meristem morphogenesis. Finally, we have established novel tools, which give us direct access to the genomic markup of subpopulations of cells within the shoot apex.

Selected publications since 2013
Number of peer-reviewed articles 2013-2017: 22, number of citations 2013-2017: 264, h-index (2013-2017): 10, total h-index: 27 (according to Thomson Reuters).

Pfeiffer, A., Janocha, D., Dong, Y., Medzihradszky, A., Schöne, S., Daum, G., Suzaki, T., Forner, J., Langenecker, T., Rempel, E., Schmid, M., Wirtz, M., Hell, R., and Lohmann J.U. (2016) Integration of light and metabolic signals for stem cell activation at the shoot apical meristem. eLife *5*, e17023.

Schuster, C., Gaillochet, C., and Lohmann, J.U. (2015) Arabidopsis HECATE genes function in phytohormone control during gynoecium and fruit development. Development *142*, 3343-50.

Daum, G., Medzihradszky, A., Suzaki, T., and Lohmann J.U. (2014) A mechanistic framework of noncell autonomous stem cell induction in Arabidopsis. Proc. Nat. Acad. Sci. USA *111*, 14619-24.

Schuster, C., Gaillochet, C., Medzihradszky, A., Busch, W., Daum, G., Krebs, M., Kehle, A., and Lohmann, J.U. (2014) A regulatory framework for shoot stem cell control integrating metabolic, transcriptional, and phytohormone signals. Developmental Cell *28*, 438-49.

Lampropoulos, A., Sutikovic, Z., Wenzl, C., Maegele, I., Lohmann, J.U. *, and Forner, J. (2013) GreenGate – a novel versatile, and efficient cloning system for plant transgenesis. PLOS One 8, e83043. *corresponding author

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Fields of Interest
Environmental plasticity of plants: light sensing, signal transduction, transcriptional networks, stem cell regulation, molecular genetics, genome editing

Brief summary of work since 2013
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.
My goal is to understand how plants integrate the light signal and process this information to trigger morphological adaptations and developmental transitions.
My research applies whole genome and transcriptome sequencing to characterize genome-wide transcriptional networks that are at the core of light signal transduction. Additionally we include molecular genetics and live cell imaging to analyze the environmental influence on shoot stem cell regulation. Our studies identified the TARGET OF RAPAMYCIN (TOR) kinase as a new signaling component within this pathway that integrates light and also metabolic signals.

Major contributions since 2013
Analysis of genome-wide transcriptional networks
Massive gene expression changes are induced in plants only few minutes after the perception of light. At the core of the light-responsive gene regulatory network sits a small family of transcriptions factors, called PIFs that directly interact with the plant's light receptors. Since PIFs are known to bind a widely present DNA motive, the so-called G-box, a long-standing question has been, how the individual transcription factors achieve specificity in their gene regulatory function. We used genome-wide binding studies paired with transcriptome analysis to map the contribution of four PIF family members to the regulation of target genes (Pfeiffer et al., 2014; Zhang et al., 2013). To further address this question of specificity in transcriptional regulation we more recently studied also genome wide binding sites of transcriptional co-activators, which often act in concert with transcription factors by modifying their cis-acting function (Marín-de la Rosa et al., 2015). These studies are a valuable resource to understand how transcription factors regulate their target genes.

Environmental influence on shoot stem cell regulation
While several mechanisms of plant stem cell control have been studied under artificial constant conditions, the knowledge about how environmental stimuli can influence the underlying regulatory system is still limited. Tracing cell behavior in the shoot apical meristem (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 (Pfeiffer et al., 2016). Both signals are transduced to the SAM through activation of the TOR kinase, which is known to be an evolutionary highly conserved nutrient sensor and potent regulator of mRNA translation and metabolism. Our results suggest that via light-activation of the TOR

kinase, plants can anticipate how much energy will be available and efficiently tune their metabolism and translation machinery to the surrounding light conditions.

Genome editing tools
Our recent research questions have been addressed by molecular genetics and live cell imaging. However, the generation of homozygous reporter lines in different genetic backgrounds has been time consuming. Within the past two years, we successfully established and implemented novel genome editing tools including TALENs and CRISPR-Cas9 (Forner et al., 2015; Pfeiffer et al., 2016), and consequently were able to speed up our research process remarkably.

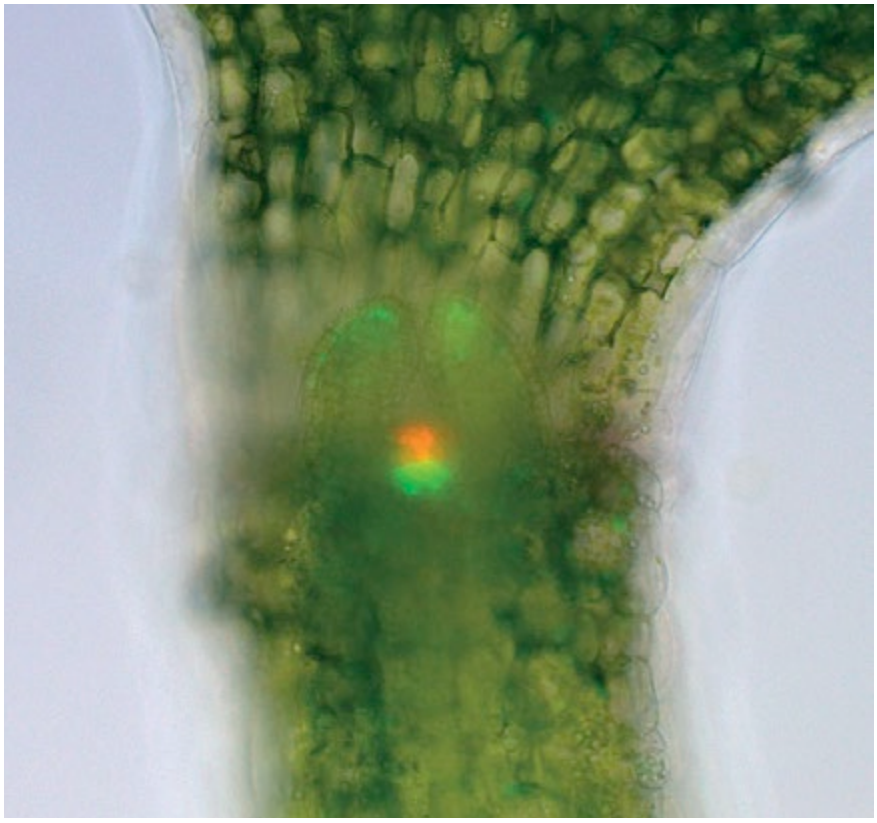

Figure 1
Shoot apical meristem of a young Arabidopsis seedling. Stem cells and cells of the stem cell niche are marked in red and green, respectively.

Planned research and new directions
The shoot stem cell pool is the origin of the entire above ground plant tissue and therefore has to coordinate environmental conditions with developmental programs throughout a plant's life. Preliminary data however showed, that the light stimulus itself is not perceived and processed in the shoot apex, but rather in more distant plant organs. One future research line will therefore aim to identify the molecular components involved in transducing the light stimulus to the SAM. Additionally we want to characterize the role of different plant tissues in perceiving, processing and transducing the light signal to the shoot tip. We also want to follow up on the question how nutrient and light signaling are intertwined to regulate stem cell activity. We identified the TOR kinases as a potent integrator of both signals, but it is still unknown how this is achieved mechanistically. Future work will concentrate on identifying components activating the TOR kinase in response to light and also analyze how TOR is connected to the hormonal network underlying stem cell regulation.

While light and nutrients were shown to have promoting effects on the stem cell niche, we propose that seedlings germinating in darkness actively suppress stem cell activity to save valuable resources. In the past, several mutants have been identified that induce new organs in the shoot regardless of the light environment. We plan to identify how the causative genes are involved in stem cell regulation and whether these mechanisms of stem cell repression are also relevant under other abiotic stress conditions like heat, cold or drought.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 8, number of citations 2013-2017: 278, h-index (2013-2017): 6, total h-index: 8 (according to Thomson Reuters).

Pfeiffer, A., Janocha, D., Dong, Y., Medzihradszky, A., Schöne, S., Daum, G., Suzaki, T., Forner, J., Langenecker, T., Rempel, E., et al. (2016). Integration of light and metabolic signals for stem cell activation at the shoot apical meristem. Elife 5, 1–21.

Marín-de la Rosa, N., Pfeiffer, A., Hill, K., Locascio, A., Bhalerao, R.P., Miskolczi, P., Grønlund, A.L., Wanchoo-Kohli, A., Thomas, S.G., Bennett, M.J., et al. (2015). Genome Wide Binding Site Analysis Reveals Transcriptional Coactivation of Cytokinin-Responsive Genes by DELLA Proteins. PLoS Genet. 7, e1005337.

Forner, J., Pfeiffer, A., Langenecker, T., Manavella, P., and Lohmann, J.U. (2015). Germline-Transmitted Genome Editing in Arabidopsis thaliana Using TAL-Effector-Nucleases. PLoS One 10, e0121056.

Pfeiffer, A., Shi, H., Tepperman, J.M., Zhang, Y., and Quail, P.H. (2014). Combinatorial complexity in a transcriptionally centered signaling hub in Arabidopsis. Mol. Plant 7, 1598–1618.

Zhang, Y., Mayba, O., Pfeiffer, A., Shi, H., Tepperman, J.M., Speed, T.P., and Quail, P.H. (2013). A quartet of PIF bHLH factors provides a transcriptionally centered signaling hub that regulates seedling morphogenesis through differential expression-patterning of shared target genes in Arabidopsis. PLoS Genet. 9, e1003244.

2.17 PROF. DR. ALEXIS MAIZEL
DEVELOPMENTAL PLASTICITY OF PLANTS

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Fields of Interest
Developmental and cell biology, plant, root, imaging, quantitative analysis.

Brief summary of work since 2013
The focus of my lab is to understand how, at the cell scale, plants are able to robustly shape their organs during post-embryonic development. As model, my lab studies the formation of lateral root in *Arabidopsis thaliana*. Lateral roots are formed throughout the plant life from the primary root and develop from a discrete number of founders that proliferate and organise in a dome shaped primordium with a stereotyped tissue organisation.

Since 2013, our work has focused on the application of light sheet fluorescence microscopy (LSFM), a type of microscopy that enables imaging with minimal photodamage, to capture at high spatial and temporal resolution the formation of lateral root. LSFM allowed us to describe and quantitatively analyse at an unprecedented level how lateral root founder cells and the overlying surrounding tissues actively collaborate during lateral root initiation and outgrowth. In particular, how the founder cells swell prior to entering division, how the overlying endodermal layer shrinks and how the primordium transition from a bilateral to a radially symmetric structure. We also combined quantitative analysis and mathematical modelling to elucidate how the typical layered organisation of the primordium comes to exist.

In parallel, we have pursued our efforts to understand the relationships between small RNA mediated mRNA degradation and mRNA quality control processes interact in plant cells. Combining genetic analysis, transcriptomics and cell biology, we could show that mRNA decapping prevents the entry of aberrant endogenous mRNA into the small RNA pathway, therefore protecting cells from the formation of rogue silencing RNAs with potentially deleterious effects.

Major contributions since 2013
1) Morphodynamics of lateral root formation in *Arabidopsis thaliana*
A fundamental question in biology is how multicellular organisms use the genetic information of their cells to reproducibly generate a complex three-dimensional shape. In Arabidopsis, the root system consists of an embryo derived primary root and of lateral roots (LRs) that are formed post-embryonically. Because LRs derive from a limited number of homogeneous founder cells that proliferate to form a primordium (LRP) with stereotyped dome shape and a characteristic organisation in layers, it is an exquisite system to understand the mechanisms of plant morphogenesis.

For the last 6 years, my lab has pioneered the use of light sheet fluorescence microscopy (LSFM) for live imaging of plant development in close-to-natural growth conditions and at the organ, cellular and sub-cellular scales. LSFM selectively illuminates a small portion of the specimen while collecting only the light emitted at the current focal resulting in lower

phototoxicity and bleaching. LSFM has become increasingly popular in developmental studies as it provides detailed time-resolved information on the behaviour of all cells over the entire duration of the morphogenetic process.

Since 2013, we employed LSFM to analyse at high resolution LR formation. We imaged and quantified the change of shape of the founder cells and overlying endodermis during LR initation. We could show that the pericycle cells swell and the endodermis shrinks. We showed that LRP morphogenesis is not a stereotypical process leading from one stage to the next and that there is no unique sequence of cell divisions during LRP development. Several patterns of cell division are associated with the same final morphological structure. We combined quantitative analysis and computer modelling, to identify the principles underpinning the emergence of this layered organisation. We showed that some founders take a dominant role and that the first division of the founders is asymmetric, tightly regulated, and has a critical impact on the correct subsequent formation of a layered structure. Our results highlight the importance of the cell geometry in determining the orientation of the plane of division and show that the apical growth pattern and mechanical constraints determine the order of appearance of these layers.

Main associated publications: Lucas et al. (2013) | Vermeer et al. (2014) | von Wangenheim et al. (2016).

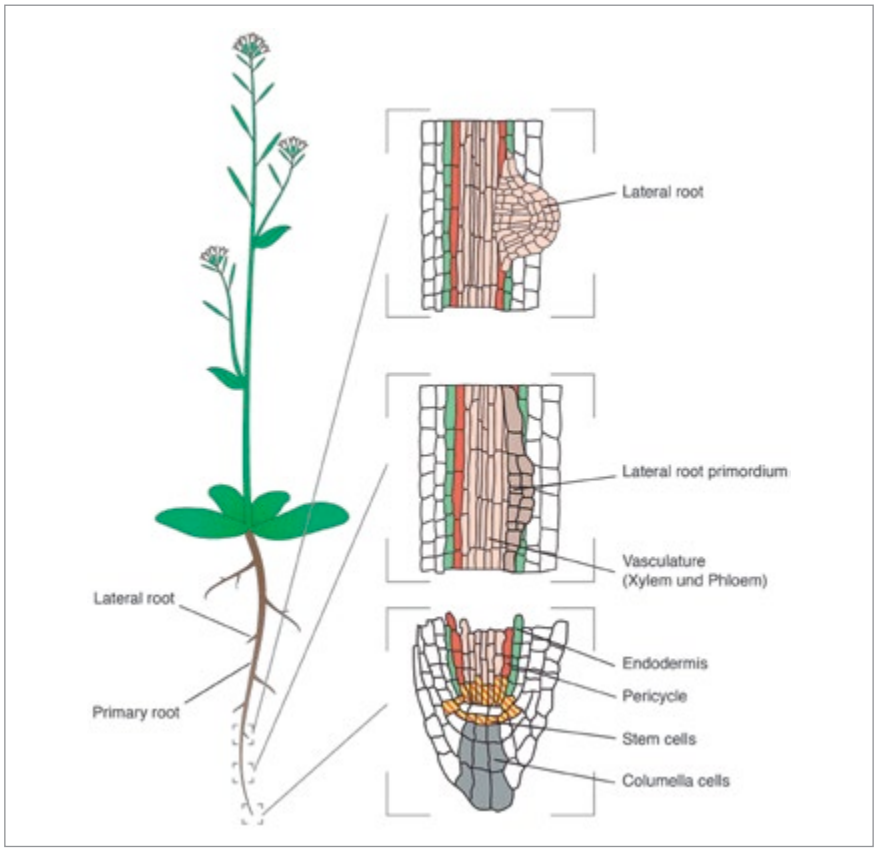

Figure 1
The root system of the model plant *Arabidopsis thaliana*. Lateral roots are formed from the primary root throughout the whole plant life by the specification at regular interval of founder cells that derive from the pericycle, the layer surrounding the vasculature. These founder cells proliferate to form the lateral root primordium which will emerge from the primary root.

2) Functional and spatial relationships between RNA silencing and RNA turnover in plant cells

RNA turnover is an integral part of eukaryotic regulation of gene expression. It regulates RNA abundance and also degrades dysfunctional transcripts. RNA turnover needs to be tightly controlled to only target specific RNA. The 5' cap and the 3' poly(A) tail, contribute to distinguish a functional mRNA from a dysfunctional transcript, thus protecting mRNAs from exoribonucleases, ensuring mRNAs stability and facilitating translation. Post-transcriptional gene silencing (PTGS) is a conserved mechanism, which destroys RNAs

originating from pathogen infection, high levels of transgene expression or select endogenous mRNAs. During PTGS single stranded RNA is transformed into double-stranded (ds) RNA by RDR6 and SGS3, and subsequently processed into small interfering (si)RNAs that guide ARGONAUTE1-dependent mRNA target cleavage. As the two pathways act on the same mRNA population, how is their action is coordinated? It was previously shown that transgene-PTGS is boosted when RNA turnover is impaired but there were no evidence supporting that endogenous mRNAs are processed analogously.

In the last five years, we explored the functional and spatial relationship between RNA turnover and PTGS in Arabidopsis. First, we show that mutations in three components of the decapping machinery, DCP1, DCP2 and VCS, provoke an increase of RDR6-dependent transgene PTGS. We also show that a mutation in RDR6 suppresses the lethality of dcp2 and vcs null alleles. Third, we uncover the existence of a new class of siRNAs (rqc-siRNA) produced by hundreds of endogenous mRNAs upon decapping impairment. rqc-siRNA production from a subset of these mRNAs depends on their RDR6-mediated conversion to dsRNA. Finally, we observe that although appearing as distinct bodies, P- and siRNA-bodies often are spatially associated and display concordant, actin-dependent, movement in the cytoplasm. Together, our data suggest that P-body-localized decapping of endogenous mRNAs deters dysfunctional mRNAs from entering the siRNA-body localized PTGS pathway, and, as such, circumvents the production of rqc-siRNAs, which could direct the sequence-specific degradation of functional cellular mRNAs. Our results reveal the importance of a careful balance of RNA turnover and RNA silencing processes for maintaining transcrip- tome integrity and, consequently, proper plant development.

Main associated publications: Moreno et al. (2013) | Martínez de Alba et al. (2015).

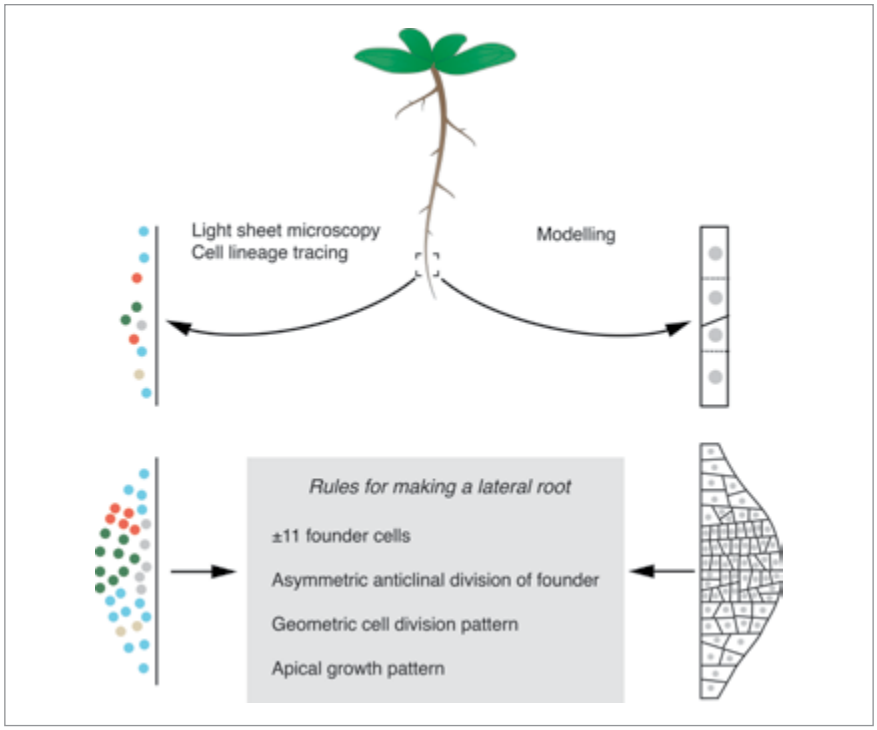

Figure 2
Morphodynamics of lateral root development. Lateral roots branch off the primary root in Arabidopsis seedlings. The Maizel lab used a combined experimental and computational approach to define the rules that underlie lateral root formation. By using light-sheet microscopy (left), high-resolution datasets were captured that describe the entire process in 3D, and cell lineages were traced from these datasets (left; colored dots represent nuclei). In parallel, models were built to simulate lateral root development in silico (right). The combination of these approaches allowed distilling a set of rules that explain development. A small set of founder cells divides asymmetrically, after which cells follow a simple, geometric division rule.

Planned research and new directions

In the coming years, we will expand our work on the cellular basis of lateral root morphogenesis to identify the principles that underpin the emergence of specific cell identities and the formation of a new stem cell niche during LR morphogenesis. We will pursue our efforts to combine LSFM live imaging and quantitative methods to analyse how auxin flow and signalling, cytoskeleton, asymmetries in cell growth and cell division coordinate to specify a robust pattern of cell identities. We will establish a time- and space-resolved atlas of specific cell identity acquisition and link it to pattern of cell growth, cytsokeleton dynamics and division and auxin signalling. The group recently acquired a multi-view light sheet microscope coupled with a 2-photon laser that in addition to providing superior image quality allows to perform cell ablation together with LSFM. This will allow us to monitor the impact on perturbation on auxin signaling patterns, cytoskeleton cell division orientation and cell identity emergence on LR morphogenesis.

Together with the group of E. Gaquerel in the context of the CellNetworks EcTop6, we are investigating the metabolic reprogramming associated with LR formation. We identify the metabolic signature of the primary root gating lateral root priming and extract the metabolic shift specifically associated with lateral root formation. Our preliminary results indicate a prominent role of primary glucose metabolism and a key role for the TOR kinase in licensing LR formation. The detailed molecular mechanisms will be studied.

Concerning the RNA silencing aspect of our research, in the coming year we will focus on elucidating the molecular specificity of the ARGONAUTE7 action and subcellular routing. We have previously shown that AGO7 associates with endomembranes and accumulates in cytoplasmic foci and that this localisation is important for its function. What determines the association of AGO7 with endomembranes is unknown. We are currently mapping the residues that are essential for its specific ability to trigger ta-siRNAs production and control its subcellular targeting. Our preliminary results indicate that the N-terminal end of AGO7 contains essential residues. We performed a yeast 2 hybrid screen with this N-terminal domain and identified a protein of unknown function (LOR6) as a specific interactor. The characterisation of the role of this protein is currently ongoing.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 17, number of citations 2013-2017: 234, h-index (2013-2017): 8, total h-index: 17 (according to Thomson Reuters).

Wangenheim, von, D., Fangerau, J., Schmitz, A., Smith, R.S., Leitte, H., Stelzer, E.H.K., and Maizel, A. (2016). Rules and Self-Organizing Properties of Post-embryonic Plant Organ Cell Division Patterns. Curr Biol 26, 439–449.

Martínez de Alba, A.E., Moreno, A.B., Gabriel, M., Mallory, A.C., Christ, A., Bounon, R., Balzergue, S., Aubourg, S., Gautheret, D., Crespi, M.D., et al. (2015). In plants, decapping prevents RDR6-dependent production of small interfering RNAs from endogenous mRNAs. Nucleic Acids Research 43, 2902–2913.

Vermeer, J.E.M., Wangenheim, von, D., Barberon, M., Lee, Y., Stelzer, E.H.K., Maizel, A., and Geldner, N. (2014). A spatial accommodation by neighboring cells is required for organ initiation in Arabidopsis. Science 343, 178–183.

Lucas, M., Kenobi, K., Wangenheim, von, D., Voß, U., Swarup, K., De Smet, I., Van Damme, D., Lawrence, T., Péret, B., Moscardi, E., et al. (2013). Lateral root morphogenesis is dependent on the mechanical properties of the overlaying tissues. PNAS 110, 5229–5234.

Moreno, A.B., Martínez de Alba, A.E., Bardou, F., Crespi, M.D., Vaucheret, H., Maizel, A., and Mallory, A.C. (2013). Cytoplasmic and nuclear quality control and turnover of single-stranded RNA modulate post-transcriptional gene silencing in plants. Nucleic Acids Research 41, 4699–4708.

2.18 PROF. DR. GISLENE PEREIRA
MOLECULAR BIOLOGY OF CENTROSOMES AND CILIA

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Fields of Interest
Cell cycle regulation, asymmetric cell division, mitotic checkpoints, centrosome biology, ciliogenesis

Brief summary of work since 2013
Microtubule organising centres (MTOCs) play an important role in the temporal and spatial organisation of the microtubules of the bipolar spindle (that segregates the sister chromatids to opposite poles of the cell during mitosis) as well as in the formation of astral microtubules, which are involved in spindle orientation in polarised cells. Furthermore, specialised sub-structures of mammalian MTOCs, named centrioles, serve as a template for the formation of the basal body, from which cilia and flagella are assembled. My lab is interested in understanding the molecular role of MTOC-associated components in cell cycle control and during the process of cilia formation. We are using *Saccharomyces cerevisiae* as a genetically tractable model system to investigate how MTOC-associated signalling coordinates spindle orientation/cytokinesis with chromosome segregation. Using mammalian ciliated cells, we are investigating how ciliogenesis is controlled on a molecular level. In the past five years, we identified and investigated molecular players involved in cell cycle control/cytokinesis and cell polarity establishment. In addition, we identified and characterised components required for the initial steps of primary cilia biogenesis in mammalian cells.

Major contributions since 2013
Control of genome integrity: Spindle Position Checkpoint (SPOC) and Cytokinesis. Many polarised cells, such as budding yeast and stem cells, orient the mitotic spindle along a defined polarity axis. In budding yeast, the plane of cell division is determined at early stages of the cell cycle. Therefore, mitotic spindle orientation is critical for the correct segregation of one set of chromosomes to the daughter cell during mitosis. In case of spindle mis-orientation, cells stop the process of mitotic exit and cytokinesis until the spindle is correctly oriented. This cell cycle delay is triggered by the spindle position checkpoint (SPOC) – a surveillance mechanism that senses spindle mis-orientation. In SPOC-deficient cells, spindle mis-orientation results in chromosome mis-segregation and cell death, emphasising the importance of this checkpoint for the genome integrity. In the past five years, we dissected the molecular mechanisms that control SPOC activation. We could show that the yeast MTOC (named as spindle pole body, SPB) constitutes a platform for the phospho-regulation of the SPOC component, Bfa1 (Caydasi, et al., MBoC 2014; Gryaznova et al., eLife 2016). We uncover the Cdc14-Early Release (FEAR)-Network as a signaling pathway that counteracts SPOC activation (Caydasi et al., Nat Commun. 2017). In addition, we identified the conserved cyclin-dependent kinase (CDK) and cell polarity components as novel regulators of Bfa1 and mitotic exit, respectively (Caydasi et al., Nat Commun. 2017). Together, our work provides the molecular basis of the current model of SPOC signaling.

Cytokinesis (the final division of mother and daughter cells) must be tightly regulated with other mitotic events in order to avoid errors in chromosome segregation. Using biochemical and genetic approaches, we uncover novel mechanisms that control cytokinesis through timely phosphorylation/dephosphorylation of key cytokinetic components (Meitinger et al., MBoC 2013 and 2017; Lombardi et al., Curr. Biol. 2013).

Negative cell polarity cues. Cell polarisation is a fundamental process that establishes a single orientation within a cell. It is key for many processes including asymmetric cell division (e. g. in stem cells), cell migration and tissue integrity. Defects in cell polarisation contribute to developmental disorders as well as tumour development. Using budding yeast cells, we uncovered an elaborated mechanism that controls cell polarisation at the level of the conserved GTPase Cdc42. We show that a protein complex composed of four proteins (Nba1, Nis1, Nap1 and Gps1) binds to discrete sites at the plasma membrane and prevents Cdc42 polarisation at these sites. This inhibitory mechanism of cell polarisation is key to avoid that cells use the same site during subsequent cell divisions. In their absence, cell polarisation at the same site of cell division leads to chromosome instability and reduced replicative aging (Meitinger et al., Cell 2014).

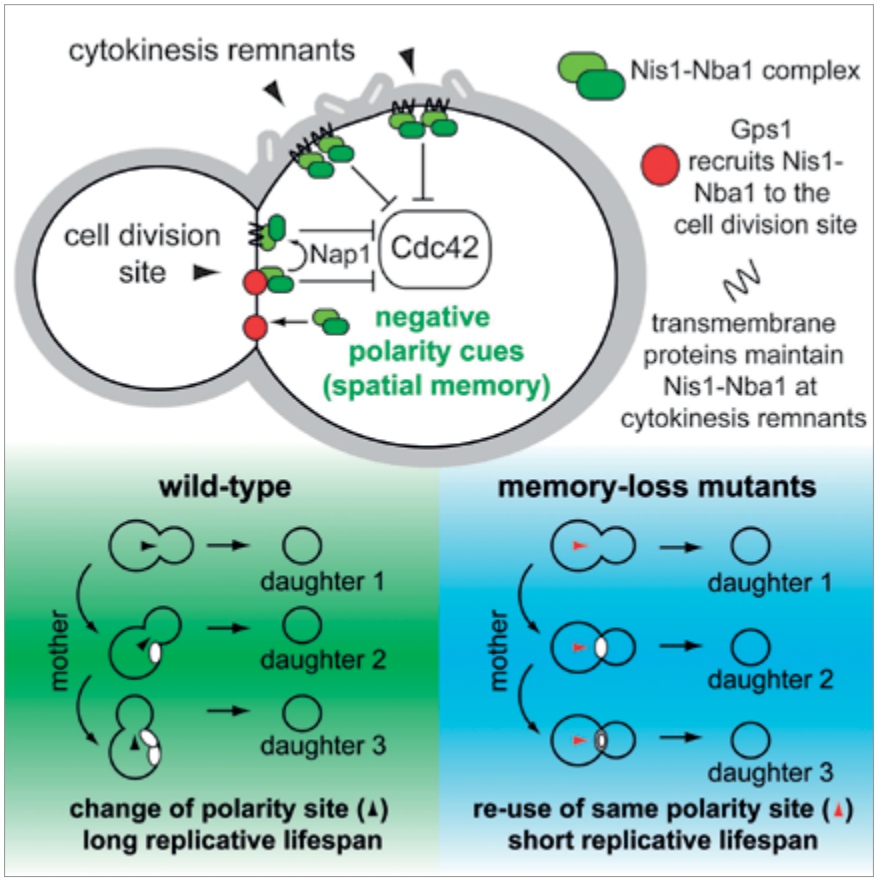

Figure 1
Memory cues inhibit Cdc42 polarisation to prevent chromosome mis-segregation and replicative aging. During mitosis, a complex of proteins that include Nis1-Nba1, Nap1 and Gps1 accumulates at the cell division site. Two of these proteins, Nis1-Nba1, remain associated with the cell division site for many generations, where they inhibit repolarisation of the GTPase Cdc42. This mechanism establishes a new cell polarity axis away from the previous site of cell division and it is key for accuracy of chromosome segregation and replicative lifespan. In the absence of the above-mentioned components (memory-loss mutants), cells dye at younger ages due to genetic instability (from Meitinger et al., Cell 2014).

Control of cilia biogenesis. The MTOC of mammalian cells, named as the centrosome, gives rise to cilia in differentiated cells. Cilia are evolutionary conserved microtubule based organelles, which are present in almost every cell type of the human body. Cilia play essential functions in embryonic development as well as in tissue homeostasis in adulthood. The physiological significance of cilia is further highlighted by a variety of human diseases, which are linked to ciliary defects, such as polycystic kidney disease, retinal degeneration, obesity, diabetes and cancer. Moreover, work in the past years revealed that receptors of important signalling pathways such as the Hedgehog and Wnt pathways are located at the primary (non-motile) cilium. Our lab is interested in understanding how the centriole converges into the basal body of cilia (initial steps of ciliogenesis). Using a high-throughput approach, we screened for kinases that are required for cilia formation. Among the kinases we identified, we show that the microtubule and affinity kinase, MARK4, is required for the initiation of axoneme extension (Kuhns et al., JCB 2013). Furthermore, we show that two novel proteins (SSX2IP and WDR8) are present in large cytoplasmic protein complexes (centriolar satellites), which are required for vesicular docking and cilia membrane extension at early stages of ciliogenesis (Klinger et al., MBoC 2014; Kurtulmus et al., JCS 2016).

Planned research and new directions

Using a combination of biochemical and genome wide approaches, we will search for novel SPOC components. Our aim is to provide a comprehensive overview of the components involved in SPOC and uncover how SPOC senses spindle mis-orientation on a molecular level. Building on our work in budding yeast, we started to investigate whether centrosome asymmetry and SPOC are conserved in stem cells. For this, we are using isolated neural and intestinal mouse stem cells growing on 2D cultures and 3D-matrixes (organoids). We will focus our future work on conserved mechanisms that coordinate spindle orientation with cell cycle progression.

Using human and murine ciliated cells, we aim to dissect how cilia formation is initiated and how ciliogenesis is coordinated with cell cycle progression. The main outstanding questions that we are interested in investigating are (1) which molecular players are involved in the formation of the basal body, (2) what are the signals that control the start of ciliogenesis and which components are regulated (transition of centriole to basal body), (3) how does the loss of cilia influence cell cycle progression and (4) how do mutations in components of cilia cause disease and contributes to tumour maintenance.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 12, number of citations for articles published between 2013-2017: 100, h-index (2013-2017): 6, total h-index: 20 (according to Thomson Reuters).

Caydasi AK, Khmelinskii A, Duenas-Sanchez R, Kurtulmus B, Knop M and Pereira G (2017). Temporal and compartment-specific signals co-ordinate mitotic exit with spindle position. Nat. Commun., doi:10.1038/ncomms14129.

Gryaznova, Y., Caydasi, A.K., Malengo, G., Sourjik, V. and Pereira G. (2016). A FRET-based study reveals site-specific regulation of spindle position checkpoint proteins at yeast centrosomes. eLife. published ahead of print (eLife 2016;10.7554/eLife.14029).

Meitinger, F., Khmelinskii, A., Morlot, S., Kurtulmus, B., Palani, S., Andres-Pons, A., Hub, B., Knop, M., Charvin, G. and Pereira, G. (2014). A memory system of negative polarity cues prevents replicative aging. Cell, 159: 1056-1069.

Lombardi, I.M.*, Palani, S.*, Meitinger, F., Darieva, Z., Hofmann, A., Sharrocks, A. and Pereira, G. (2013). Lre1 direclty inhibits the NDR/Lats kinase Cbk1 at the cell division site in a phosphorylation dependent manner. Curr. Biol., 23:1736-1745. * Co-first authors.

Kuhns, S., Schmidt, K.N., Reymann, J., Gilbert, D.F., Neuner, A., Hub, B., Carvalho, R., Wiedemann, P., Zentgraf, H., Erfle, H., Klingmüller, U., Boutros, M. and Pereira, G. (2013). The microtubule affinity regulating kinase MARK4 promotes axoneme extension during early ciliogenesis. J. Cell Biol., 200:505-522.


WIRBELTIERE
MITTELEUROPAS

2.19 PROF. DR. GABRIELE ELISABETH POLLERBERG
DEVELOPMENTAL NEUROBIOLOGY

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Fields of Interest
Cellular and molecular processes underlying growth and navigation of axons during development and regeneration of the higher vertebrate nervous system (retina of mouse and chick embryo); roles of cell adhesion molecules and their interaction partners in these processes

Brief summary of work since 2013
We investigate the cellular and molecular processes underlying growth and navigation of axons during development and regeneration. In particular we analyse the roles of cell adhesion molecules of the immunoglobulin superfamily (IgSF-CAM) in the context of their extra- and intracellular interactions. In the last years we focussed on ALCAM, a small IgSF-CAM present in the plasma membrane of growing axons and growth cones (i.e. the sensory structure at the axon tip). We could show that activation of ALCAM triggers intracellular signalling cascades leading to an invasion of microtubules into the peripheral domain of the growth cone and enhanced axon growth. As microtubule dynamics are regulated by microtubule-associated proteins (MAPs), we analysed axon growth in a MAP k.o. mouse and could show severe axonal misrouting. We also investigated the crosstalk between ALCAM and the cortical cytoskeleton and revealed that activation of ALCAM leads to a dramatic stiffening of the plasma membrane. In addition, we created an artificial cortical cytoskeleton allowing for the investigation of the physical properties of the ALCAM-anchorage to the cortical cytoskeleton in a readily accessible and highly defined system. We could identify a linker protein which anchors ALCAM to the cortical cytoskeleton; this interaction is surprisingly reduced when ALCAM is activated and the linker protein phosphorylated. We moreover identified an axon guidance molecule to interact with ALCAM. It co-migrates with ALCAM in the plasma membrane and is co-internalized upon experimentally induced clustering; first experiments indicate that this interaction regulates the growth cone response to the guidance molecule.

Major contributions since 2013
During development and regeneration of the nervous system, neurons send out axons with a motile sensory structure at their tip, the growth cone. Proteins in the growth cone's plasma membrane interact with molecules in the environment and thereby trigger intracellular molecular modulations ensuring appropriate steering reactions and ultimately the correct target-finding of the axon. We are interested in cell adhesion molecules of the immunoglobulin superfamiliy (IgSF-CAMs); in particular, we study ALCAM in the context of its intracellular interaction partners and signalling targets (Pollerberg et al., 2013). One of the down-stream signalling targets are the microtubules; we therefore analysed the dynamics of microtubules in retinal ganglion cell growth cones, monitoring them by an optimized long-term, high frequency time-lapse-microscopy protocol. For this, we cloned novel, less toxic fluorescent fusion proteins which selectively bind to the microtubule plus tips. We

could show that – upon activation of ALCAM by experimentally induced clustering – microtubules invade the peripheral growth cone domain, in particular into its tip, accompanied by an almost twofold enhancement of axon growth. We also analysed k.o. mice, which lack a microtubule-associated protein (MAP), and found an abnormal axon elongation and fasciculation; the most drastic effect however is the severe misrouting of retinal ganglion cell axons at the optic nerve head of the developing retina (Figure 1), which is strongly reminiscent to the misrouting we observe caused by absence of functional ALCAM.

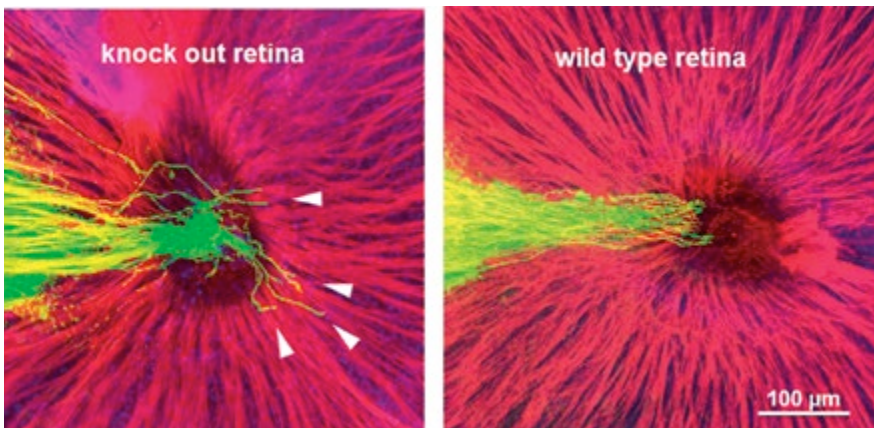

Figure 1
Central region of the embryonic retina where the retinal ganglion cell axons (red) enter into the optic nerve (darker area). Labelling of axon subpopulations (green) reveals that in MAP k.o. mice, axons are misrouting to the opposite retina side (arrow heads).

In a project funded by the Cell Networks Excellence Cluster, we were able to show that activation of ALCAM also has an effect on the physical properties of the plasma membrane: Its stiffness is increased almost fivefold as revealed by atomic force microscopy (AFM) measurements (in collaboration with J.P. Spatz, Heidelberg University). Within this project we moreover started to create an artificial cortical cytoskeleton using nano-gold-patterns as a scaffold to which short actin filaments are coupled. Via a linker protein, ALCAM is coupled to this artificial cortical cytoskeleton allowing to study the impact of this anchorage on ALCAM´s adhesive properties, e. g. (Figure 2). We originally hypothesized this linker protein to bind to ALCAM's intracellular domain based on our finding that one distinct ALCAM spacing (70 nm distance) is incompatible with efficient axon growth, conceivably as the substrate binding sites are not in register with the linker protein binding sites. Analysis of the intracellular domain of ALCAM revealed two binding sites for this linker protein, and we could indeed demonstrate its binding by bimolecular fluorescence complementation (BiFC) assays. Various mutations (by exchange / deletions of amino acids) of the binding sites on ALCAM completely abolish the BiFC signal, revealing the capacity of the linker protein to bind to each of the two binding sites independently (Figure 3). Interaction of ALCAM with the linker protein could moreover be shown by a highly significant Pearson correlation coefficient (PCC) which is surprisingly reduced upon activation of ALCAM. In addition, we performed acceptor photobleaching fluorescence resonance energy transfer (apFRET) experiments which showed the interaction of the linker protein with ALCAM in living cells. Also by this technique, stimulation of ALCAM results in a lowered interaction with the linker protein. Since we found reduced PCC values for the phosphorylated form of the linker protein, its binding to ALCAM appears to depend on the phosphorylation state of the linker protein.

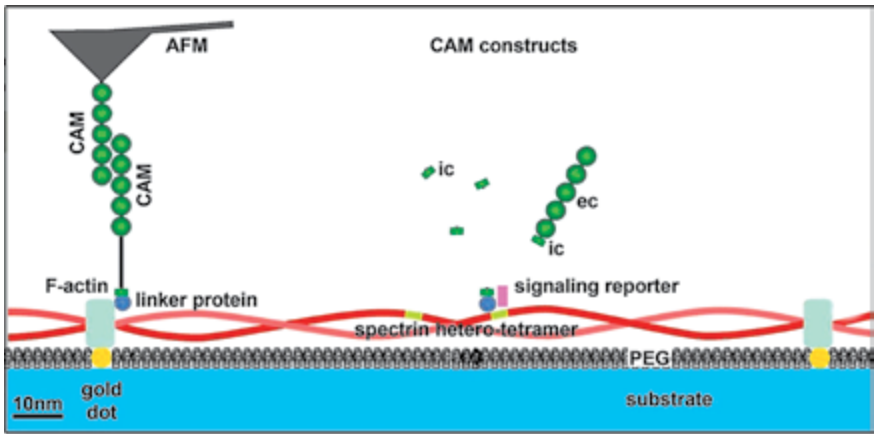

Figure 2
An artificial cortical cytoskeleton created on a gold dot scaffold (yellow) with short actin filaments (grey), spectrin heterotetrameres (red), linker proteins (blue) and full-length or domain-tailored CAMs (green) allows e. g. for atomic force measurements.

Searching for novel interaction partners of ALCAM, we identified an axon guidance protein which interacts with the intracellular domain of ALCAM. This finding was confirmed by fluorescence correlation spectroscopy (FCS) analyses of the two intracellular domains and also by immuno-affinity co-purification. Moreover, we could show that clustering of the axon guidance protein causes a co-migration and co-endocytosis of ALCAM, revealing that the two proteins indeed interact in the plasma membrane of living cells (neuroblastoma cells transfected with both proteins).In addition we could demonstrate the co-expression of the two proteins in the developing retina and co-presence in the growth cone plasma membrane. To elucidate the functional role of this interaction for growing retinal ganglion cell axons, we developed a technique for the application soluble compounds to retinal explants in cell culture, succeeding in a fast distribution of the applied protein without any unspecific disturbance of the advancing growth cones. First results indicate that a negative axonal response is evoked by applying the activator of the axon guidance molecule (as a soluble fusion-protein) and that this response can be down-regulated by simultaneous stimulation of ALCAM; this is an important aspect also for the optimisation of ALCAM-based implants designed to enhance axonal regeneration.

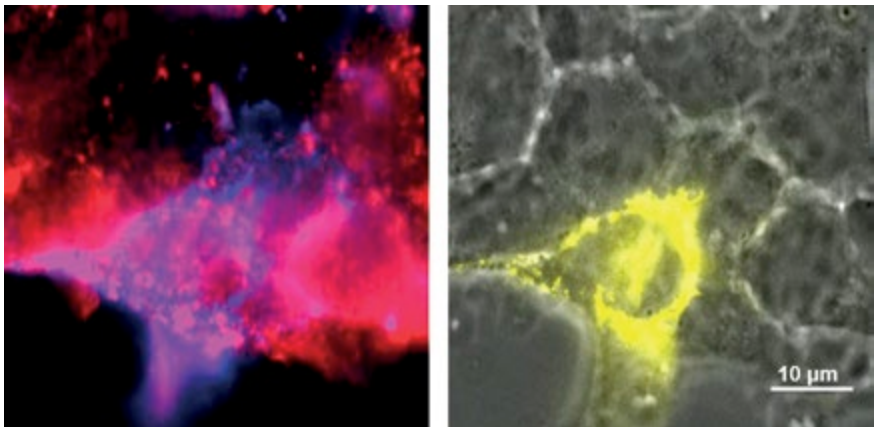

Figure 3
ALCAM (blue) and its linker protein (red), eached fused to one half of YFP, interact in HEK cells resulting in a bimolecular fluorescence complementation (BiFC) signal (yellow) shown on the right merged with the phase contrast micrograph (grey).

Planned research and new directions
Our major aim is the investigation of the roles of CAMs in concert with their interaction partners for growing axons, not only since we are familiar with this research field but also as it has a strong potential for both basic and translational studies. We aim at understanding not only of the molecular interactions per se but also their functional relevance for axon growth, both during development and regeneration; for the latter, we already started to strengthen our studies of adult axons. We hence plan to investigate the role of ALCAM's anchorage to the cytoskeleton via the identified linker protein for the growth of axons. For this, we recently established a technique allowing (for the first time worldwide) for the transfection of mouse retinal ganglion cells which is compatible with axon extension on a defined substrate. We are therefore now for example able to knock down the linker protein by siRNA transfection and monitor the impact of the disturbed ALCAM-cytoskeleton anchorage on axon growth with or without ALCAM stimulation. Moreover, we will monitor and analyse this interaction by high resolution microscopy, taking advantage of the excellent imaging facilities on the campus (NIC, Heidelberg e. g).

With respect to the interaction of ALCAM with the identified axon guidance molecule, we plan to analyse the mechanism how ALCAM stimulation might attenuate the negative axon response. One possible mechanism could be the co-endocytosis followed by the co-degradation of both molecules causing a depletion of (one of) the molecules in the plasma membrane; we already established the quantification of these processes. We moreover want to find out whether this molecular interaction interferes with the ability of retinal ganglion cell axons to avoid the guidance protein and/or to prefer ALCAM by making use of improved substrate choice assays which we developed and optimized. We are also aiming at the application of our findings by performing translational studies, designing optimized biomimetic implants presenting CAMs and at the same time regulating their interaction partners, thereby more efficiently enhancing axonal regeneration (in collaboration with the Neuroregeneration Section, Heidelberg University Hospital). In this context, we now want to find out how our recent findings – both the anchorage of ALCAM to the cytoskeleton and ALCAM's capacity to modulate the negative axon response to environmental cues – can be implemented into the design of novel, further improved implants (a second patent application is planned).

Selected publications since 2013
Number of peer-reviewed articles 2013-2017: 1, number of citations for articles published between 2013-2017: 43, h-index (2013-2017): 3, total h-index: 21 (according to Thomson Reuters).

Pollerberg GE, Thelen K, Theiss MO, Hochlehnert BC. (2013) The role of cell adhesion molecules for navigating axons: Density matters. Mech Dev. 2012:10.1016


TrpS/TrpR S1.7/R2.2,17,22,7
cytosolic S1.9/R2.1,3,10,12,14

2.20 PROF. DR. THOMAS RAUSCH
PLANT MOLECULAR PHYSIOLOGY

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Fields of Interest
Dynamic adaptation of plant primary and secondary metabolism to environmental and developmental cues. Characterization of regulatory mechanisms, ranging from transcriptional to post-translational controls. *Arabidopsis* as model system, but strong focus on crop plants (chicory, grapevine, *Miscanthus*).

Brief summary of work since 2013
In response to changes in the environment plants have to rewire the interaction of metabolism with development. Our research has focused on dynamic changes at the interface between stress-modulated metabolism and functionally related developmental adaptations. To elucidate the underlying molecular mechanisms our experimental approaches included mutant analysis, genotypic variants, and transgenic plants, leveraging genomic and transcriptomic NGS and integrating gene expression and metabolite profiling, functional promoter activation studies, protein biochemistry and cell biology. Research topics included the transcriptional regulation of complex suites of primary and secondary metabolism and the control (and manipulation) of glutathione biosynthesis. Important results included: 1) Identification of chicory R2R3MYB transcription factors (TFs) involved in the regulation of fructan metabolism under stress exposure; 2) Comprehensive characterization of the multi-layered TF network regulating lignin biosynthesis in *Miscanthus*; 3) Identification and characterization of two R2R3MYB TFs regulating stilbene biosynthesis in grapevine; 4) Development of a pulsed stress exposure treatment for boosting flavonol accumulation in Arabidopsis via attenuation of a negative feedback loop; 5) Priming tobacco plants for defense via ectopic expression of a bifunctional (bacterial) enzyme for glutathione biosynthesis; 6) Elucidation of the link between homo dimerization and redox-mediated post-translational activation of glutamylcysteine ligase; and 7) Identification of plant abscisic acid as a colonization-stimulating factor for the mutualist fungus *Piriformospora indica*. In summary, our research has contributed to a deeper understanding of fundamental regulatory circuits but has also opened new perspectives for practical application in plant breeding and biotechnology.

Major contributions since 2013

Novel insight into the transcriptional regulation of stilbene biosynthesis in grapevine
Plant stilbenes are phytoalexins that accumulate in a small number of plant species, including grapevine *(Vitis vinifera)*, in response to biotic and abiotic stresses and have been implicated in many beneficial effects on human health. We have identified and functionally characterized two R2R3-MYB-type transcription factors (TFs) from grapevine, which regulate the stilbene biosynthetic pathway. These TFs, designated MYB14 and MYB15, are co-expressed with STS genes, both in leaf tissues under biotic and abiotic stress and in the skin and seed of healthy developing berries during maturation. In transient gene reporter assays, MYB14 and MYB15 were demonstrated to specifically activate the promoters of STS genes, and the ectopic expression of MYB15 in grapevine hairy roots resulted in increased STS expression and in the accumulation of glycosylated stilbenes *in planta*. The results demonstrate the involvement of MYB14 and MYB15 in the transcriptional regulation of stilbene biosynthesis in grapevine.

Impact of pulsed UV-B stress exposure on plant performance: How recovery periods stimulate secondary metabolism while reducing adaptive growth attenuation
While the molecular response to continuous UV-B exposure (Cuv) has been elucidated in substantial detail, the dynamic response to multiple stress exposures interrupted by recovery periods has received less attention. In our study, the response of Arabidopsis plantlets to Cuv was compared to that of pulsed UV B exposure (Puv). Remarkably, Puv induced substantially higher sinapyl and flavonol (S+F) accumulation than Cuv, whereas adaptive growth attenuation was alleviated. During Puv treatment, transcript level of AtMYB4 (a repressor-type R2R3-MYB transcription factor) displayed a conspicuous pulse regime-related pattern. In a myb4 mutant, Puv and Cuv caused identical accumulation of S+F, reaching the same value as observed in Puv-treated WT plants. These observations and the ability of AtMYB4 to repress AtMYB12- or AtMYB111-mediated activation of target gene promoters (pCHS, pFLS) indicate that in WT plants the impact of recovery periods is mediated by alleviated AtMYB4 feedback control. The observed benefit from Puv is expected to stimulate a reevaluation of commercial plant production practices.

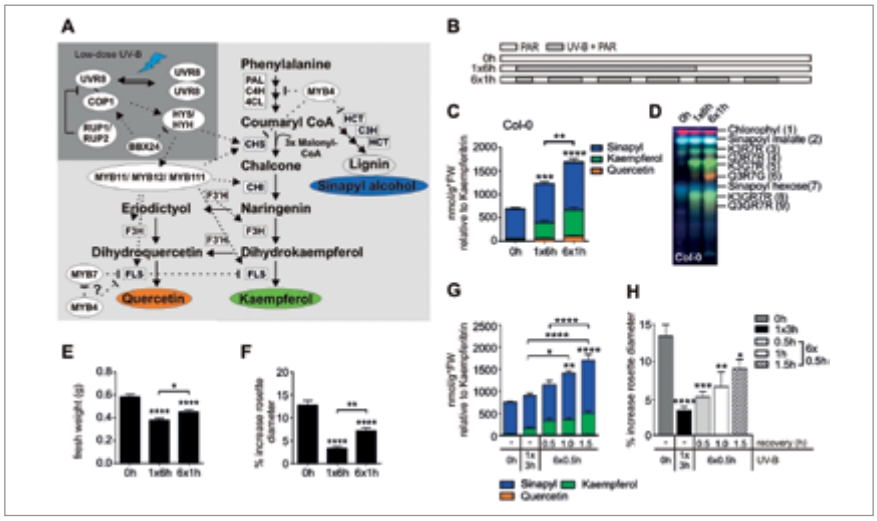

Figure 1
UV-B stress on Arabidopsis: Multiple (pulsed) stress exposure boosts accumulation of secondary metabolites

First identification of chicory R2R3-MYB transcription factors selectively activating promoters of fructan exohydrolase genes
In the biennial Cichorium intybus, inulin-type fructans accumulate in the taproot during the first year. Upon cold or drought exposure, fructans are degraded by fructan exohydrolases, affecting inulin yield and degree of polymerization. While stress-induced expression of 1-FEH genes has been thoroughly explored, the transcriptional network mediating these responses had remained unknown. Several R2R3-MYB transcriptional regulators were analyzed for their possible involvement in 1-FEH regulation. CiMYB3 and CiMYB5 selectively enhanced promoter activities of fructan exohydrolase genes (1-FEH1, 1-FEH2a

and 1-FEH2) without affecting promoter activities of fructosyltransferase genes. CiMYB5 displayed co-expression with its target genes in response to different abiotic stress and phytohormone treatments. The results indicate that CiMYB5 and CiMYB3 act as positive regulators of the fructan degradation pathway in chicory.

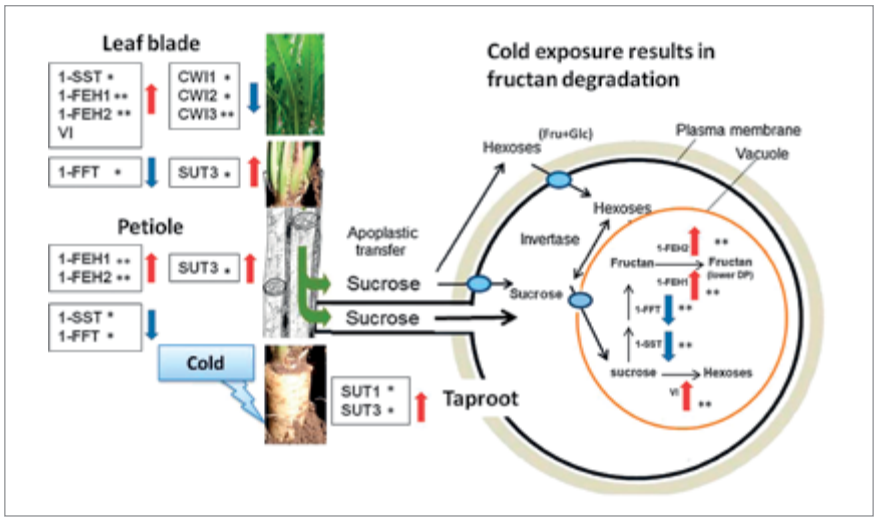

Figure 2
Cold stress-induced degradation of fructans in chicory taproot

Regulation of secondary cell wall biosynthesis and patterning by a NAC transcription factor from Miscanthus
We have identified a *Miscanthus* transcription factor related to SECONDARY WALL-ASSOCIATED NAC DOMAIN1, which acts as a master switch for the regulation of secondary cell wall formation and lignin biosynthesis. Ms*SND1* is expressed in tissues undergoing secondary cell wall formation, together with its potential targets. Consistent with this observation, Ms*SND1* was able to complement the secondary cell wall defects of the *Arabidopsis snd1 nst1* double mutant, and transient expression of Ms*SND1* in tobacco leaves was sufficient to trigger patterned deposition of cellulose, hemicellulose and lignin reminiscent of xylem elements. MsSND1 was shown to regulate directly and indirectly the expression of a broad range of genes involved in secondary cell wall formation, including MYB transcription factors that regulate only a subset of the SCW differentiation program. Our results indicate that MsSND1 is a transcriptional master regulator orchestrating secondary cell wall biosynthesis in *Miscanthus*.

An imposed glutathione-mediated redox switch primes for defense against bacterial pathogens
We have explored the impact of elevated GSH content on the cytosolic redox potential and on early defense signaling at the level of mitogen-activated protein kinases (MAPKs), as well as on subsequent defense reactions. Wild-type (WT) *Nicotiana tabacum* L. and transgenic high-GSH lines (HGL) were transformed with the cytosol-targeted sensor GRX1-roGFP2 to monitor the cytosolic redox state. Unexpectedly, HGLs displayed an oxidative shift in their cytosolic redox potential and an activation of two MAPKs (WIPK, SIPK). This activation occurred in the absence of change in free SA content, but was accompanied by constitutive up-regulation of defense genes. When HGL plants were challenged with adapted or non-adapted *Pseudomonas syringae* pathovars, the cytosolic redox shift was further amplified and the defense response was increased, indicating a priming effect. The results revealed that in tobacco MAPK and SA signaling may operate independently, both possibly being modulated by the GSH redox potential.

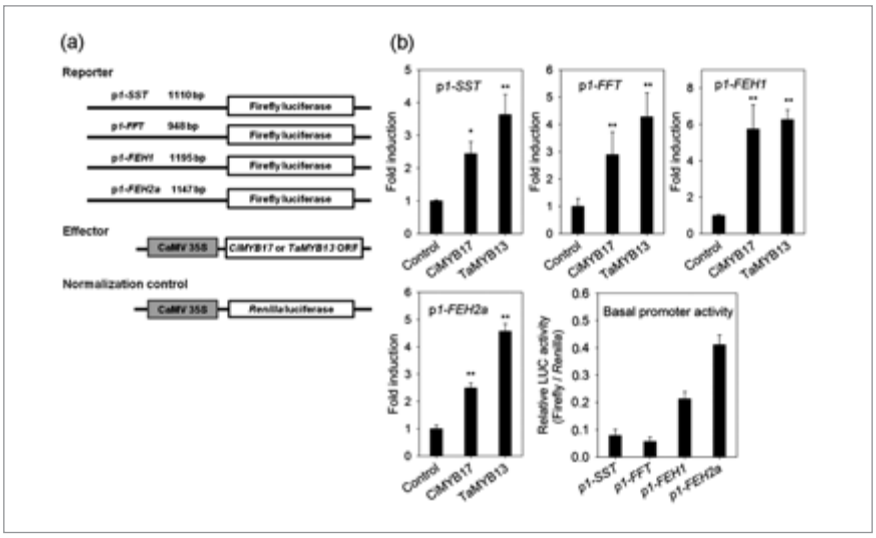

Figure 3
Chicory R2R3-MYB transcription factor CiMYB17 activates promoters of fructosyltransferase genes *(1-SST, 1-FFT)* and fructan exohydrolase genes *(1-FEH1, 1-FEH2a/b)*

Planned research and new directions

For the period 2017-2020 (retirement in 2020), the focus will be on the following research lines: i) Chicory: Exploring the interaction of R2R3-MYB TFs with other stress-mediating TFs (AP2/ERF superfamily) during fructan synthesis and degradation; ii) *Miscanthus*: Elucidating the interplay of activating and repressing TFs in the control of target genes involved in lignin biosynthesis; iii) Identification of genes encoding enzymes for stilbene metabolism in grapevine; iv) Boosting plant secondary metabolism by pulsed stress exposure regimes: Transfer of gained knowledge from Arabidopsis to vegetable crops and medicinal plants.

Fructan metabolism in chicory: The fruitful collaboration with our industrial partner (Südzucker AG) on regulatory aspects of fructan metabolism in chicory will be continued. First results indicate that in addition to the characterized R2R3-MYB TFs, members of the AP2/ERF superfamily are involved in stress-mediated regulation of fructan-active enzymes (FAZYs: fructosyltransferases and fructan exohydrolases). This work will include a comparison of different chicory genotypes (i.e. high/low inulin yield and/or degree of polymerization).

Lignin biosynthesis in *Miscanthus*: The regulation of lignin biosynthesis in *Miscanthus sinensis* via a multilayered TF network, launched in 2015 with support from the MWK (Bioeconomy BW consortium), will be further explored by focusing on the impact of environmental factors and genotype. Also, the role of repressor-type R3R3-MYB TFs will be addressed (MsMYB31/42). With respect to metabolic target genes, the role of different laccase isoforms will be investigated.

Stilbene metabolism in grapevine: The goal is to identify additional target genes of transcription factors VvMYB14 (or VvMYB15) assumed to encode enzymes for stilbene modification, transport or metabolism. Regulation of these genes and functional characterization of the encoded proteins will be explored in collaboration with the research group of Prof. Dr. Jochen Bogs (DLR-Rheinpfalz, guest professor at COS).

Boosting secondary metabolism via pulsed stress exposure: As our exploratory study on the impact of pulsed stress exposure regime on secondary metabolism in Arabidopsis has revealed an increased secondary metabolite accumulation, the potential of this approach will now be evaluated for selected vegetable crops and medicinal plants. A first study has been initiated with *Catharanthus roseus* (effect of pulsed UV-B exposure on catharanthine accumulation).

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 12, number of citations 2013-2017: 140, h-index (2013-2017): 5, total h-index: 34 (according to Thomson Reuters).

Wei, H., Zhao, H., Su, T., Bausewein, A., Greiner, S., Harms, K., and Rausch, T. (2017) Chicory R2R3-MYB transcription factors CiMYB5 and CiMYB3 regulate fructan 1-exohydrolase expression in response to abiotic stress and hormonal cues. Journal of Experimental Botany, erx210, https://doi.org/10.1093/jxb/erx210 2017

Wei H, Bausewein A, Greiner S, Dauchot N, Harms K, Rausch T (2017) CiMYB17, a stress-induced chicory R2R3-MYB transcription factor, activates promoters of genes involved in fructan synthesis and degradation. New Phytologist 215 (1), 281–298.

Peskan-Berghöfer T, Vilches-Barro A, Müller TM, Glawischnig E, Reichelt M, Gershenzon J, Rausch T (2015) Sustained exposure to abscisic acid enhances the colonization potential of the mutualist fungus Piriformospora indica on Arabidopsis thaliana roots. New Phytologist 208 (3), 873-886.

Matern S, Peskan-Berghoefer T, Gromes R, Vazquez Kiesel R, Rausch T (2015) Imposed glutathione-mediated redox switch modulates the tobacco wound-induced protein kinase and salicylic acid-induced protein kinase activation state and impacts on defense against Pseudomonas syringae. J Exp Botany 66 (7), 1935-1950.

Höll J, Vannozzi A, Czemmel S, D'Onofrio C, Walker AR, Rausch T, Lucchin M, Boss PK, Dry IB, Bogs J (2013) The R2R3-MYB transcription factors MYB14 and MYB15 regulate stilbene biosynthesis in Vitis vinifera. Plant Cell 25 (10), 4135-4149.

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Fields of Interest
Fructan and sucrose metabolism, greenhouse gas production in plants, Cell wall polysaccharides

Brief summary of work since 2013
About 15 % of all higher plants accumulate fructans, which perform multiple physiological functions *in planta*, but they are also a valuable commodity for humans with a broad range of applications. In a collaborative research effort with the Südzucker AG, we explore the molecular physiology of sucrose and fructan metabolism in chicory, a fructan-synthesizing higher plant. During the growth season, chicory accumulates linear fructans (inulin-type) in its taproot, providing the primary industrial source for inulin production. The goals of this project are (i) to gain new insight into the molecular mechanism behind the dynamic regulation of fructan pool sizes and composition, and (ii) to provide relevant information for chicory breeding towards improved inulin content and composition.

Major contributions since 2013

The expression of Fructan Active Enzymes, Cell Wall Invertases and Sucrose Transporters is linked to Fructan profiles in growing taproot of Cichorium intybus (Chicory) and depends on hormonal and environmental cues (Wei et al., 2016)
In chicory taproot, the inulin-type fructans serve as a carbohydrate reserve. Inulin metabolism is mediated by fructan active enzymes (FAZYs): sucrose:sucrose 1-fructosyl-transferase (1-SST; fructan synthesis), fructan:fructan-1-fructosyltransferase (1-FFT; fructan synthesis and degradation), and fructan 1-exohydrolases (1-FEH1/2a/2b; fructan degradation). In developing taproot, fructan synthesis is affected by source-to-sink sucrose transport and sink unloading. We determined the expression of FAZYs, sucrose transporter (SUT) and cell wall invertase (CWI) isoforms, vacuolar invertase and sucrose synthase in leaf blade, petiole, and taproot of young chicory plants and compared with taproot fructan profiles for the following scenarios: (i) N-starvation, (ii) abscisic acid (ABA) treatment, (iii) ethylene treatment (via ACC), and (iv) cold treatment. Both N-starvation and ABA treatment induced an increase in taproot oligofructans. However, while under N-starvation this increase reflected *de novo* synthesis, under ABA treatment gene expression profiles indicated a role for both *de novo* synthesis and degradation of long-chain fructans. Distinct SUT and CWI expression profiles were observed, indicating a functional alignment of SUT and CWI expression with taproot fructan metabolism under different source-sink scenarios.

CiMYB17, a stress-induced chicory R2R3-MYB transcription factor, activates promoters of genes involved in fructan synthesis and degradation (Wei et al., 2017)
On the level of regulatory networks the role of R2R3-MYB transcription factors in FAZY regulation was explored via bioinformatic identification of R2R3-MYBs (using RNAseq database), studies of co-expression of these factors with target genes, *in vivo* transient transactivation assays of FAZY target promoters (dual luciferase assay), and a yeast one-hybrid assay investigating the specificity of the binding of these factors to cis-elements.

The chicory MYB transcription factor CiMYB17 activated promoters of 1-SST and 1-FFT by binding to a specific DNA-motif. Unexpectedly, CiMYB17 also activated promoters of fructan exohydrolase genes. The stimulatory effect on promoter activities of sucrose transporter and cell wall invertase genes points to a general role in regulating the source-sink relationship.

Co-induction of CiMYB17 with 1-SST and 1-FFT (and, less consistently, with 1-FEH1/2) in nitrogen-starved or abscisic acid (ABA)-treated chicory seedlings and in salt-stressed chicory hairy roots supports a role in stress-induced fructan metabolism, including *de novo* fructan synthesis and trimming of pre-existing fructans, whereas the reduced expression of CiMYB17 in developing taproots excludes a role in fructan accumulation under normal growth conditions.

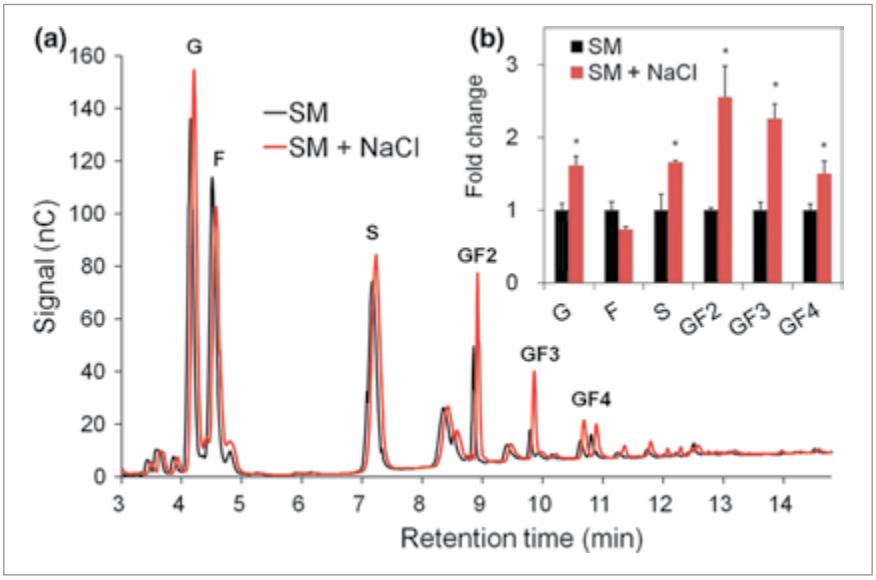

Figure 1
Impact of salt treatment (100 mM NaCl) on the fructan composition of chicory hairy root cultures (CiHRCs). (a) Representative sugar and fructan profiles of control (standard medium (SM)) and 24-h salt-treated (SM + 100 mM NaCl) CiHRC samples. (b) Quantitative analysis of short-chain fructans from control and salt-treated hairy roots: glucose (G), fructose (F), sucrose (S), 1-kestotriose (GF2), 1-1-kestotetraose (GF3) and 1,1,1-kestopentaose (GF4).

Abiotic methanogenesis from organosulphur compounds under ambient conditions (Althoff et al., 2014)
We also perform a research project on methane (CH_4) formation in plants. This reaction obviously takes place under aerobic conditions in contrast to the long known methane formation by microbes that live in oxygen-free environments. In this context we proposed a mechanism of abiotic methanogenesis from organosulphur compounds under ambient conditions. This novel chemical route via sulphoxidation of methyl sulphides is ubiquitous in the environment, and might mimic methane formation in living aerobic organisms.

Planned research and new directions
Future research will focus on regulatory aspects of fructan metabolism, mainly on transcriptional control of FAZY gene expression. As first results indicate that in addition to the characterized R2R3-MYB TFs, also members of other transcription factor families (e. g. the AP2/ERF superfamily) are involved in stress-mediated regulation of FAZYs, we will include those in future analysis. Future work will include TF network analysis with chicory plants grown under field conditions as well as comparison of different chicory genotypes with a focus on the traits inulin yield and degree of inulin polymerization.

Results from this research will provide fundamentally new insight into fructan metabolism and may open new routes for biotechnology.

The studies on production of the greenhouse gas methane under aerobic conditions by plants will be extended to the greenhouse gas nitrous oxide (N_2O) which also is readily produced under aerobic conditions in plants. Studies starting with different plant species from *in vitro* culture up to experiments on the ecosystem level (in cooperation) will help to estimate the proportion of plant N_2O production on global natural terrestrial sources.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 9, number of citations 2013-2017: 54, h-index (2013-2017): 3, total h-index: 20 (according to Thomson Reuters).

Wei, H., Zhao, H., Su, T., Bausewein, A., Greiner, S., Harms, K., and Rausch, T. (2017) Chicory R2R3-MYB transcription factors CiMYB5 and CiMYB3 regulate fructan 1-exohydrolase expression in response to abiotic stress and hormonal cues.

Journal of Experimental Botany, erx210, https://doi.org/10.1093/jxb/erx210 2017

Wei, H., Bausewein, A., Greiner, S., Dauchot, N., Harms, N., and Rausch, T. (2017) CiMYB17, a stress-induced chicory R2R3-MYB transcription factor, activates promoters of genes involved in fructan synthesis and degradation New Phytologist 215 (1), 281-298

Wei, H., Bausewein, A., Steininger, H., Su, T., Zhao, H., Harms, K., Greiner, S., and Rausch, T., (2016) Linking Expression of Fructan Active Enzymes, Cell Wall Invertases and Sucrose Transporters with Fructan Profiles in Growing Taproot of Chicory (Cichorium intybus): Impact of Hormonal and Environmental Cues Fontiers in Plant Science 7

Wolf, S., van der Does, D., Ladwig, F., Sticht, C., Kolbeck, A., Schürholz, AK., Augustin, S., Keinath, N., Rausch, T., Greiner, S., Schumacher, K., Harter, K., Zipfel, C., and Höfte, H. (2014) A receptor-like protein mediates the response to pectin modification by activating brassinosteroid signaling

PNAS 111(42): 15261-15266

Althoff, F., Benzing, K., Comba, P., McRoberts, C., Boyd, D.R., Greiner, S., and Keppler, F. (2014). Abiotic methanogenesis from organosulphur compounds under ambient conditions. Nature Communications 5.

2.21 PROF. DR. KARIN SCHUMACHER
PLANT DEVELOPMENTAL BIOLOGY

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Fields of Interest
pH- and ion-homeostasis, proton-pumps, membrane transport, protein trafficking, vacuole biogenesis, genetically encoded sensors

Brief summary of work since 2013
The endomembrane system of higher plants has evolved several unique features that serve the needs imposed by their sessile lifestyle and reflect the plasticity of growth and development at the cellular level. The identity of the individual eukaryotic endomembrane compartments is not only characterized by their respective protein ensembles but also by their luminal pH. Luminal acidification is driven by the V-ATPase, a rotary nano-engine that energizes secondary active transport and is essential for diverse pH-dependent trafficking events in the secretory and endocytic pathways. Due to this dual function in transport and trafficking the V-ATPase is of pivotal importance for cellular homeostasis and over the past years, we have made substantial progress in understanding the structure, function and regulation of the V-ATPase in the model plant Arabidopsis. The trans-Golgi network/early endosome (TGN/EE), the central sorting hub for protein trafficking in higher plants and the vacuole are two compartments that are characterized by the presence of specific V-ATPase isoforms and we have made several major contributions to understanding their functional identity, dynamics and biogenesis. Moreover, we have implemented the use of genetically-encoded sensors for pH and Ca^{2+} and are making use of these tools in dissecting how the V-ATPase is integrated in diverse cellular and metabolic networks.

Major contributions since 2013

Vacuole biogenesis
The success of land plants is closely linked to the evolution of a large central vacuole that not only enables plants to buffer changes in the availability of essential nutrients and to detoxify the cytosol when challenged by harmful molecules but most importantly allows cells to fill large volumes at low metabolic cost. By taking up most of the cell volume vacuoles act as a hydrostatic skeleton that in combination with the cytosol and the cell wall provides turgor pressure, the driving force underlying cell growth and reversible changes in cell volume. Despite their manifold and essential function of vacuoles, the molecular mechanisms underlying their biogenesis have not been determined. We have shown that the ER plays a major role in vacuole biogenesis (Viotti et al., 2013) and have since established suitable tools that allow us to visualize and manipulate the formation of ER-derived provacuoles (Fig.1).

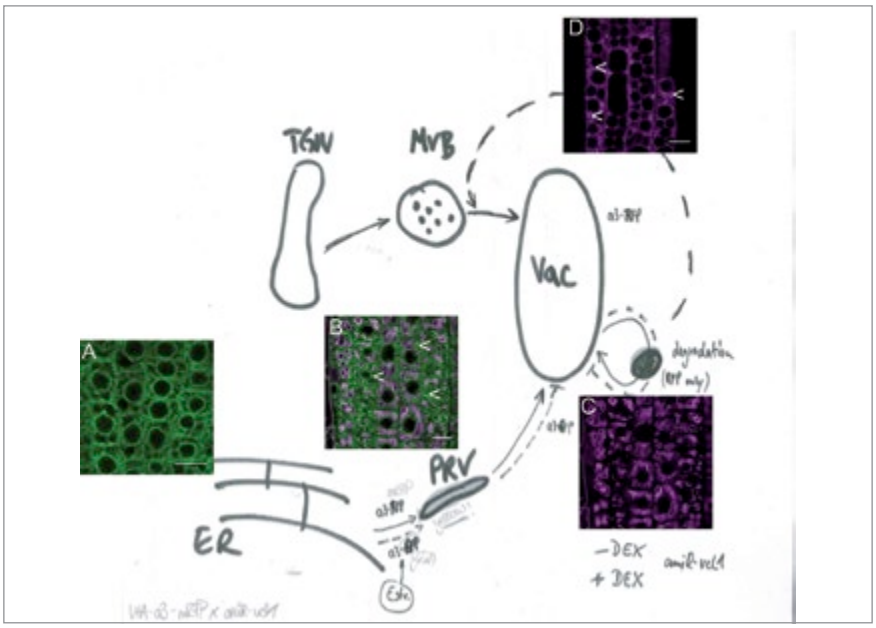

Figure 1
Vacuole biogenesis starts with a subdomain of the (A) ER being converted into a (B) provacuole (PRV, arrowheads) that eventually fuses with a pre-existing vacuole (C). Knock-down of a subunit of the HOPS-complex leads to depletion of VHA-a3-RFP from the tonoplast and accumulation in punctate structures (D, arrowheads) representing either provacuoles or degradative compartments.

Vacuolar proton-pumps and cold acclimation

The geographical distribution of plant species as well as the growth and yield of crop plants are limited by their tolerance to low temperatures. Whereas plants from temperate regions increase their freezing tolerance during exposure to low but non-freezing temperatures in a process termed cold acclimation, chilling-sensitive plants fail to do so. Damage to cellular membranes is the major cause of freezing injury in chilling-sensitive plants and inactivation of the vacuolar H^+-ATPase (V-ATPase) leading to cytosolic acidification has been reported to be one of the primary events after cold exposure. We have shown that cold acclimation in Arabidopsis involves an increase in V-ATPase activity that requires the presence and activity of a second enzyme, the vacuolar H^+-PPase (Kriegel et al., 2015) and might involve regulation by phosphorylation (Figure 2).

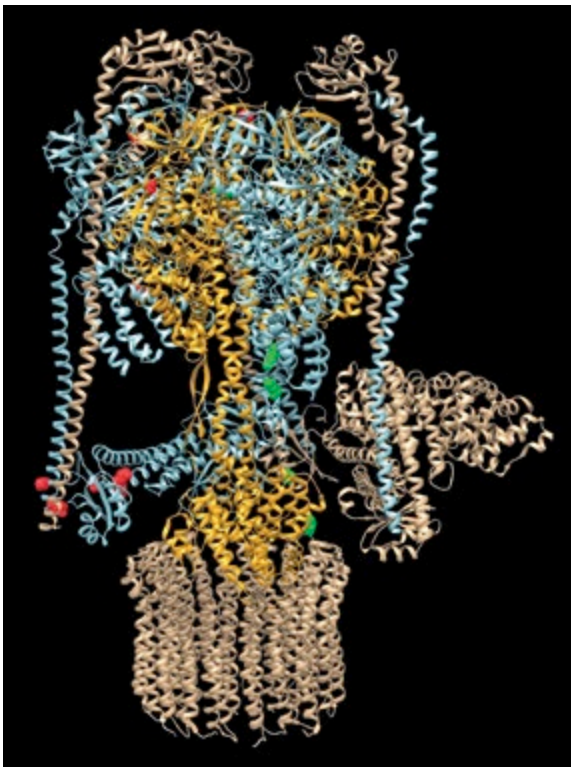

Figure 2
The phosphorylation status of the V-ATPase changes during cold acclimation. Residues with decreased phosphorylation after cold acclimation are highlighted in red, residues with increased phosphorylation are shown in green.

pH-homeostasis in the endomembrane system
Adaptation of the cellular protein-repertoire according to the developmental program and in response to environmental changes requires a highly specific targeting and trafficking machinery. In plants, the trans-Golgi network/early endosome (TGN/EE) is the central dynamic hub in which exo- and endocytic trafficking pathways converge and thus specificity of cargo routing needs to be achieved. The identity of the TGN/EE is determined by the presence and activity of the V-ATPase and luminal pH is a key determinant for protein trafficking (Figure 3, Luo et al., 2015). In this context we have identified the V-ATPase targeting domain in VHA-a1 and have shown that overexpression of this domain causes dominant-negative effects pointing to a pH-sensing function as well as a direct interaction with the core trafficking machinery. Moreover, we have shown that the precise TGN/EE-localization of the V-ATPase is dependent on the presence of two members of the ClC-family of anion transporters pointing to a crucial yet mechanistically unresolved connection between ion homeostasis, protein trafficking and membrane fusion. Together with the group of Ursula Kummer we have established a mathematical model that allows us to test predictions which players along with the V-ATPases are criticial for pH-homeostasis.

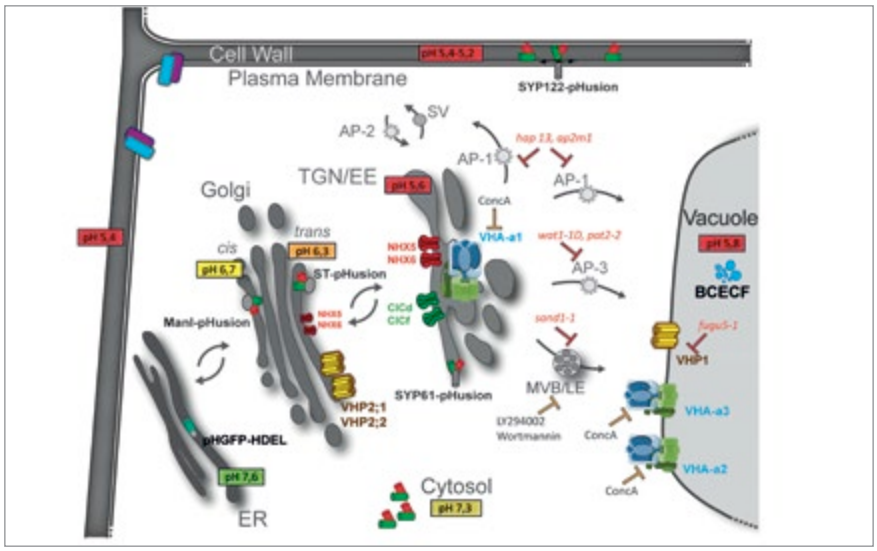

Figure 3
pH-map of the endomembrane system in Arabidopsis root cells as established by the use of genetically-encoded pH-sensors targeted to individual compartments or the use of a pH-sensitive dye in case of the vacuole.

Planned research and new directions

Goals for our research in the coming years are:
1. Mapping functional subdomains of the TGN/EE
Using super-resolution pH-imaging in combination with a comprehensive set of markers for subdomains of the TGN/EE will allow us to determine how such a highly dynamic compartment that directs much of the cells protein flux can maintain its identity by a core set of proteins that appears static.

2. Elucidate the mechanism of provacuole formation
We have proposed that provacuoles are derived from subdomains of the ER and will identify the molecular machinery involved in fission of provacuoles from the ER and fusion with pre-existing vacuoles by using proteomics as wells as forward and reverse genetic approaches.

3. Interplay of the vacuolar proton-pumps during cold acclimation
We will characterize the molecular interaction of V-ATPase and V-PPase during cold acclimation as well as the function of their reversible modification via S-acylation. Moreover, we are performing a comprehensive analysis of the role of vacuolar H^+-pumps during cold acclimation via genetically encoded sensors (pH and ATP/ADP ratio) as well as by metabolomics, proteomics and lipidomics approaches.

4. How does the vacuole control cell shape?
We have identified a molecular link between vacuoles and the cytoskeleton that is crucial for cell shape that dramatically affects tissue architecture of the leaf spongy mesophyll. The reduction of leaf size observed in mutants lacking vacuolar proton-pumps is not caused by the reduced cell size but by a dramatic reduction of intercellular space that reduces CO_2-diffusion. We will determine if tissue architecture is controlled by ambient CO_2-concentration and will perform a screen for mutants with reduced leaf air space.

Selected publications since 2013
Number of peer-reviewed articles 2013-2017: 15, number of citations 2013-2017: 2400, h-index (2013-2017): 10, total h-index: 35 (according to Thomson Reuters).

Kriegel, A., Andres, Z., Medzihradshzky, A., Krüger, F., Scholl, S., Delang, S., Patir-Nebioglu, G., Gute, G., Yang, H., Murphy, A., Peer, W. A., Pfeiffer, A., Krebs, M., Lohmann, J. U., Schumacher, K. (2015). Job Sharing in the Endomembrane System: Vacuolar Acidification Requires the Combined Activity of V-ATPase and V-PPase. Plant Cell 27: 3383–3396.

Luo, Y., Scholl, S., Doering, A., Zhang, Y., Irani, N. G., Di Rubbo, S., Neumetzler L., Krishnamoorthy, P., Van Houtte, I., Mylle, E., Bischoff, V., Vernhettes, S., Winne, J., Friml, J., Stierhof, Y.-D., Schumacher, K.*, Persson, S., Russinova, E. (2015). V-ATPase activity in the TGN/EE is required for exocytosis and recycling in Arabidopsis. Nature Plants 1, e15094.

Keinath, N. F., Waadt, R., Brugman, R., Schroeder, J. I., Grossmann, G., Schumacher, K., and Krebs, M. (2015). Live Cell Imaging with R-GECO1 Sheds Light on flg22- and Chitin-Induced Transient [Ca(2+)]cyt Patterns in Arabidopsis. Mol Plant 8, 1188–1200.

Andrés, Z., Pérez-Hormaeche, J., Leidi, E. O., Schlücking, K., Steinhorst, L., McLachlan, D. H., Schumacher, K., Hetherington, A. M., Kudla, J., Cubero, B., Pardo, J (2014). Control of vacuolar dynamics and regulation of stomatal aperture by tonoplast potassium uptake. Proc Natl Acad Sci USA 111, E1806–E1814

Viotti, C., Krüger, F., Krebs, M., Neubert, C., Fink, F., Lupanga, U., Scheuring, D., Boutte, Y., Frescatada-Rosa, M., Wolfenstetter, S., Sauer, N., Hillmer, S., Grebe, M., Schumacher, K. (2013). The endoplasmic reticulum is the main membrane source for biogenesis of the lytic vacuole in Arabidopsis. The Plant Cell, 25, 3434–3449.

PROJECT LEADER
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Fields of Interest
Ca^{2+} signaling, genetically-encoded fluorescent sensors, live cell imaging, vacuoles, proton pumps, membrane transport, pH homeostasis, nitrate homeostasis

Brief summary of work since 2013
Transient changes of cytosolic free Ca^{2+} concentrations ($[Ca^{2+}]_{cyt}$), represent a fundamental concept in signaling in all eukaryotic cells. In plants, the second messenger Ca^{2+} is associated with nearly all aspects of plant development and plant-environmental interactions. To facilitate the analysis of Ca^{2+} dynamics in plant cells, we optimised procedures for Ca^{2+} imaging in intact seedlings, which allowed us to identify so far unresolved single-cell Ca^{2+} oscillations in intact leaves. Moreover, we developed protocols to calibrate Ca^{2+} indicators in intact plant tissue which is critical to correlate $[Ca^{2+}]_{cyt}$ changes with the biochemical activity of the respective Ca^{2+} decoding proteins.

Major contributions since 2013
Fluctuating environmental conditions trigger diverse metabolic and developmental adaptation reactions in plants. The underlying signalling mechanisms rely on transient concentration changes of the ubiquitous second messenger Ca^{2+}. Although Ca^{2+} signalling is involved in nearly all aspects of plant development and plant-environmental interactions, the question is how multiple Ca^{2+} signals lead to diverse and specific output reactions. Specificity in Ca^{2+} signalling depends on multiple factors and starts already during signal encoding, as the duration, amplitude and frequency of the Ca^{2+} signal can be stimulus-dependent. In addition, specificity is conferred at the level of signal decoding that is mediated by a plethora of Ca^{2+} binding proteins that differ in their expression, localisation and Ca^{2+} binding affinities (Figure 1). The different properties of these Ca^{2+} sensors are critical for the differential signal processing and determine the specificity of Ca^{2+}signalling pathways. To understand how Ca^{2+} signalling regulates many physiological and developmental processes in plants, it is important to i) identify and characterise stimulus-coupled transient $[Ca^{2+}]_{cyt}$ patterns in intact tissues with high spatio-temporal resolution and ii) quantify absolute $[Ca^{2+}]_{cyt}$ changes to correlate $[Ca^{2+}]_{cyt}$ patterns directly with the activity of Ca^{2+} regulated target proteins. To address these important questions, we employed several strategies to analyse Ca^{2+} dynamics in plant cells using genetically-encoded fluorescent indicators (GEFIs) for Ca^{2+}.

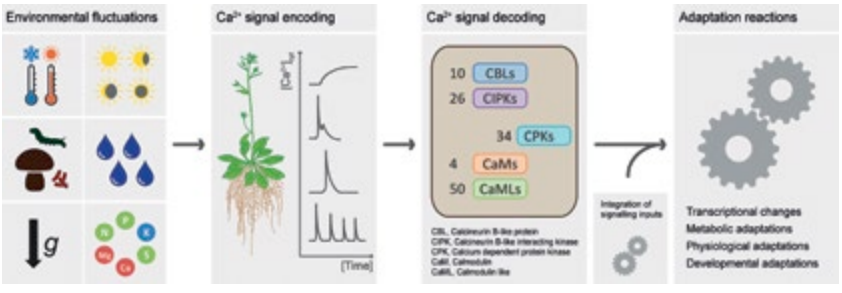

Figure 1
Ca^{2+} signalling in plant environmental interactions. Fluctuating environmental conditions trigger transient $[Ca^{2+}]_{cyt}$ changes that differ in amplitude and frequency in a stimulus-dependent way. These $[Ca^{2+}]_{cyt}$ signals are decoded by different classes of Ca^{2+} sensor proteins that translate the information into downstream responses which result in specific physiological and developmental adaptation reactions. Ca^{2+} signals are integrated with other signal transduction mechanisms that involve production of reactive oxygen species (ROS), pH changes, changes in hormone concentration and membrane potential changes. Depicted are numbers of different classes of Ca^{2+} sensor proteins from Arabidopsis.

We generated a robust imaging platform that allowed us to monitor GEFI changes in a minimally invasive manner and over a broad spatio-temporal range (Behera et al., 2013; Krebs and Schumacher, 2013; Krüger et al., 2013). Simultaneously, we established Ca^{2+} imaging with the red-emitting intensity-based Ca^{2+} indicator R-GECO1, which exhibits superior performance in Arabidopsis compared to previously used Ca^{2+} indicator Yellow cameleon 3.6 (YC3.6). Due to its increased sensitivity, we were able to visualise so far unresolved single-cell Ca^{2+} oscillations in intact leaves after stimulation with bacterial and fungal effector molecules (Keinath *et al.*, 2015). More recently, we fused intensity-based Ca^{2+} indicators to a reference fluorescent protein. With this approach, we generated ratiometric Ca^{2+} indicators that combine the high dynamic range of single-FP Ca^{2+} indicators with the advantages of ratiometric imaging. (Waadt *et al.* 2017). To be able to correlate $[Ca^{2+}]_{cyt}$ patterns with the activity of Ca^{2+} regulated target proteins we developed protocols for *in vivo* calibration of Ca^{2+} indicators in Arabidopsis root tips. Based on this, we are now able to quantify absolute $[Ca^{2+}]_{cyt}$ changes which is critical in order to place Ca^{2+} decoding proteins into a specific developmental program or a physiological signalling pathway (Waadt et al. 2017).

Planned research and new directions

In the past years, we have continuously optimized tools and protocols for Ca^{2+} imaging in plant cells. However looking at $[Ca^{2+}]_{cyt}$ changes alone is not enough to untangle complex signaling pathways. There is growing evidence that cellular signaling events involving Ca^{2+} as a second messenger are tightly interconnected with other signal transduction mechanisms that involve production of reactive oxygen species (ROS), pH changes, changes in hormone concentration and membrane potential changes (Figure 1). Therefore, our future efforts will focus on extending the repertoire of GEFIs to establish multiparameter imaging. This type of analyses allows to follow several signaling pathways in parallel and in real-time. Simultaneous analyses of Ca^{2+} and pH, ROS, hormones or electrical signals will help us to gain new insight into the order of events, the dynamics of interaction or the inter-dependent behavior of the respective signaling pathways. Implementation of this technique into mutants that lack vacuolar proton pumps or proton-coupled transporters will allow us to unravel their function in signaling and adaptation in response to biotic and abiotic stress conditions.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 9, number of citations 2013-2017: 192, h-index (2013-2017): 6, total h-index: 12 (according to Thomson Reuters).

Waadt, R., Krebs, M., Kudla, J. and Schumacher, K. (2017). Multiparameter imaging of calcium and abscisic acid and high-resolution quantitative calcium measurements using R-GECO1-mTurquoise in Arabidopsis. New Phytologist, in press.

Kriegel, A., Andrés, Z., Medzihradszky, A., Krüger, F., Scholl, S., Delang, S., Patir-Nebioglu, M.G., Gute, G., Yang, H., Murphy, A.S., et al. (2015). Job Sharing in the Endomembrane System: Vacuolar Acidification Requires the Combined Activity of V-ATPase and V-PPase. The Plant Cell 27, tpc.15.00733.

Wang, Y., Dindas, J., Rienmüller, F., Krebs, M., Waadt, R., Schumacher, K., Wu, W.H., Hedrich, R., and Roelfsema, M.R.G. (2015). Cytosolic Ca^{2+} Signals Enhance the Vacuolar Ion Conductivity of Bulging Arabidopsis Root Hair Cells. Molecular Plant 8, 1665–1674.

Keinath, N.F., Waadt, R., Brugman, R., Schroeder, J.I., Grossmann, G., Schumacher, K., and Krebs, M. (2015). Live Cell Imaging with R-GECO1 Sheds Light on flg22- and Chitin-Induced Transient $[Ca^{2+}]_{cyt}$ Patterns in Arabidopsis. Molecular Plant 8, 1188–1200.

Krebs, M., and Schumacher, K. (2013). Live cell imaging of cytoplasmic and nuclear Ca^{2+} dynamics in Arabidopsis roots. Cold Spring Harbor Protocols 2013, 776–780.

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GLYCOBIOLOGY

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Fields of Interest
Glycobiology, membrane and glycoproteins; protein O-mannosyltransferases and functions of O-mannosyl glycans of yeast and mammals; protein quality control in the endoplasmic reticulum; unfolded protein response; yeast cell wall; ER and cell wall stress response.

Brief summary of work since 2013
Glycosylation is an abundant posttranslational modification of proteins entering the secretory pathway. Among the diverse types of glycosylation, N-glycosylation and O-mannosylation are evolutionarily conserved, essential in fungi and animals, and underlie the pathophysiology of severe congenital disorders with diverse clinical presentations in humans. Using yeast and mammalian model systems our group aims to unravel the molecular mechanism of the initial steps of protein O-mannosylation in the endoplasmic reticulum (ER) and the molecular functions of O-mannosyl glycans. In the recent past, we showed that in baker´s yeast protein O-mannosyltransferases efficiently mannosylate nascent polypeptide chains which enter the ER through the Sec61 translocon complex. Protein O-mannosyltransferases interact with oligosaccharyltransferase which initiates N-glycosylation at the translocon and even compete for glycosylation acceptor sites of target proteins. To elucidate the full repertoire of O-mannosylated proteins we developed mass spectrometry-based approaches which allowed us to describe the first yeast O-mannose glycoproteome and resulted in the identification of new mammalian O-mannosylated proteins including the cell adhesion molecules cadherins. We further established poly- and monoclonal O-mannosyl glycan directed antibodies. Applying these novel »glyco«-tools, our group demonstrated that O-mannosylation is an abundant protein modification of the murine brain and impacts on cadherin-mediated cell adhesion during mouse embryonic development as well as in human gastric carcinoma. Very recent data indicate that the absence of protein O-mannosyltransferases affects N-glycosylation of cadherins suggesting a mutual influence of these types of glycosylation also in mammals.

Major contributions since 2013
Protein O-mannosylation is initiated at the lumenal side of the ER membrane, where the first mannose is transferred from an activated lipid-linked mannosyl donor to substrate glycoproteins (Figure 1). This reaction is catalyzed by a conserved family of protein O-mannosyltransferases (PMTs). In baker´s yeast O-mannosylation is the only type of protein glycosylation beside N glycosylation, making *Saccharomyces cerevisiae* an ideal eukaryotic model to study the molecular mechanism and functions of this essential posttranslational modification (reviewed in Loibl and Strahl, 2013). In yeast, secretory and membrane proteins can be N-glycosylated, O-mannosylated or both. We recently demonstrated that N-glycosylation and O-mannosylation can be initiated at the Sec61 translocon complex and that oligosaccharyltransferase and protein O-mannosyltransferases (PMTs) even compete for glycan acceptor sites (Loibl et al. 2014). In an important step towards the full understanding of protein O-mannosylation we established the first yeast O-mannose

glycoproteome. O-Mannosylated glycopeptides and glyco-sites were monitored by higher-energy collision-induced dissociation/electron-transfer dissociation (HCD/ET-D)-based MS/MS. We identified over 500 *O*-glycoproteins from all subcellular compartments for which over 2,300 *O*-mannosylation sites were mapped (Halim et al., 2015, Neubert et al., 2016). We discovered hitherto unknown O-mannosyl glycans on nuclear, cytoplasmic and mitochondrial proteins. The type of nucleocytoplasmic proteins and the localization of identified O-mannose residues resemble that of O-GlcNAcylation which is an essential process in all eukaryotic cells with the exception of yeast. Our results shed light on the longstanding conundrum how yeast survives without the nucleocytoplasmic O-GlcNAcylation system (Halim et al., 2015). Furthermore, we identified O-mannosyl glycans on 26% of the proteins that enter the secretory pathway and are targets of PMT-based O-mannosylation. For the vast majority of these proteins, O-mannosylation has been directly demonstrated for the first time. The analysis of these 293 proteins including 2221 individual O-mannosylation sites revealed a high complexity of this glycosylation (Neubert et al., 2016). In the close proximity of N-glycosylation acceptor sites, O-mannosylation is impeded. Using a recently established microsomal system (Loibl et al., 2014), we could verify the mutual influence of these modifications. Our finding that for instance major mediator and effector proteins of ER protein quality control are natural O-mannosylation targets provide an entirely novel view on the mutual dependence between protein glycosylation and folding, and adds a hitherto unrecognized layer of regulation in ER protein homeostasis (Neubert et al., 2016).

Protein O-mannosylation is an essential protein modification in fungi and animals. In human, it plays a critical role in the pathomechanisms of congenital muscular dystrophies with brain and eye malformation. The most severe forms such as Walker-Warburg-Syndrome are frequently caused by defects in the POMT1-POMT2 protein O-mannosyltransferase complex. In baker´s yeast the PMT family is redundant and the different members show specificity towards acceptor proteins (reviewed in Loibl and Strahl, 2013).

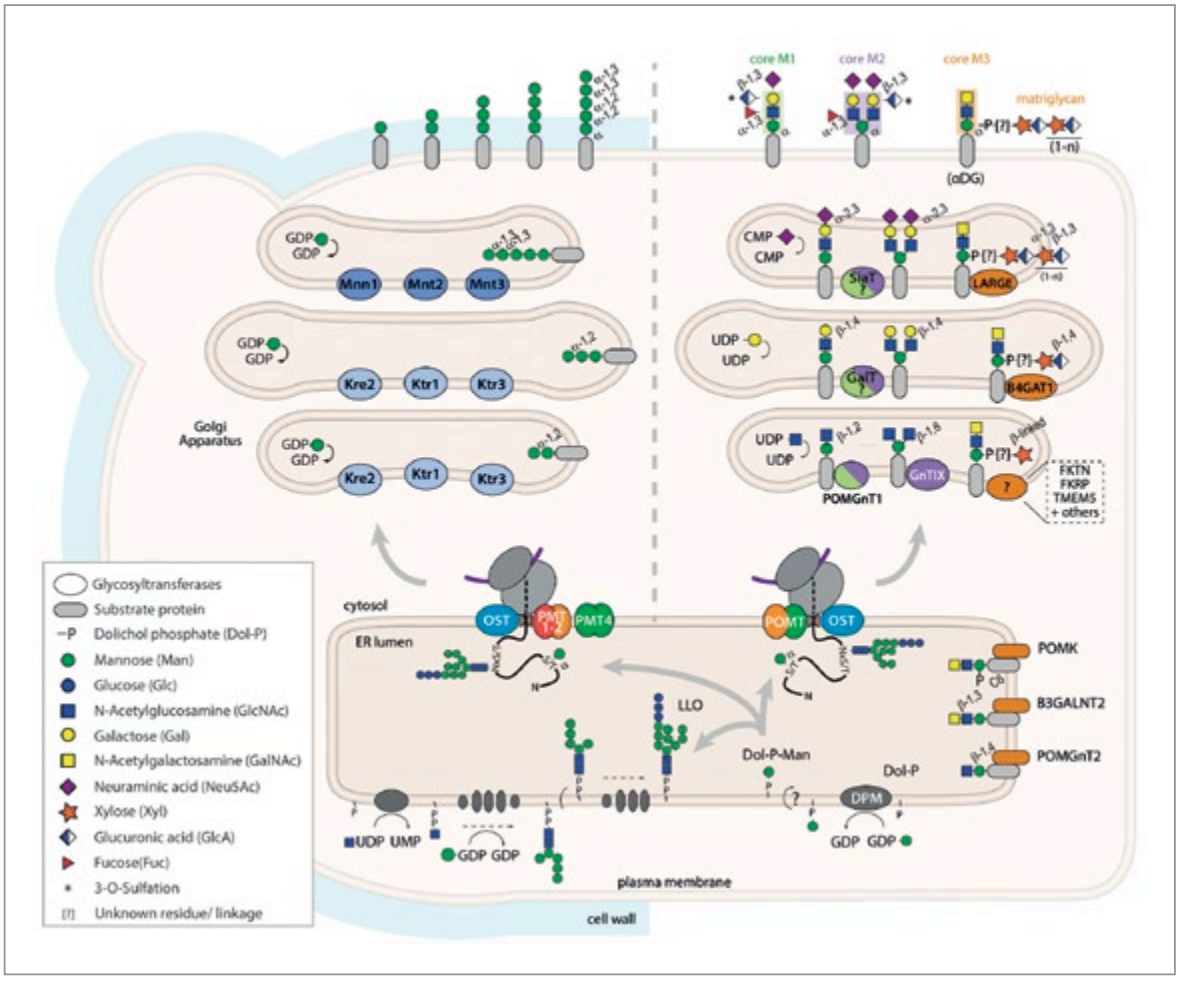

Figure 1
The biosynthetic pathway of *O*-mannosyl glycans in baker's yeast and mammals. From review Neubert and Strahl (2016).

We took advantage of *in vitro* O-mannosyltransferase activity assays specific for different PMT-family members and demonstrated major functional and biochemical similarities between the human POMT complex and the POMT1 orthologue from baker´s yeast, Pmt4. We mimicked Walker-Warburg-Syndrome -associated POMT1 amino acid exchanges in yeast Pmt4. *In vivo* and *in vitro* analyses proved the mutants catalytically inactive as their human counterparts. Our results define yeast Pmt4 as a model to study this class of essential enzymes (Bausewein et al., 2016).

A major bottleneck in the analysis of O-mannosylated peptides and proteins was the lack of O-mannosyl glycan directed antibodies. We established rabbit monoclonal antibodies directed against the epitope Thr(α1-Mannose) and verified the specificity (Bartels et al., 2016). Using this tool, we demonstrated that mono O-mannosyl glycans occur ubiquitously throughout the murine brain but are especially enriched at inhibitory GABAergic neurons (Figure 2) and at the perineural nets. In addition, our mass spectrometry-based approach led to the identification of glycoproteins from the murine brain that bear single O-mannose residues (Bartels et al., 2016). Among the candidates identified are members of the cadherin and plexin superfamilies and the perineural net protein neurocan. In addition, we identified neurexin 3, a cell adhesion protein involved in synaptic plasticity, as a new O-mannosylated glycoprotein. In combination, our results demonstrate that mono O-mannosyl glycans are a frequent protein modification at least in the murine brain.

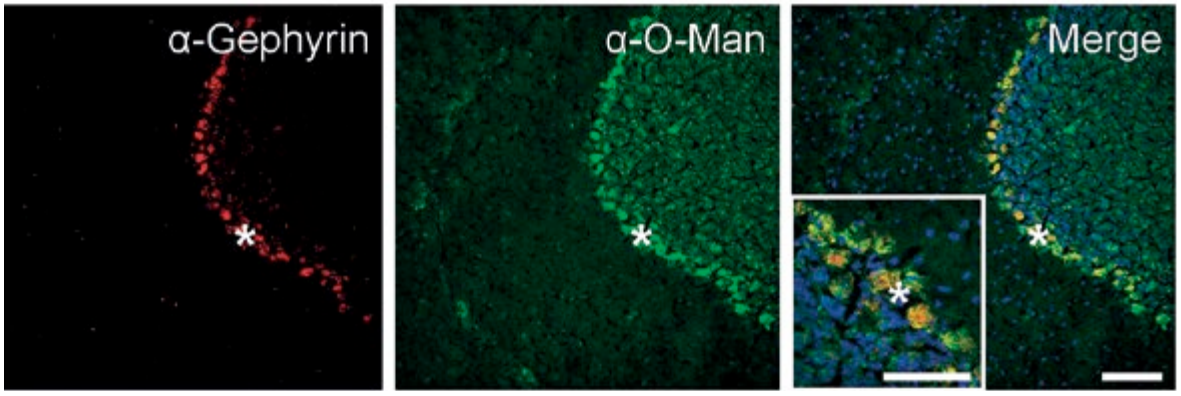

Figure 2
Mono O-mannosyl glycan staining of inhibitory neurons of the cerebellum from wild-type murine brain. Co-staining of gephyrin, an inhibitory synapse protein from GABAergic neurons and the α-O-Man antibody on cryosections. Scale bars = 50 µm. From: Bartels et al. (2016).

Taking advantage of the monoclonal Thr(α1-Mannose) directed antibody, we could establish a link between human cancer and O-mannosylation in collaboration with S. Pinho (University of Porto) (Carvalho et al., 2016). This study further revealed that the inhibition of mammalian protein O-mannosyltransferases negatively affects E-cadherin-mediated cell-cell adhesion, via unexpected crosstalk between O-mannosylation and the biosynthesis of individual N-glycan structures on this important adhesion molecule.

Planned research and new directions

Our recent work revealed a multi-layered coordination of O-mannosylation and N-glycosylation in yeast and mammals including humans. It is still unclear however, whether in mammals the observed effect is restricted to E-cadherin or whether N-glycosylation is generally affected by a decrease in O-mannosylation, and what mechanisms are behind this coordinated interplay. To address these questions, we now aim to study mammalian O-mannosylation and its relation to N-glycosylation at the operating, regulatory and functional levels. This work will performed in the frame of Research Unit 2509 »The concert of dolichol-based glycosylation: from molecules to disease models«, for which I am the coordinator.

Evidence is accumulating that in baker's yeast protein O-mannosylation is also crucial for ER quality control and the unfolded protein response. However, the molecular mechanism and components of the so-called unfolded protein O-mannosylation system are still poorly

understood. The fact that the O-mannose glycoproteome revealed major mediator and effector proteins of ER quality control and unfolded protein response on the side of PMT substrates potentially adds a hitherto unrecognized layer of regulation in ER homeostasis. Over the next few years we wish to unravel the role of protein O-mannosylation for ER quality control especially during ER stress conditions and gain a mechanistic understanding of the unfolded protein O-mannosylation pathway using baker´s yeast as a model (SFB 1036, project 11 »The role of protein O-mannosylation in the ER quality control and the unfolded protein response«).

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 11, number of citations 2013-2017: 106, h-index (2013-2017): 5, total h-index: 28 (according to Thomson Reuters).

Bartels MF, Winterhalter PR, Yu J, Liu Y, Lommel M, Möhrlen F, Hu H, Feizi T, Westerlind U, Ruppert T, and Strahl S. (2016) Protein O-Mannosylation in the Murine Brain: Occurrence of Mono-O-Mannosyl Glycans and Identification of New Substrates. PLoS One. 11, e0166119. doi: 10.1371/journal.pone.0166119.

Bausewein D, Engel J, Jank T, Schoedl, M, and Strahl, S. (2016) Functional similarities between the protein O-mannosyltransferases Pmt4 from baker's yeast and human POMT1. J Biol Chem. 291, 18006-18015. doi: 10.1074/jbc.M116.739128.

Neubert P, Halim A, Zauser M, Essig A, Joshi HJ, Zatorska E, Larsen IS, Loibl M, Castells-Ballester J, Aebi M, Clausen H, and Strahl S. (2016) Mapping the O-Mannose Glycoproteome in Saccharomyces cerevisiae. Mol Cell Proteomics 15, 1323-1337. doi: 10.1074/mcp.M115.057505.

Loibl M, Wunderle L, Hutzler J, Schulz BL, Aebi M, and Strahl S. (2014) Protein O-mannosyltransferases associate with the translocon to modify translocating polypeptide chains. J Biol Chem. 289, 8599-8611.

Lommel M, Winterhalter PR, Willer T, Dahlhoff M, Schneider MR, Bartels MF, Renner-Müller I, Ruppert T, Wolf E, and Strahl S. (2013). Protein O-mannosylation is crucial for E-cadherin-mediated cell adhesion. Proc Natl Acad Sci U S A. 110, 21024-21029.

2.23 PROF. DR. JOACHIM WITTBRODT
DEVELOPMENTAL BIOLOGY/PHYSIOLOGY

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Fields of Interest
Molecular and developmental genetics, population genomics, genome environment interactions, genetics of individuality, adult stem cells, eye development and differentiation, evolution, neuronal networks, transcriptional networks.

Brief summary of work since 2013
We delineated factors involved in retinal growth and regeneration and the underlying transcriptional networks. A particular focus was on the role of retinal stem and progenitor cells and their role in establishing and maintaining the perfect shape of the eye which is fundamental for its functional homeostasis. We have shown that the neuroretina is creating shape out of itself via a programmed behavior of neuroretinal stem cells. The pigmented epithelium on the other side arises from the same stem cell niche and passively follows the lead (and shape) implemented by the action of the neuroretinal stem cells. Our computational model for retinal growth is complemented by functional insights originating from clonal gain and loss of function analyses of key players governing the activity of retinal stem and progenitor cells. We have employed stochastic activation of transcriptional modules that couple *in vivo* indicators with the gain or loss of function of key pathway components (e.g. of wnt signaling).

Our massive progress in Crispr/Cas technology was instrumental for the establishment of genetically validated conditional paradigms that now allow addressing the acute loss of key players (e.g. Rx genes). Those are of high interest since they appear to facilitate life-long growth of the retina in teleosts and their targeted inactivation has furthered our understanding of vertebrate retinal size control. Another striking feature of teleost eyes retinae is their apparently unlimited regenerative capacity. We carefully compared different species and took advantage of the loss of retinal regeneration in medaka (similar to human) to identify key factors that, when targeted to Mueller glia cells in the retina, reinstate the regenerative capacity.

Major contributions since 2013
Addressing the role of individual key genes in retinal development, growth and regeneration we initially focussed on the level of the population (tissue, organ). However, we soon realized that the function is only understood, when analysed on the level of the individual cell in the context of the population.

Scaling that insight, we had been initiating a large project already 2005 in collaboration with Ewan Birney (EBI) and have identified an unstructured medaka population in collaboration with K. Naruse from the NIBB in Okazakai, Japan in 2010. This was the starting point for systematic inbreeding (Spivakov et al., 2014) in collaboration with Felix Loosli at KIT and we have now sequenced the genome more than 100 inbred lines (backcross generation 10 or higher, with E. Birney).

While the genetic resource was getting on its way, we in parallel established the resources for systematic quantitative analyses, with a focus on high resolution morphometrics (Figure 1), the heart, pharmacogenomics, stem cells and regeneration as well as behavioural analyses. We have established efficient and quantitative phenotyping pipelines adapted to high throughput.

To immediately tackle genome/environment interactions we will retrieve the transcriptomes of eight organs per line in two replicates of males and females for all 120 lines under summer and winter conditions. A total number of almost 8000 samples has been extracted and is currently prepared for quantitative analysis by RNA seq.

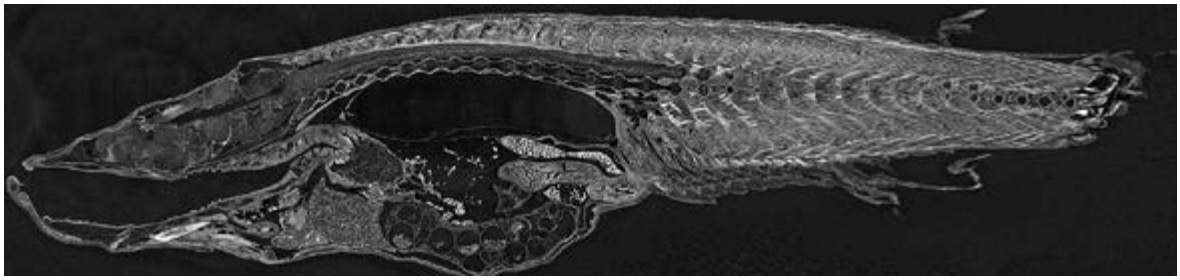

Figure 1
Micro CT analysis of an adult medaka male. Virtual sagittal section through a full 3D dataset of an adult medaka male fixed and stained with PTA. Note the excellent resolution also of soft tissues facilitating automated segmentation and morphometric analysis.

Role of Retina specific homeobox genes in defining retinal stem cells
The continuously growing retina in fish ideally facilitates addressing establishment and maintenance of a stem cell niche. We have addressed players that control retinal stem cells on the transcriptional level using the retina specific homeobox-containing transcription factor Rx2 as a proxy for retinal stem cells (Centanin et al., 2014). On the one hand, we had identified upstream regulators of Rx2, defining the identity of retinal stem cells (Reinhardt et al., 2015). On the other hand, we have tackled the role of Rx2 and developed and employed an improved iDamID protocol (Gutierrez et al., 2016) to identify downstream mediators of its activity. The partial functional redundancy due to the overlapping expression of its close paralog Rx1 and their pleiotropic function also at early stages of development has posed a serious challenge that we mastered by gain and loss of function studies followed by clonal analyses. Our results indicated that the modulation of Rx2 expression immediately impacts on the features of the affected cells. Rx2 overexpression even drives even differentiated retinal neurons into a stem cell like fate. In contrast, the repression or Rx2 within stem cells triggers their arrest and sends them into dormancy. We generated and analyzed Rx2, Rx1 and Rx1/2 mutants that now allow to validate the Rx2 downstream cascade of direct target genes.

Clonal analysis of wnt signaling
Life-long proliferation and differentiation of retinal progenitor cells into the proper numbers of terminally differentiated neurons of the mature retina allows addressing the tight coordination of both, proliferation and differentiation of progenitor cells. Here we addressed the impact of the Wnt signaling pathway by clonal interference and quantitation of downstream reporters. This allowed delineating the relative contribution of canonical Wnt signaling to proliferation and differentiation of specific progenitor cells along the differentiation cascade. The quantitative analysis will contribute to our model of retinal growth and differentiation to predict and test the impact of Wnt signaling in other contexts.

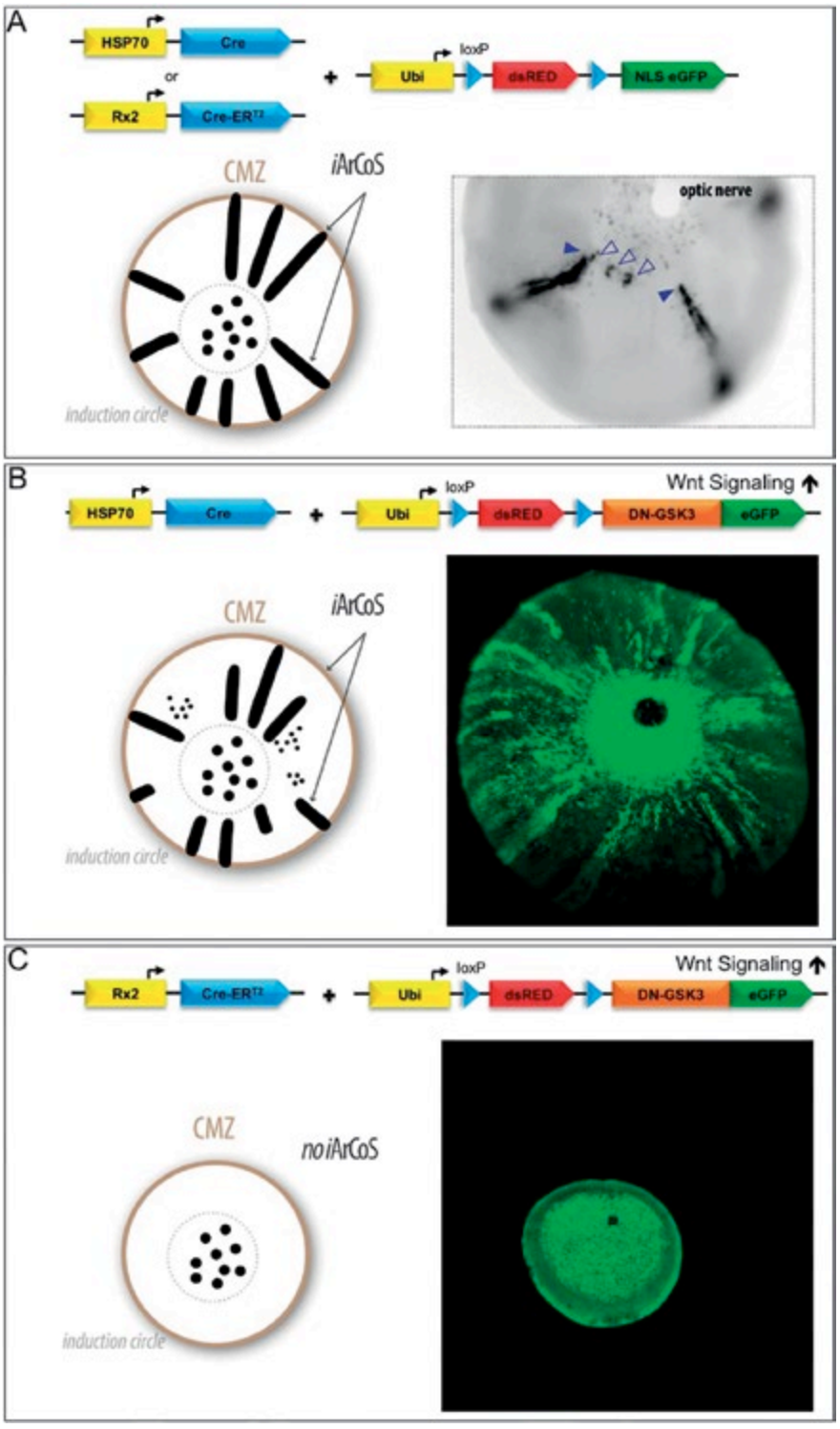

Figure 2
Impact of clonal gain of wnt signaling on ArCoS shape. A) Upon stochastic heat shock or tamoxifen induced recombination of the Gaudi-cassette (red switch green; RSG), three events can be observed: 1) terminally differentiated cells in the central retina will shift their color (salt and pepper staining close to the optic nerve). In the absence of proliferation, cells and GFP will remain static during growth. 2) Progenitor cells are apparent in response to heat shock Cre activation and leave a footprint (open arrowhead) due to their limited proliferative potential. 3) ArCoS arise from recombination events in stem cells of the CMZ (arrowheads). These continuous lines increase slowly but consistently in their width and length to cope with circumferential and axial growth of the eyecup. B) Upon ubiquitous heat shock induced upregulation of Wnt signaling by stochastic activation of a dominant negative GSK3 more ArCoSs and footprints arise. ArCoSs expressing DN-GSK3 often terminate earlier and start later compared to wt-ArCoSs. C) ArCoSs cannot be induced when Wnt is activated in retinal stem cells (by Rx2::CreERT2). Activation of Wnt in the photoreceptors (second domain of rx2), however, did not alter their fate and served as recombination control (Möller and Wittbrodt unpublished). Data on wt-ArCoS adapted from (Centanin et al., 2014)

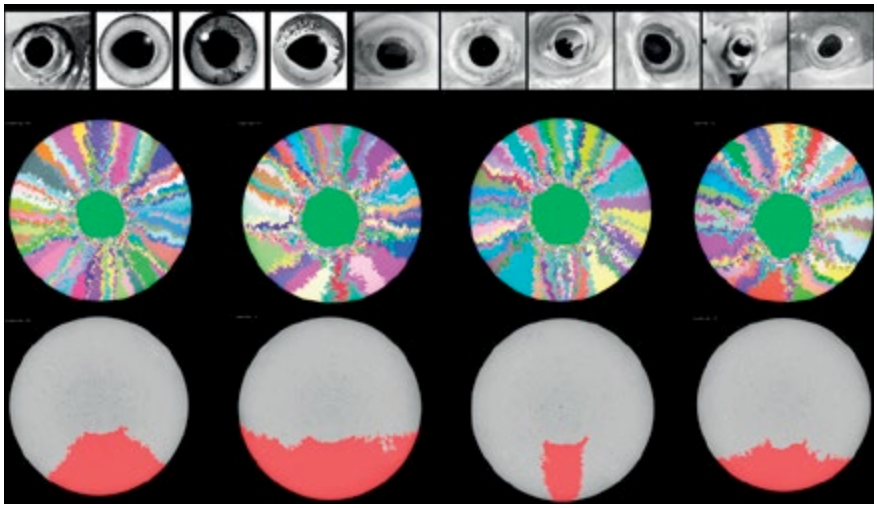

Figure 3
Altered stem cell division properties in the ventral NR affect retinal topology. Schematic illustrating how the position of the embryonic retina shifts during growth. Upper panels: Simulation examples embryonic retina in green. Lower panels: modulation of division plan and number of cell cycles shifts embryonic retina. Red area outlines cells affected by modulation.

Modeling of 3D retinal growth

Morphogenesis of complex organs requires coordination of anatomically and functionally distinct tissues. The precise 3D shape required for a functioning eye makes the eyes of teleosts an ideal system to explore how tissues coordinate growth and maintain the shape of an organ in functional homeostasis. Here, neural retina (NR) and retinal pigmented epithelium (RPE) arise from distinct stem cells in a single bipartite stem cell niche, the ciliary marginal zone (CMZ). Differentiated retinal cells grow little in size, retain their position without cell rearrangement, and have negligible death rates. Therefore, the stem cells in the CMZ must coordinate proliferation rates with organ growth. However, it was unknown how this coordination occurs and whether stem cells play a role in maintaining organ shape during post-embryonic growth. By comparing clonal lineage data in three concentric eye tissues of the teleost medaka (*Oryzias latipes*) – NR, RPE, and the surrounding choroid – with computational simulations of a 3D agent-based model, we show that NR stem cells orchestrate both, growth and shape in post-embryonic eye morphogenesis. All three eye tissues follow distinct growth strategies; clonal patterns in the RPE and the choroid show they passively follow the lead of the NR. We highlight how a minimal target node for evolution – the proliferation of NR stem cells – can be exploited to adapt whole-organ morphogenesis in a complex vertebrate organ.

Regeneration and repair

To identify key factors that confer regenerative capacities to non-regenerative species is of pivotal relevance for basic research and translational approaches. We have uncovered a differential response in retinal regeneration between medaka (*Oryzias latipes)* and zebrafish (*Danio rerio*). In contrast to zebrafish, medaka Müller glia (olMG) cells behave like progenitors and exhibit a restricted capacity to regenerate the retina. After injury, olMG cells proliferate but fail to self-renew and ultimately only restore photoreceptors.

In our injury paradigm, we observed that in contrast to zebrafish, proliferating olMG cells do not maintain *sox2* expression. Sustaining *sox2* expression in olMG cells confers regenerative responses similar to those of zebrafish MG (drMG) cells. Thus, a single, cell-autonomous factor reprograms olMG cells and establishes a regeneration-like mode. Our results position medaka as an attractive model to delineate key regeneration factors (e. g. Lust et al., 2016) with translational potential.

Planned research and new directions
In the coming years, I plan to merge the population genomics approaches and the studies on stem cells and regeneration to address their relevance in the context of environmental conditions. We will expand the panel in its width and will establish new and complementary inbred lines. In parallel we will integrate new features into the existing panel and will employ Crispr/Cas to engineer a unique and highly efficient PhiC31 landing site (Kirchmaier et al., 2013) into a fully accessible locus (UBI-one) in each of the 120 inbred lines. This will facilitate the use of established assays in a fully comparable context, in the absence of different position effects either in the individual fish lines or in cell lines derived from them.

We will take advantage of the population genomics resource and establish further high throughput phenotyping pipelines to quantify regenerative and stem cell related phenotypes. One particular focus will be on the interplay between stem cells and the immune system, an unexpected and tight interconnection that we recently uncovered.

Another novel aspect of our work in the present years related to the medaka heart and the contribution of genetic and environmental factors to heart function at post-embryonic stages. GWAS on heart phenotypes is most advanced and we will use the heart as model for validating the relative contribution of individual SNPs to the heart phenotypes. We will first take advantage of newly established tools to map regulatory elements from human to medaka (Dolle et al., 2015), in particular now incorporating the information provided by the sequenced genome of bridging species like the spotted gar.

Taken together our approaches will be directed towards ultimately allow addressing the relative contribution of multiple factors and will be a first step towards addressing the genetics of individuality in different contexts.

Selected publications since 2013
Number of peer-reviewed articles 2013-2017: 39, number of citations 2013-2017: 421, h-index (2013-2017): 13, total h-index: 44 (according to Thomson Reuters).

Collaborative manuscripts involving different COS groups are indicated by *

Inoue, D., Stemmer, M., Thumberger, T., Ruppert, T., Bärenz, F., Wittbrodt, J., and Gruss, O.J. (2017). Expression of the novel maternal centrosome assembly factor Wdr8 is required for vertebrate embryonic mitoses. Nat Commun *8*, 14090.

*Lust, K., Sinn, R., Pérez Saturnino, A., Centanin, L., and Wittbrodt, J. (2016). De novo neurogenesis by targeted expression of atoh7 to Müller glia cells. Development *143*, 1874–1883.

*Reinhardt, R., Centanin, L., Tavhelidse, T., Inoue, D., Wittbrodt, B., Concordet, J.-P., Martinez-Morales, J.-R., and Wittbrodt, J. (2015). Sox2, Tlx, Gli3, and Her9 converge on Rx2 to define retinal stem cells in vivo. Embo J *34*, 1572–1588.

*Heermann, S., Schütz, L., Lemke, S., Krieglstein, K., and Wittbrodt, J. (2015). Eye morphogenesis driven by epithelial flow into the optic cup facilitated by modulation of bone morphogenetic protein. Elife *4*.

Spivakov, M., Auer, T.O., Peravali, R., Dunham, I., Dolle, D., Fujiyama, A., Toyoda, A., Aizu, T., Minakuchi, Y., Loosli, F., Felix Loosli, Naruse, K., Birney, E., and Wittbrodt, J. (2014). Genomic and Phenotypic Characterization of a Wild Medaka Population: Towards the Establishment of an Isogenic Population Genetic Resource in Fish. G3 (Bethesda) *4*, 433–445.

PROJECT LEADER
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Fields of Interest
Aquatic ecology and toxicology; embryo toxicity; teratogenicity; genotoxicity; neurotoxicity; endocrine disruption; histopathology; cytopathology; river and lake restoration.

Brief summary of work since 2013
Efforts have been undertaken to reduce environmental contamination and to improve water quality. A huge body of regulations has been installed, and environmental compartments are now subject to regular control. However, despite such improvement, fish populations still show deficits. The reasons being manifold, specifically acting anthropogenic trace contaminants are being considered as candidate reasons. Since current surveillance is mainly based on acute toxicity testing and combination effects are likely to be overseen, there is a need to develop more subtle procedures to elucidate trace contaminants. Given that animal-free testing receives increasing attention, fish testing should be replaced by cell culture and embryo testing. Our major goal is to develop a test battery based on fish cells and embryos that allows the detection of multiple endpoints.

Major contributions since 2013
Teratogenicity testing with fish: Establishment and optimization of staining techniques to visualize cartilage and bone formation in early zebrafish development (Strecker et al. 2013). Investigations into effects of solvents and emulsifiers (Kais et al. 2013) as well as into the permeability of the zebrafish chorion (Pelka et al. 2017).

Neurotoxicity testing: Within BMBF-RiSKWa, Tox-Box was launched to enhance the existing GOW-concept through development of end point-related testing strategies (genotoxicity, neurotoxicity, germ cell damage, endocrine effects; Eckhardt et al. 2017, Grummt et al. 2013). Together with classical acetylcholinesterase testing (Kais et al. 2015), retina, olfactory epithelium and neuromasts were investigated as model for toxicity assessment (Braunbeck et al. 2015, Stengel et al. 2017).

Endocrine disruption: With the maturity index, a novel tool to quantify sex determination and sex reversal of fish (Baumann et al. 2013) has been established for female (Baumann et al. 2014a) and male fish gonads (Baumann et al. 2014b).

Biotransformation capacities in fish cells and fish embryos: Normalization of EROD activity to cellular metabolic activity by a modified MTT assay and introduction of metabolic cell equivalents based on MTT data rather than protein contents allowed to significantly improve EROD quantification and cytotoxicity testing in fish-based systems (Heinrich et al. 2014).

Genotoxicity testing: Two-generation exposure was used to elucidate the environmental relevance of genotoxic effects in fish (Faßbender & Braunbeck 2013).

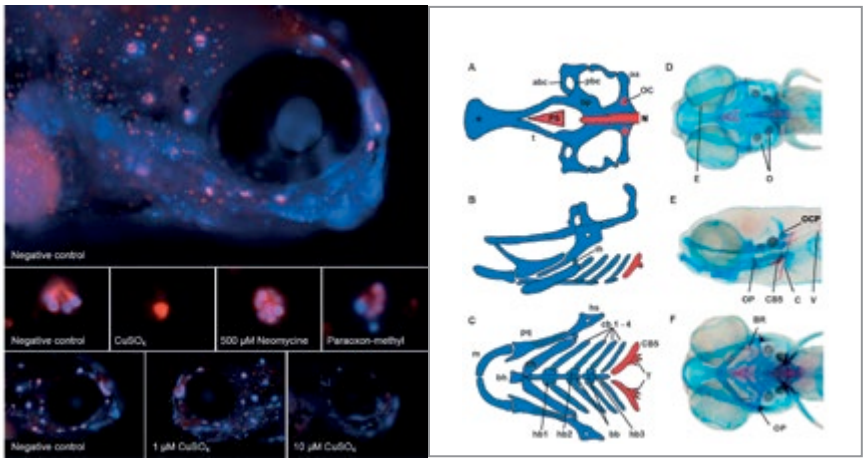

Figure 1
In vivo visualization of neuromasts in the head region of a 96 hpf zebrafish embryo: 30 min double-staining with 0.005 % DASPEI (pink) as a marker of mitochondria-rich cells and 0.1% DAPI (blue). Among potential positive controls (copper sulfate, neomycin, paraoxon-methyl), 96 h exposure to copper sulfate leads to the most prominent concentration-dependent decline in stainable neuromasts (from Braunbeck et al. 2015).

Figure 2
Zebrafish (Danio rerio) neurocranium (A), lateral view of all head cartilages (B) as well as the pharyngeal skeleton (C) at 144 hpf, modified from Kimmel et al. (2001). D – F show photomicrographs of stained zebrafish larvae focused to the corresponding cartilage and bone elements. Cartilages are stained blue (alcian blue) and bones red (alizarin red; from Strecker et al. 2013).

Sediment toxicity testing based on fish embryo toxicity testing: As a spin-off of former collaborations, screening programs based on zebrafish embryo testing have been implemented in Brazil and New Zealand (Bluhm et al. 2104, Boehler et al. 2017, Heinrich et al. 2017, Otte et al. 2013, Seiler et al. 2014, Schulze et al. 2014, 2015, Suarez-Rocha et al. 2015).

Substance-specific toxicity testing with fish cells and embryos focused on estrogenic compounds (Baumann et al. 2013, 2014a, b), polynuclear aromatic hydrocarbons (Heinrich et al. 2014, Otte eta. 2013, Seiler et al. 2014, 2015, Schulze et al. 2014) and microplastic particles (Batel et al. 2016).

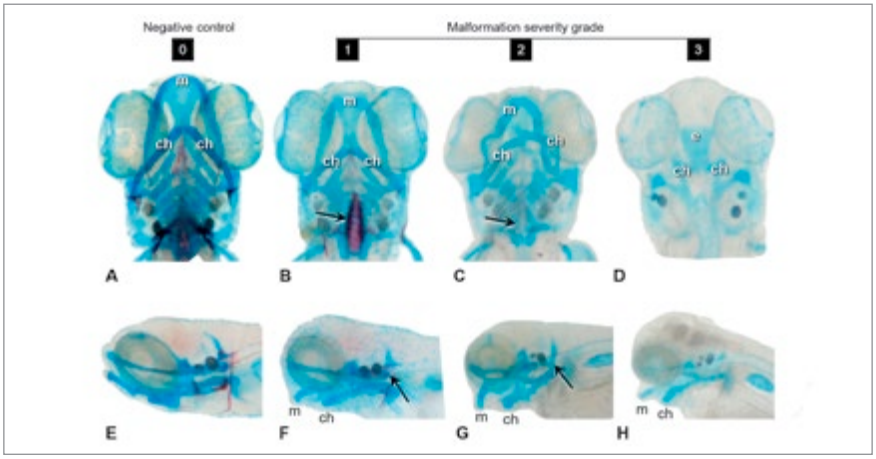

Figure 3
Photomicrographs of zebrafish (Danio rerio) head after exposure to various concentrations of disulfiram. Cartilages are stained blue (alcian blue) and bones red (alizarin red; from Strecker et al. 2013).

Planned research and new directions

Future directions of research will continue our efforts to develop the zebrafish embryo as a general model in ecotoxicology and toxicology. Further refinement of the fish embryo test will be directed towards attempts to at least partially replace teratogenicity testing with mammalian species. For this end, e.g., further investigations designed to more comprehensively describe the bioactivation potentials of fish embryos will be necessary. Additional endpoints to be incorporated into the fish embryo testing strategy cover more specific mechanisms of bioactivation, teratogenicity, neurotoxicity and immunotoxicity.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 28, number of citations 2013-2017: 239, h-index (2013-2017): 9, total h-index: 42 (according to Thomson Reuters).

Batel, A., Linti, F., Scherer, M., Erdinger, L. and Braunbeck, T. (2016). Transfer of benzo[a]pyrene from microplastics to *Artemia nauplii* and further to zebrafish via a trophic food web experiment – CYP1A induction and visual tracking pf persistent organic pollutants. Environ. Toxicol. Chem. 35, 1656-1666.

Braunbeck, T., Kais, B., Lammer, E., Otte, J., Schneider, K., Stengel, D. and Strecker, R.T. (2015). The fish embryo test (FET): origin, applications, and future. Environ. Sci. Pollut. Res. 22, 16247-16261.

Busquet, F., Strecker, R., Rawlings, J.M., Belanger, S.E., Braunbeck, T., Carr, G.J., Cenijn, P., Fochtman, P., Gourmelon, A., Hübler, N., Kleensang, A., Knöbel, M., Kussatz, C., Legler, J., Lillicrap, A., Martínez-Jerónimo, F., Polleichtner, C., Rzodeczko, H., Salinas, E., Schneider, K.E., Scholz, S., van den Brandhof, E.-J., van der Ven, L.T.M., Walter-Rohde, S., Weigt, S., Witters, H. and Halder, M. (2014). OECD validation study to assess intra- and inter-laboratory reproducibility of the zebrafish embryo toxicity test for acute aquatic toxicity testing. Reg. Tox. Pharm. 69, 496-511.

Otte, J.C., Keiter, S., Faßbender, F., Higley, E.B., Suares Rocha, P., Brinkmann, M., Wahrendorf, D.-S., Manz, W., Wetzel, M.A., Braunbeck, T., Giesy, J.-P., Hecker, M. and Hollert, H. (2013). Contribution of priority PAHs and POPs to Ah receptor-mediated activities in sediment samples from the River Elbe Estuary, Germany. PLoS ONE 8(10), e75596.

Scholz, S., Sela, E., Blaha, L., Braunbeck, T., Galay-Burgos, M., García-Franco, M., Guinea, J., Klüver, N., Schirmer, K., Tanneberger, K., Tobor-Kapłon, M., Witters, H., Belanger, S., Benfenati, E., Creton, S., Cronin, M.T.D., Eggen, R.I.L., Embry, M., Ekman, D., Gourmelon, A., Halder, M., Hardy, B., Hartung, T., Hubesch, B., Jungmann, D., Lampi, M.A., Lee, L., Marc Léonard, M., Küster, E., Lillicrap, A., Luckenbach, T., Murk, A.J., Navas, J.M., Peijnenburg, W., Repetto, G., Salinas, E., Schüürmann, G., Spielmann, H., Tollefsen, K.E., Walter-Rohde, S., Whale, G., Wheeler, J.R. and Winter, M.J. (2013). A European perspective on alternatives to animal testing for environmental hazard identification and risk assessment. Regul. Toxicol. Parmacol. 67, 506-530.

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Fields of Interest
Stem Cells and their Niches, Coordination of Growth Within and Among Vertebrate Organs, Lineage Relations, Phenotypic Variation, Fish Genetics and Metabolism.

Brief summary of work since 2013
Fish are growth machines: they grow during their entirely post-embryonic life, well beyond acquiring sexual maturation. Permanent growth comes with major challenges: new cells need to be incorporated to already functional organs, and the extent of growth of each organ has to match that of the entire fish. In my lab, we focus on post-embryonic stem cells and their coordination within and among different organs. We use the neuromasts of the lateral line and the fish gills to address stem cell mediated coordination at different scales. Our work on the neuromasts (Figure 1) explores the activity of stem cells in real time and in their endogenous niche. Our work on the gills (Figure 2) focuses on the short- and long-range coordination of independent stem cells.

Major contributions since 2013
Tools for lineage analysis. We have generated a living toolkit (Centanin et al., 2014) which consists of transgenic lines expressing LoxP-mediated fluorescent proteins (Gaudi^{RSG}, Gaudi^{BBW}, Gaudi^{LxBBW}), and inducible drivers (Gaudi$^{Ubiq.iCRE}$, Gaudi$^{Hsp70.Cre}$). These were used in collaboration with Jochen Wittbrodt to address stem cells in different organs, (Centanin et al. 2014, Lust et al. 2016, Aghaallaei et al. 2016).

Life-long organogenesis. The posterior lateral line (pLL) system adapts to the growing fish body by generating new neuromasts – new entire organs. We found that in medaka, individual cells leave the organ, group with cells coming from a neighbour neuromast and migrate to their final location to start differentiation (Seleit et al, 2017). We also found that, while most fish use two primordia to set up two pLLs, medaka utilise just one primordium to generate two parallel pLLs (Figure 1)(Seleit et al, 2017), which constitutes a fascinating model to explore the evolution of developmental modules.

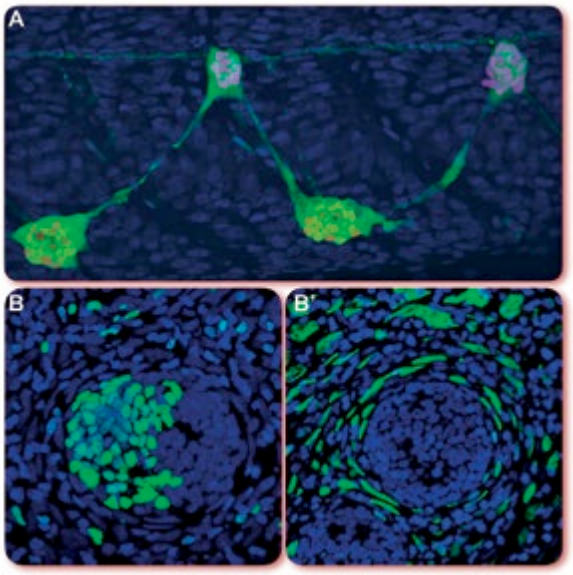

Figure 1
Independent Lineages in the Neuromasts of the Lateral Line. (A) The lateral line in composed of neuromasts, which are exposed to the surface and allow 4D imaging. (B-C) The neuromasts contain two independent lineages, the one including neural stem cells, support cells and neurons (B) and the other consisting on neuromast border cells (C).

Generation of composite organs. Most organs consists of cells from different embryonic origins. We found that neuromasts are composed of two independent lineages: a neural lineage and a border cell lineage (Figure 1). Applying a 4D approach, we revealed that the neural lineage induces the transformation of skin epithelial cells to form border cells, which operates as a structural niche (Seleit, Kraemer et al, 2017). We are currently exploring the molecular cues mediating fate-transformation and niche induction.

Permanent Growth. We are using medaka gills to understand the rationale of stem cell-mediated growth. We have identified fate-restricted growth stem cells that locate to the growing tip of filaments (Figure 2), and homeostatic stem cells with a different location. We are exploring molecular differences between these two pools of stem cells.

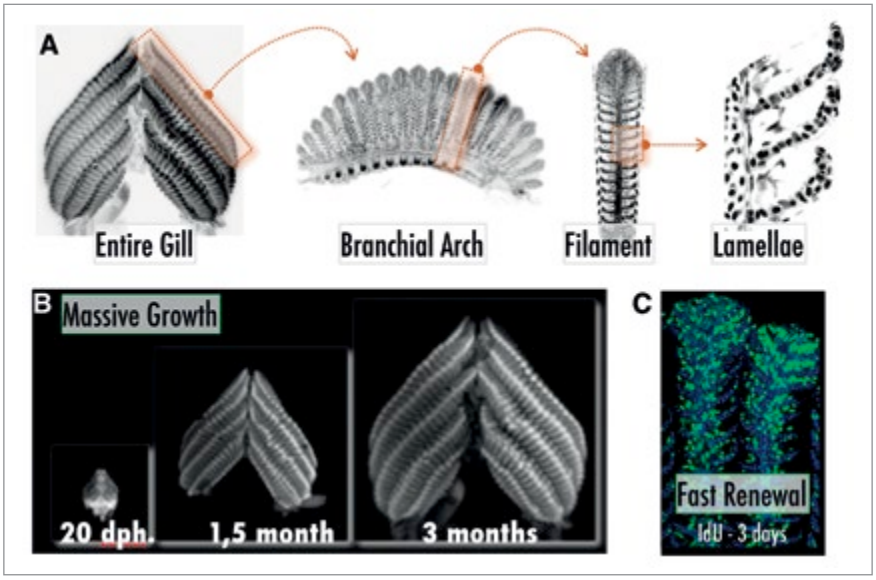

Figure 2
Massive Growth in the Fish Gills. Medaka gills (A, *left*) are composed of four pairs of branchial arches (the right most anterior is highlighted). Each branchial arch (A, *middle left*) contains an ever-increasing number of filaments (A, *middle right*). Lamellae (A, *right*) are the functional respiratory unit of the organ, and they protrude from the internal core of the filament. The post embryonic gill grows massively (B) and display a fast turn over rate (C) from proliferating cells distributed along the longitudinal axis of the filament.

Planned research and new directions

Fish constitute an animal model perfectly suited to tackle how stem cells drive coordinated growth, and to identify molecular cues that might disrupt that coordination. We have genetic tools to explore many aspects of stemness, and will combined them with live imaging and experimental embryology. Our main focus will be on short and long range coordination among stem cells, and we will perform RNA seq to characterise growth coordination molecularly within the gills and the neuromasts. An unexplored aspect of both systems is their dramatic phenotypic variation when fish are grown at different conditions (temperature, salinity, water flow), and we plan to use them to tackle stem cell mediated developmental plasticity.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 9, number of citations 2013-2017: 65, h-index (2013-2017): 5, total h-index: 11.

Seleit, A., Krämer, I., Riebesehl, B.F., Ambrosio, E.M., Stolper, J.S., Lischik, C.Q., Dross, N., Centanin, L. (2017) Neural stem cells induce the formation of theior physical niche during organogenesis. eLife.29173

Seleit, A., Krämer, I., Ambrosio, E., Dross, N., Engel, U., and Centanin, L. (2017). Sequential organogenesis sets two parallel sensory lines in medaka. Development 144, 687–697.

Lust, K., Sinn, R., Pérez Saturnino, A., Centanin, L.#, and Wittbrodt, J.# (2016). De novo neurogenesis by targeted expression of atoh7 to Müller glia cells. Development 143, 1874–1883. # co-corresponding

Reinhardt, R.*, Centanin, L.*#, Tavhelidse, T., Inoue, D., Wittbrodt, B., Concordet, J.P., Morales, J.R.M., and Wittbrodt, J.# (2015). Sox2, Tlx, Gli3, and Her9 converge on Rx2 to define retinal stem cells in vivo. The EMBO Journal 34, 1572–1588. *equal contribution, # co-corresponding.

Centanin, L.#, Ander, J.J., Hoeckendorf, B., Lust, K., Kellner, T., Kraemer, I., Urbany, C., Hasel, E., Harris, W.A., Simons, B.D., et al. (2014). Exclusive multipotency and preferential asymmetric divisions in post-embryonic neural stem cells of the fish retina. Development 141, 3472–3482. # co-corresponding

2.24 DR. SEBASTIAN WOLF
INDEPENDENT RESEARCH GROUP PLANT CELL WALL SIGNALLING

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Fields of Interest
Plant cell biology, plant development, cell wall, signal transduction, hormone signalling, cell mechanics, mechanical signalling

Brief summary of work since 2013
How the development of complex multicellular organisms is accomplished through coordinated cell proliferation, differentiation, and growth is a fundamental biological question. In animals, stem cell maintenance and cell identity specification are in part controlled by the extracellular matrix (ECM). The rigid, yet dynamic ECM of plants, the cell wall, is known to guide morphogenesis through selective restriction of cell expansion. To control growth, but also to respond to extrinsic perturbations, cell wall state is believed to be under constant surveillance by cell surface receptors connected to intracellular signalling. However, very little is known about how cell wall properties are sensed, how signals are transduced, and how cell wall-mediated feedback signalling intersects with the regulatory mechanisms of plant development. Recently, we discovered a pathway connecting cell wall surveillance with the well-described signalling pathway for growth-regulatory brassinosteroid hormones. Central to this signalling integration is a plasma membrane-localized receptor protein, which associates with the cell wall and is able to interact with the brassinosteroid receptor complex, promoting its activity. This allows cells to integrate information pertaining to the state of the ECM with intracellular growth regulation. However, the role of ECM surveillance goes beyond ensuring cell wall homeostasis during growth. For example, stress responses, cell differentiation, and cell fate decisions are affected by cell wall signalling, suggesting broad-range functions of cell wall-mediated feedback. Consistent with this, we discovered distinct, cell type-specific responses to a change in cell wall state, demonstrating the plasticity of the regulatory wiring integrating growth, differentiation, and cell fate.

Major contributions since 2013
Cell expansion in plants is mediated by regulated and selective loosening of the cell walls, which comprises breaking of load-bearing bonds and subsequent displacement of cell wall polymers. In this view, cell wall integrity is challenged by growth itself. Thus, cell wall homeostasis mechanisms must ensure that the individual cell wall polymers are present in the correct amount and modification state, tailored for any given developmental state. Recently, we have shown that feedback signalling from the wall can modulate the outputs of the well-known brassinosteroid (BR) signalling pathway, ensuring cell wall homeostasis and integrity. When cell wall integrity is compromised, BR signalling is rapidly activated.

This, in turn, results in altered expression of cell wall modifying enzymes and, ultimately, in altered cell wall properties. We have identified a receptor-like protein (RLP44) which mediates integration of cell wall and BR signalling in response to cues in the wall. Mutants in RLP44 are affected in development and show hypersensitivity to cell wall-related stress conditions. Expression of RLP44 is sufficient to activate BR signalling and this activation is mediated by direct interaction of RLP44 with both the regulatory co-receptor BAK1 and the BR receptor protein BRI1, possibly in a ternary complex. Conversely, RLP44 is post-translationally regulated through phosphorylation by the BR receptor complex, adding another layer of feedback control to fine-tune cell wall-triggered BR signalling. Phosphorylation is required for the function of RLP44 as non-phosphorylated mutant versions are not able to rescue RLP44 loss-of-function mutants. Interestingly, phosphorylation controls the subcellular localization of the protein through interference with its internalization. Consequently, mutant versions of RLP44 that mimic the phosphorylated state are hyperactive and constitutively present at the plasma membrane, the location of BR receptor complexes actively involved in ligand perception and signalling.

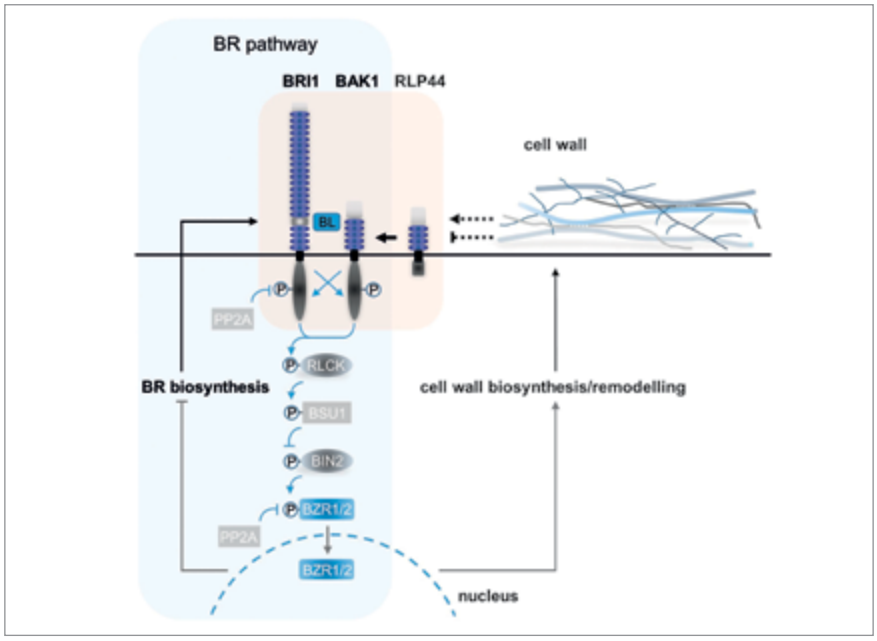

Figure 1
Model of signalling integration between RLP44-mediated cell wall and BR signalling. Upon triggers from the cell wall, RLP44 is capable of interacting with the BR receptor complex composed of BRI1 and BAK1. This leads to enhanced BR signalling strength and transcriptional activation of cell wall biosynthesis and remodelling genes. RLP44 is not itself part of BR signalling, but provides lateral input into the pathway, presumably incorporating feedback information about the state of the cell wall. Therefore, RLP44 is potentially able to uncouple, at least temporally, BR signalling strength from the negative feedback on BR biosynthesis exerted by the transcription factors BZR1 and BES1/BZR2. This molecular circuitry is thus capable of ensuring cell wall homoeostasis during growth but also of tuning BR signalling strength according to feedback signals reporting on, for example, the cell wall state as a readout for the differentiation status of the cell (modified from Wolf 2017).

One of the consequences of activated BR signalling is the promotion of the biosynthesis of the pectin component of the cell wall, together with the upregulation pectin-modifying enzymes. As it was discovered in conditions limiting the activity of those enzymes and is able to physically associate with cell wall pectins, RLP44 seems to form the core of a feedback mechanism ensuring cell wall homeostasis. We assume that RLP44 is sequestered by the wall when pectate binding sites are available, but is released form the wall when those binding sites become limiting. In this situation, RLP44 would be free to interact with, and promote the activity of, the BR receptor complex (Figure X). Recently, we could reveal a surprising physiological role of this feedback mechanism: the maintenance of cell fate. Mutants of RLP44 and BRI1 show premature differentiation of procambial cells into tracheary elements adjacent to existing xylem in the root stele, suggesting that RLP44-mediated signalling controls tissue boundaries. Strikingly, this role is independent of classical BR signalling outputs and target genes, demonstrating the involvement of a BR receptor-dependent, but BR signalling-independent pathway in the maintenance of cell fate. Accordingly, RLP44 is able to interact with other members of the large family of plant receptor-like kinases known to be involved in development and presumably responsible for cell fate maintenance. In this view, RLP44 not only integrates cell wall and BR signalling, but also bridges receptor-like kinase pathways previously thought to be independent to fine-tune developmental outcomes.

Consistent with the emerging theme of an involvement of cell wall signalling in the control of cell fate, we discovered that the response to cell wall perturbation varies greatly between tissues. The identification of feedback signalling mechanisms often requires using brute-force approaches to overcome the phenotypic buffering capacity of homeostatic mechanisms and compensatory responses. To this end, we have started to devise a flexible, tissue-specific, and inducible expression system based on trans-activation that can be easily adapted to a large number of desired cell wall modifications. We have established transgenic driver lines using, in total, >20 tissue-specific promoters, and >10 responder lines expressing cell wall effectors, which together will be made available as a community resource. Using this inducible, cell type-specific expression system in the root, we classified the responses to cell wall perturbation on the organ, tissue, and cellular level, and revealed that the organization of the stem cell niche in the root is particularly dependent on tight control of cell wall properties. Interestingly, this particular phenotype seems unrelated to the pectate-RLP44-BR receptor signalling module we previously identified. This suggests the existence of additional pathways integrating the perception of cell wall state of mechanical signalling with the maintenance of cell identity.

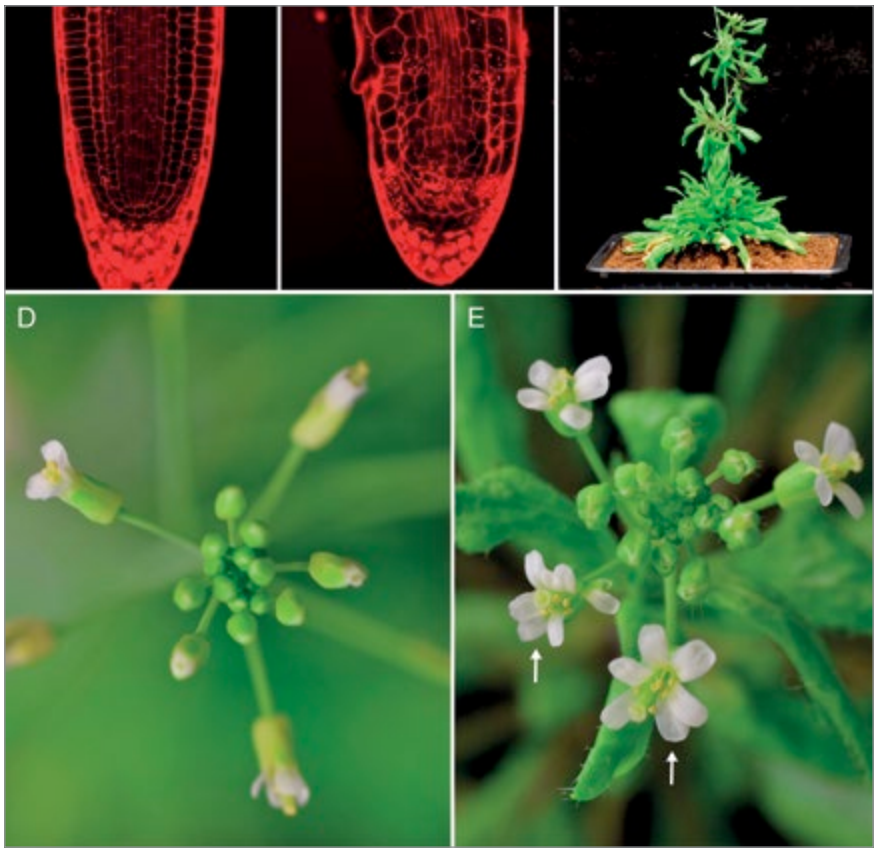

Figure 2
Control of cell wall properties is required for normal plant development. Inducible expression of a cell wall-modifying protein in xylem pole pericycle cells (B) results in ectopic formation of protoxylem (arrows) and reduction of meristem size in the root compared to the control (A). (C-E) Above-ground development shows severe defects such as an overproliferating vegetative meristem (C), delay of floral transition, and supernumerary floral organs (E, arrows) compared to the wildtype control (D).

Planned research and new directions

Our long term goal is to define a mechanistic framework of cell wall signalling-mediated control of cell wall homeostasis, cellular mechanics, growth coordination and cellular behaviour. Using a variety of cell biological and genetic methods, we study how the cell wall controls signalling to the cell interior and characterize the consequences of these signalling processes at the cell and tissue level. In addition, using genetic screens and various biochemical approaches, we want to identify novel components of cell wall signalling to gain a molecular understanding of the cell wall and its function in the control of development adapted to environmental conditions.

Building on our recent discoveries, we want to decipher how cues from the wall are processed by intracellular networks and how cell identity impacts on the response to this cues. Conversely, we intend to explore to which extent feedback signalling form the extracellular matrix contributes to the regulation of cell fate, concentrating on the root and shoot stem cell niche. To this end, we will investigate cell autonomous and non-cell autonomous responses to cell wall cues using cell type-specific transcriptome analyses. We will follow cell identity signature based on transcriptome data, as well as life-imaging of cell identity markers to reveal how cell wall-related signalling intersects with cell fate maintenance. We expect this approach to provide information about the cell identity regulators targeted by cell wall signalling as well as reveal potential integration with known pathways. Moreover, we will identify candidate loci putatively involved in the response to cell wall alteration and cell identity maintenance.

In addition, we will continue our work on RLP44-mediated signalling, focusing on i) elucidating the mechanism of cell wall perception and the consequences of cell wall interaction for signalling outputs, ii) deciphering the biochemical events during RLP44-induced BR receptor activation, and iii) revealing the signalling outputs of signalling pathway integration mediated by RLP44. We intend to extend this approach to RLP44 relatives which might act redundantly or together with a distinct spectrum of plasma membrane receptors.

Independent of these projects, we continue to be interested in identifying novel components of cell wall signalling through forward and reverse genetic methods. We have recently adopted a candidate approach testing evolutionarily conserved membrane-bound proteins with putative cell wall interaction domains in parallel with our efforts to identify genes involved in cell wall signalling pathways from suppressor screens in cell wall-challenged genetic backgrounds.

Selected publications since 2013

Number of peer-reviewed articles 2013-2017: 6, number of citations 2013-2017: 666, h-index (2013-2017): 14, total h-index: 14 (according to Thomson Reuters).

Golfier P, Volkert C, He F, Rausch T, Wolf S (2017). Regulation of secondary cell wall biosynthesis by a NAC transcription factor from Miscanthus. Plant Direct. DOI: 10.1002/pld3.24

Wolf S (2017). Plant cell wall signalling and receptor-like kinases. Biochem J. 474, 471-492.

Pacheco-Villalobos D, Diaz-Moreno SM, van der Schuren A, Tamaki T, Kang YH, et al. (2016). The Effects of High Steady State Auxin Levels on Root Cell Elongation in Brachypodium. Plant Cell 28: 1009-1024

Wolf S[1], van der Does D, Ladwig F, Sticht C, Kolbeck A, et al. (2014). A receptor-like protein mediates the response to pectin modification by activating brassinosteroid signaling. Proc Natl Acad Sci U S A 111:15261-6

Wolf S, Hofte H. (2014). Growth Control: A Saga of Cell Walls, ROS, and Peptide Receptors. Plant Cell 26:1848-56

1 = co-corresponding author

CORE FACILITIES

3

3.1 NIKON IMAGING CENTER AT HEIDELBERG UNIVERSITY
LIGHT MICROSCOPY FACILITY

The Nikon Imaging Center is lead scientifically by Dr. Ulrike Engel and Prof. Dr. Thomas Holstein

Brief summary of work since 2013

The Nikon Imaging Center at Heidelberg University (NIC@Uni-HD, or NIC) offers advanced light microscopy on campus since 2005. The facility is based on a collaboration between the University and the company Nikon, where Nikon sponsors the majority of the instrumentation. We have so far trained approx. 1150 researchers and supported them in their projects. We cover a wide range of fluorescence techniques on currently 13 instruments (Table 1), which researchers can work on independently once they have received training. A team of 3 postdoctoral scientists headed by Dr. Ulrike Engel trains researcher on equipment and gives guidance in assay development and data analysis (460 training sessions in last 5 years). After initial training, researchers gain access to instrumentation 24/7 through an online booking system. In addition, we give in depth support in projects focused on cell biology and developmental biology. In the last 5 years the NIC has supported more than 100 publications.

Major contributions since 2013

The Nikon Imaging Center at Heidelberg University provides access to advanced light microscopy (for list of instrumentation see Table 1). Application range from in-vivo imaging to subcellular dynamics, FRAP and FRET. We established long term imaging in the zebrafish brain on collaboration with the group of Matthias Carl (Medical University Mannheim) that allowed us to follow neuronal migration and axonal projections over 4 days (Beretta et al. 2017, Dross et al. 2014). Long term imaging was combined with photoconversion (Beretta et al, 2016) and laser ablation of single cells or small clusters, an approach reused in another collaboration in Medaka (Seleit et al. 2017). While this and other studies were aimed at looking at the interaction of cells in a tissue, the NIC hosted many studies that looked at subcellular localization and dynamics. Spinning disk microscopy was used for virus uptake studies and viral protein binding (Herold et al. 2014, Meier et al 2013) while we used laser scanning confocal microscopy for many other studies (e.g. Wolfowicz et al. 2016, Beckmann et al. 2015, Schrenk-Siemens et al. 2015). For super-resolution, the NIC offers structured illumination with up to 4 channels (Rüthnick et al in press, Kurtulmus et al. 2016, Cerikan 2015, Engel 2014).

While the NIC belongs to the COS, it radiates beyond the boundaries of the institute, with users from the BZH, Virology, Pharmacology, IZN and groups of the DKFZ. Of the 64 groups that are currently active at the NIC, 33 belong to the medical faculty and only 16 to COS. As an imaging hub, the NIC has taken part or is part of several research initiatives since 2013: SFB 873 (Maintenance and Differentiation of Stem Cells) and the newly founded SFB1324 (Mechanisms and functions of Wnt signaling), both with many groups from the COS. The NIC is part of the excellence cluster CellNetworks and has profited from its open equipment funding calls (2013-2017: Euro 340.000). In collaboration with the SFB 1134 (Functional Ensembles) we have acquired a light sheet microscope for imaging of cleared organs and embryos. This was installed in 2017 and we are now able to acquire 3D images of entire mouse brains (Fig. 1). This system bridges the gap between low resolution whole animal imaging and laser scanning confocal microscopy.

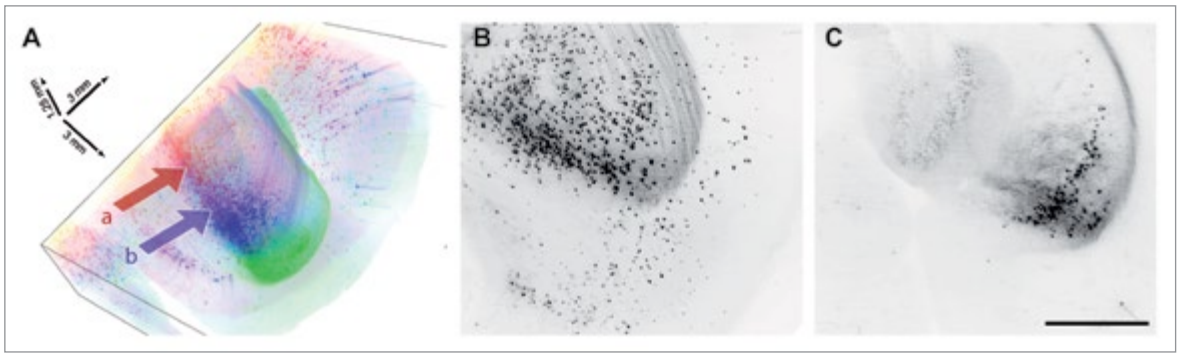

Figure 1
Light sheet image of Thy-1-GFP expressing mouse brain. The entire brain was optically cleared and imaged in a benzylethanol benzyl-benzaote mixture (BABB). (A) 3D-rendering of image stack (3mm x 3mm x 1.25 mm) with z-position shown in color. (B-C) Details at postion b and c, respectively (Projections of 100µm). Scale bar is 0.5 mm. Specimen by courtesy of Tina Sackmann (Draguhn lab).

Table 1: Microscopes available in the Nikon Imaging Center

Type	Number of instruments	Short description
Light sheet microscope	1	Light sheet microscope for specimen up to 10mm with dipping lenses equipped for clarity, BABB or for living samples in aqueous medium.
Inverted wide field fluorescence microscope	2	Automated inverted microscope for time lapse acquisition with perfect focus system (Nikon Ti2), 7 channels (multiple fluorescent proteins, FRET, Fura). Cameras include EMCCD (Andor iXon) and dual sCMOS (2 Andor sNEO on TuCAM)
TIRF	1	Total internal reflection fluorescence (TIRF) microscope with triggered acquisition and single molecule sensitivity. FRAP with 10 ms switching time to acquisition. Laser lines: 405nm, 440 nm, 488 nm, 514 nm, 561 nm, 640 nm
Wide field FRET-FLIM	1	Fluorescence Life Time Imaging Microscopy (FLIM) based on frequency domain on a sensitized CCD camera. Speed at 1000x1000 pixel resolution up to 1 fps (multiple image acquisition). Can be used for FRET pairs CFP-YFP and GFP-mCherry.
Laser scanning confocal microscope	2	Laser scanning confocal systems (Nikon C2 and Nikon A1R) on an automated inverted microscope (Ti2) with perfect focus and and multipoint acquisition. On A1R resonant mode as well as GaAsP-detectors and spectral detector are available. Laser lines: 405, 488, 514, 561 nm.
Spinning disc confocal microscopes	1	Spinning disk confocal systems with sensitive EM-CCD detection (Perkin Elmer Ultra-View ERS and Vox) on inverted microscope for high resolution cellular dynamics. Fast z-acquisition with objective piezo and 2 ms laser switching. On Vox, dual camera acquisition is available. On Ultra-View, FRAP and photoactivation is available. Laser lines: 405, 440, 488, 514, 561, 640 nm
2-photon microscope on intravital microscospe	1	2-photon system (LaVision Biotec) on upright fix-stage microscope (Nikon FN-1) for physiological deep-in-tissue observation. Detection on non-descanned all-GaAsP ultra-sensitive-PMT port (4 channels). Exciation of UV-dyes (fura) up to red fluorescent proteins (e.g. mCherry). OPO avaible for deep-red fluorophores. Equiped for 2P-FLIM.
Structured illumination for superresolution	1	Nikon structured illumination microcope (N-SIM) for multichannel imaging with a lateral resolution of 110nm. 2D-SIM, 3D-SIM and TIRF –SIM illumination modes are available. Laser lines: 405, 488, 561, 640
Biostation for time lapse microscopy	1	Environmental (37C, 5 % CO2) enclosed microscope with multipoint acquisition for fluorescence and brightfield observation over 100 hours, e.g. for evaluation of cellular phenotypes of RNAi.
Upright wide field fluorescence	1	Upright microscope (Nikon NiE) with automated z and 6-channel acquisition as well as multipoint and stitching
Automated stereomicroscope with fluorescence	1	Stereomicroscope for fluorescence imaging

Planned research and new directions

Mechanistic data by single molecule imaging or endogenous expression: The NIC aims to contribute mechanistic data by studying single molecules, or ensemble studies that rely on endogenous expression level rather than overexpression. To this end, the NIC will work together with the community to broadly spread light efficient labeling techniques (Snap-, or Helo Tag), click chemistry or novel brighter FPs (mScarlet, Turquoise3 etc), tools which are already used by experts on campus. To increase its expertise in FCS and single molecule measurements, the NIC will profit from interaction with Prof. U. Nienhaus (KIT) in a shared Z-project part of SFB1324 on mechanisms of wnt signaling. Also, our TIRF system has been upgraded to 4 lasers and a FRAP unit. This system is equipped for single molecule studies and with the addition of the FRAP is now able to perform photoactivation and sensitive protein mobility measurements (needed for example for endogenous expression in yeast).

FLIM-FRET for protein-protein interaction: We are in the process of evaluating fluorescent protein FRET pairs for high contrast in life time response. We tested this on our existing frequency domain FLIM (wide field) instrument. Currently the NIC has a 9 months loan of the Picoquant FLIM and FCS module installed on the advanced laser scanning confocal microscope.

Light sheet microscopy: In 2017 we have installed a light sheet microscope with 3 laser lines for imaging of organs and embryos. This system, which is funded by the CellNet-works equipment program and SFB1134 is particularly well suited for big specimens, which are chemically treated for optical transparency (clearing). The current system can image specimen up to 5-10 mm at cellular resolution. We see a high potential for SPIM also for subcellular dynamics. The NIC is currently evaluating user-friendly SPIMs for live imaging with subcellular resolution, such as an inverted SPIM.

Selected publications since 2013

Publications supported by the NIC (as acknowledged in article) or with co-authorship. Total publication 2013-2017: 100. Number of citations and h-index not available.

Seleit, A., Kramer, I., Ambrosio, E., Dross, N., Engel, U., and Centanin, L. (2017). Sequential organogenesis sets two parallel sensory lines in medaka. Development 144, 687-697.

Beretta, C.A., Dross, N., Guglielmi, L., Bankhead, P., Soulika, M., Gutierrez-Triana, J.A., Paolini, A., Poggi, L., Falk, J., Ryu, S., et al. (2017). Early Commissural Diencephalic Neurons Control Habenular Axon Extension and Targeting. Curr. Biol. 27, 270-278.

Wolfowicz, I., Baumgarten, S., Voss, P.A., Hambleton, E.A., Voolstra, C.R., Hatta, M., and Guse, A. (2016). Aiptasia sp. larvae as a model to reveal mechanisms of symbiont selection in cnidarians. Sci. Rep. 6, 32366.

Beckmann, A., Xiao, S., Muller, J.P., Mercadante, D., Nuchter, T., Kroger, N., Langhojer, F., Petrich, W., Holstein, T.W., Benoit, M., et al. (2015). A fast recoiling silk-like elastomer facilitates nanosecond nematocyst discharge. BMC Biol. 13, 3.

Chinen, T., Liu, P., Shioda, S., Pagel, J., Cerikan, B., Lin, T.C., Gruss, O., Hayashi, Y., Takeno, H., Shima, T., et al. (2015). The gamma-tubulin-specific inhibitor gatastatin reveals temporal requirements of microtubule nucleation during the cell cycle. Nat. Commun. 6, 8722.

OBJECTIVE APARTURE
PUMP
AIR

3.2 ELECTRON MICROSCOPY CORE FACILITY (EMCF)
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Fields of Interest
Plant cell biology, electron microscopy, high pressure freezing, vacuole biogenesis, storage protein deposition, lipid storage.

As mentioned in the previous COS-Report as a possible option for the future the Electron Microscope Facility of the COS actually fused with the campus wide Electron Microscopy Core Facility (EMCF) in 2014. The EMCF was founded originally in 2009 with funds from CellNetworks and the medical faculty. It was first located in parts of the cryo-EM preparation space in the Bioquant building, but to better facilitate the use of the EMCF it was decided to integrate the equipment for electron microscopy to a center at INF345. COS agreed to support this independent facility with equipment and personal (S. Gold, Dr. S. Hillmer) to supplement the facility. The EMCF now holds 2,5 scientific and 1,5 technical positions in total. Lab space for users was set up at the new location and the standard TEM formerly located at COS was also moved to INF345. Main technical focus at the facility was and still is on routine thin section EM, including cryo-sectioning and immunolabeling as well as negative staining. During the past years additional techniques such as scanning EM (and its sample preparation), sample preparation by high-pressure freezing and freeze substitution and more recently correlative light and electron microscopy (CLEM) as well as tomography on plastic embedded samples were established. The EMCF provides the basis for electron microscopy studies on campus both in terms of equipment and know-how. Essential services are education of the users to make optimal use of the equipment for electron microscopy and maintenance of the available microscopes as well as sample processing equipment. The educational goals are achieved by courses on biological TEM for students on different levels, initial scientific project discussions, individual user training and last but not least assistance with the interpretation of the EM images.


COS
Centre for
Organismal
Studies
Heidelberg
PEGASUS

3.3 DEEP SEQUENCING CORE FACILITIY

The Deep Sequencing Core Facility is headed by David Ibberson
david.ibberson@bioquant.uni-heidelberg.de

Introduction
The CellNetworks Deep Sequencing Core Facility was opened in September 2010 to provide access to Next Generation Sequencing technology, (NGS) for the Heidelberg University research community. The core facility is supported by the Excellence Cluster CellNetworks, the Centre for Organismal Studies (COS Heidelberg) and the Heidelberg Molecular Life Sciences (HMLS) research council.

Personnel
The core facility has had to make changes to its' personnel structure within the last two years due to the long term sickness and the eventual sad passing away of Prof. Gabriele Petersen in 2016. Currently the facility is headed by David Ibberson (MSc) with administrative and laboratory assistance coming from Claudia Loosli (former Müller). Since December 2016 the core facility has been further reinforced with a new technician Hannah Hänisch (MSc), to help with the increasing workflow we have experienced over the last two years. It is hoped that with the restructuring the core facility can better cope with the increased work flow and reduce waiting times for final results.

Core Facility Concept and Services Provided
The facility was founded with the idea not only to provide library preparation and sequencing for its' users on campus but also to offer professional advice on how to implement NGS into its' users research. This advice has been widely taken up and has in some cases lead to development of new protocols or the refinement of current »standard« protocols. In addition it is widely recommended to speak with us before a project is started to clear up any mis-conceptions about NGS which will inevitably save the researcher money and time. With regards to services, the core facility offers a mixture of services for its' users. The main day to day activities revolve around the standard library preparation methods for Illumina sequencing. However due to the high diversity in sample genome origin on campus, and the fact reagents are tailored to samples originating from human and mouse, the library preparations are often tailored to the individual group from which the samples originate.

»Standard« Library Preparations on Offer:
– RNA-Seq (as of June 2014 all libraries are strand specific)
– Small RNAseq
– Chip-Seq
– gDNA-Seq (de novo, resequencing)
– Mate Pair (2.5 – 10.0 kbp mate pair)
– Target Enrichment
– Methyl-Seq / Bisulfite Sequencing

As mentioned previously the core facility is often called upon to help design new approaches and protocols to specific questions, eg 16S rRNA amplicon sequencing of patients with Cystic Fibrosis to identify microbial community changes during therapy. Here to enhance the power of the amplicon sequencing new ways to identify sources of bias were required which led to the development of including molecular ids within the amplicon sequence before this was available commercially. In other cases the core facility has been called upon to establish new protocols which if proven successful can be applied to other research areas and thus will be taken up as part of its' sample preparation repertoire, eg GuideSeq for HIV integration sites (currently under development).

There have been other cases where logistically it is difficult for the core facility to perform the library preparation themselves, but have helped in the establishment of group specific protocols such as ChIPexo and CRACseq for the Tamas Fischer group of BZH. This has now been widely »exported« within BZH to other research groups.

Sequencing and Collaboration with the Genomics Core Facility, EMBL
Since the end of 2013 / beginning 2014 the Deep Sequencing Labor has entered a highly successful collaboration with the Genomics Core Facility, EMBL. This has been a mutually beneficial collaboration which has resulted for both parties a reduction in waiting times for sequencing results due to the higher input of samples. An optimal load on the sequencing instruments (which in some cases truly is 100 % working load), this has had the positive impact on keeping prices low when compared to an instrument in house. In addition there is a very open communication between the two facilities even prior to the collaboration which has meant the exchange of protocols, new product information and problematic issues to the benefit of our users. This collaboration does not mean that the core facility no longer performs the sequencing, on the contrary the facility is sequencing more than previously and there are days where a member of the facility will be at the EMBL sequencing.

With regards sequencing up until the end of 2015 sequencing was being performed on one of two instruments dependent upon the requirements of the user, this being the HiSeq 2500 or the MiSeq. Since Q4 of 2015 there has been the purchase of the NextSeq 500 sequencing instrument. The instrument has an extremely rapid turn-around time (when one looks at individual run times) and due to the larger data output when compared to one lane of the HiSeq 2000 it is actually in many cases cheaper than sequencing on the HiSeq, especially when a user can increase the number of samples they load onto the machine. Due to this instrument has proved highly popular during the year of 2016 to such an extent that there will be soon three such instruments in place. Additionally the NextSeq offers also in some ways more flexibility than the HiSeq a good example being assymetric reads. A downside to the switch in instrumentation has been for certain sequencing protocols extremely long waiting times, especially 125 paired end (PE) and 50 PE option on the HiSeq as these have generally been replaced by the 75 base PE on the NextSeq. This has been to such an extent that 50 PE is no longer offered and 100 PE only in special cases.

Sequencing Options on Offer:
1. NextSeq 500:
   a. Mid-Output (ca 120 million reads raw data)
      -75 and 150 PE.
   b. High Output (ca 300 – 400 million reads raw data)
   i. 75 SingleEnd (SE)
   ii. 75 and 150 PE.
2. HiSeq 2500 (ca 200 million reads raw data)
   i. 50 SE
   ii. By request 125 PE
3. MiSeq (ca 12 million reads raw data)
   -36, 150, 250 PE

User Base, Access and Fees
2016 saw the busiest year of the core facility with over 50 users and nearing 1000 samples. The users came from a spectrum of institutes (see fig. 1), and sample numbers have increased again (See fig. 2). It is anticipated that sample numbers will continue to increase, especially from the medical faculties (this impression is gained after multiple talks from group leaders from the clinics prior to Christmas 2016).

The following clientele can enroll as user of the facility:
– Members of Heidelberg University, including the Medical Faculties Heidelberg and Mannheim
– Excellence Cluster CellNetworks
– Graduate school HBIGS (Hartmut Hoffmann Berling International Graduate School of Molecular and Cellular Biology)
– HMLS (Heidelberg Molecular Life Sciences) members including DKFZ and EMBL.

The best way to contact the core facility is via e-mail: deepseqlab@bioquant.uni-heidelberg.de

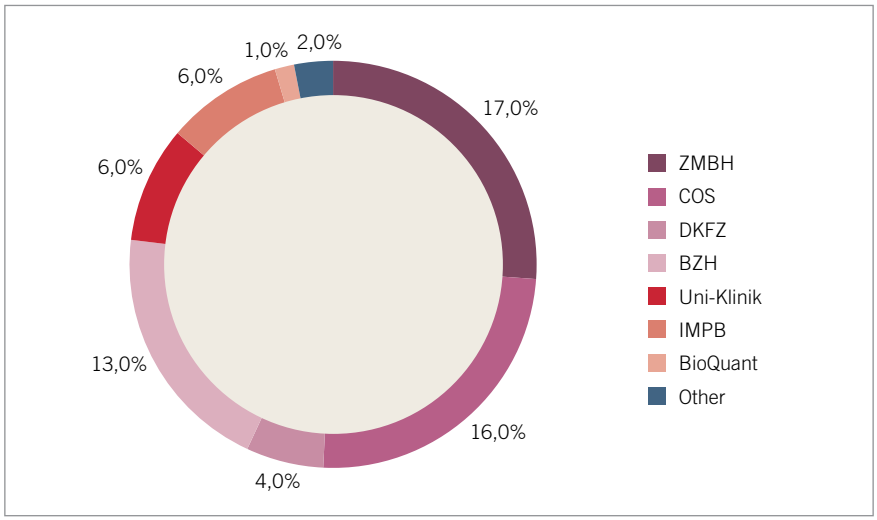

Figure 1
Chart showing sample origin by institute.

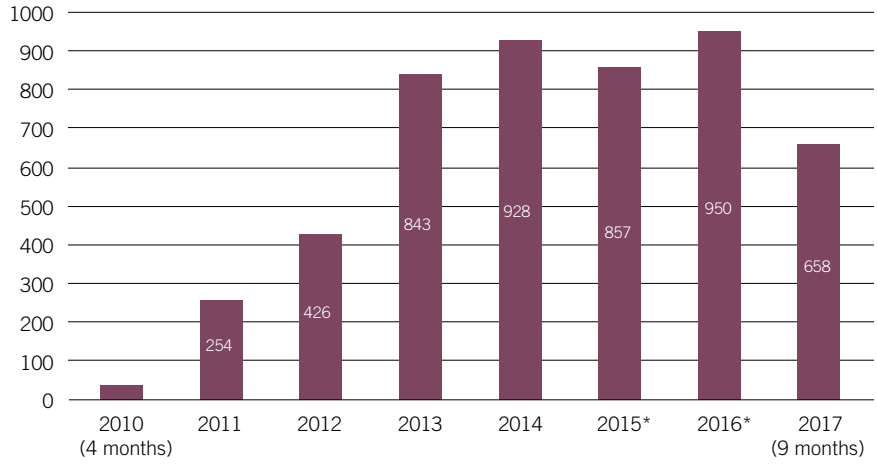

Figure 2
Chart showing the number of samples over the years.
*indicates years where multiple samples were submitted for library preparation under one submission number, this has not been included in the final numbers

The core facility unfortunately cannot operate on goodwill alone, and thus has a price structure in place to recuperate costs incurred for library preparation and sequencing. The fees can be requested from us at any time, and are regularly checked and corrected on a 6–8 month cycle.

Online Registration System

Since March 2017 the core facility has implemented an online registration system through the provider iLabs. The main aim of the system was to provide a clearer overview to the researcher as to where their samples are, ie at what stage they are in the process, and to hopefully provide a more transparent pricing structure. These two have been the main criticisms of the core facility which we are hoping through the implantation to alleviate. In addition it is hoped to provide us with a better understanding of what our customers wish for and that our customers can be better informed as to what services are on offer. To this effect so far we have had less communications regarding sample submission, and there has been less confusion as to what we can provide. We have also had very positive feedback with regards the aims of the first two points and hope this will continue to be the case.

Conclusions and Future Direction:
The core facility has become a well utilized facility since its' founding. We anticipate a continued growth and demand for NGS on campus, and are well positioned to meet this. Our main area of expertise is not the library preparation and sequencing per se but the open attitude we have towards our users. We encourage an open dialogue and are able to provide input to the experimental design required, including the design of novel solutions. With the recent introduction of the iLabs system we hope to increase our transparency and ultimately reduce the waiting times for sample preparation and data generation.

Selected Publications

Gutierrez-Triana JA, Mateo JL, Ibberson D, Ryu S, Wittbrodt J. (2016) DamIDseq and iDear: and improved method and computational pipeline to prolific chromatin binding proteins. *Development* 143(22)

A. Gumiero et al, (2016) Interaction of the cotranslational Hsp70 Ssb with ribosomal proteins and rRNA depends on its liddomain. *Nature Communications* 7

S. Boutin et al. (2015) Comparison of Microbiomes from Different Niches of Upper and Lower Airways in Children and Adolescents with Cystic Fibrosis. *PloS One*


35 30 35 30 35

3.4 METABOLOMICS CORE TECHNOLOGY PLATFORM

The Metabolomics Core Technology Platform is headed by Prof. Dr. Rüdiger Hell and managed by Dr. Gernot Poschet

FIELDS OF INTEREST
Targeted analyses, untargeted analyses, metabolite profiling, identification and quantification, flux analyses, metabolic networks, development of analytical protocols for new relevant metabolites

Brief summary of work since 2013
The Metabolomics Core Technology Platform (MCTP) was established by the Institutional Strategy of the University in the Excellence Initiative II program, together with the junior research group »Plant Defense Metabolism« (Dr. Emmanuel Gaquerel). The MCTP mission is to provide developmental and analytical services across the Heidelberg Molecular Life Sciences including the non-university institutions DKFZ, MPImF, EMBL as well KIT. It aims to improve the technical and scientific metabolomics approaches in Heidelberg, to develop new analytical tools and to provide custom-made services for research projects. MCTP accompanies projects in close cooperation from early development to publication and funding applications. It is located in COS within the Department of Molecular Biology of Plants.

Since 2013 more than 16.000 metabolite analyses were conducted for more than 60 research groups all over Heidelberg Molecular Life Sciences, clearly showing the strong utilization as well as demand. Towards the most requested targeted analyses high throughput protocols have been developed that now allow the quantitative determination of more than 160 compounds. To this end, UPLC based separations (Waters) are combined with UV/VIS, fluorescence, mass (Waters), conductivity and pulsed amperometric detectors (Thermo Scientific). For un- or semi-targeted analyses a GC-MS system (Shimadzu) was established, complemented by an UPLC system coupled to a XEVO QTof MS (Waters). The latest addition (2017) is an UPLC system coupled to Vion IMS-QTof MS (Waters) which promises high accuracy required for determinations in flux analyses.

Major contributions since 2013
With the beginning of this report period MCTP successively began to replace analytical equipment that was used in the beginning and provided by the departments of Molecular Biology and Molecular Ecophysiology of Plants (HPLCs, Fluorescence detectors, Anion Analytics) by own equipment. Funding came from applications to competitive campus wide instrument programs led by the Heidelberg Molecular Life Sciences (HMLS) Research Council, the CellNetworks Cluster of Excellence and to a small extent from faculty teaching funds due to MCTP's strong teaching activities. These instruments are mostly used for targeted analyses and at present constitute an important source of revenues to maintain the facility.

Starting in 2015 a major campaign was launched to acquire a competitive mass spectrometer for untargeted analyses and flux measurements. This demand had been addressed to MCTP and led to a joint project where players across campus contributed funds for the acquisition. With decisive support by the rectorate of the university, it became possible to collect contributions from COS, HMLS, CellNetworks, Collaborative Research Centres (SFBs) 1036 and 1118, and the National Cancer Research Centre (DKFZ), and to develop cooperation contracts that allow tax and DFG congruent operations. A Vion IMS-QTof MS (Waters) was chosen because of superior properties of mass accuracy based on the newly developed ion mobility separation feature.

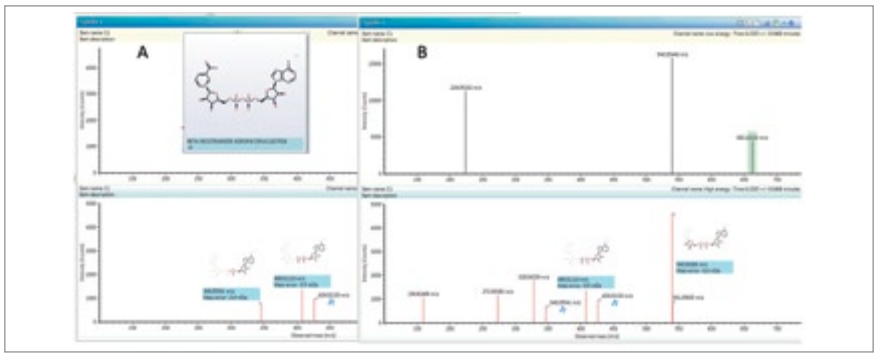

Figure 1
High and low energy spectra of NAD (Nicotinamide adenine dinucleotide) resolved (A) or unresolved (B) by ion mobility separation (IMS).

The instrument was installed in INF360 in March 2017 and will be ready for routine use in July. To keep the MCTP up to date, this kind of intramural funding but in future also extramural funding will be indispensable.

Figure 2
VION IMS-QTof MS system coupled to ACQUITY UPLC I-class system

The financial situation of MCTP largely depends on internal funding of personal by the Institutional Strategy of the Heidelberg Excellence Initiative. The extension of analytics strongly increased sample numbers and bioinformatics demands. In addition to one staff scientist and half time technician (Dr. Gernot Poschet, Eva-Maria Käshammer-Lorenz; funded by the Excellence Initiative) and one staff scientist funded by COS (Dr. Michael Büttner), a half time technician (Lorenz Nowack) and 20 % administrative staff (Birgit Maresch) were hired.

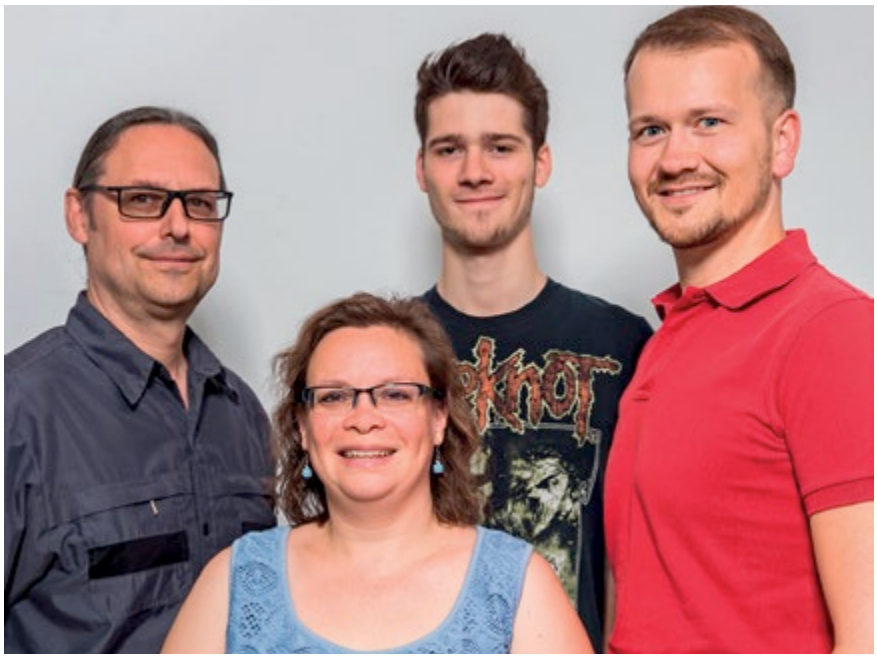

Figure 3
Group photo MCTP, from left to right: Dr. Michael Büttner, Eva-Maria Käshammer-Lorenz, Lorenz Nowack, and Dr. Gernot Poschet

The entire running costs need to be raised by user fees. To this end, a transparent cost structure was developed in accordance with the university central administration and DFG regulations. In this context, a new management system (iLab Operations Core Facility Management Software, Agilent) was applied for by eight Core Facilities together and funded by HMLS: This allows a first come, first serve organisation, tracking of samples and processing for customer and staff, data archiving and billing. MCTP is represented in the mass spectrometry panels at campus and Gernot Poschet is speaker of the HMLS core facilities, allowing for coordinated planning of new instruments and cost structures.

Planned research and new directions

With respect to personal, MCTP needs to have a core group of permanent staff of a minimum of two scientists, one engineer and one technician. The request for permanent staff results from the long training required to achieve a professional and reliable analytical level and from the simple fact that otherwise no qualified personal can be attracted or kept. The present major financial support from the Institutional Strategy of the university's excellence initiative will come to an end 30.10.2019. Until then the facility needs to be integrated into the campus wide concept of core facilities. This will be based on the next round of excellence competition and the promised sustainability of part of the initiative by the land Baden-Württemberg.

Work force for timely limited projects will be added by successful contribution to external funding schemes such as Collaborative Research Centres (CRCs = SFBs). As an example, MCTP was faced by considerable demand of customers for analyses of released glycan structures of diverse sample matrices. These can be analysed by the recently acquired high-resolution Vion LC-MS system. The missing solid-phase extraction system and a sensitive fluorescence detector were funded by HMLS research council, enabling support of the recently established DFG research unit »The concert of Dolichol-based Glycosylation: from Molecules to Disease Models« headed by Sabine Strahl (COS). MCTP currently also contributes to a central project in the renewal of CRC1118 (Reactive metabolites as a cause of diabetic complications; Medical Faculty). These approaches will be used to gain equipment for novel applications (e.g. Triple Quadrupol MS system) and possibly bioinformatics support and thus contribute to further increase the technical and scientific level of MCTP for service functions.

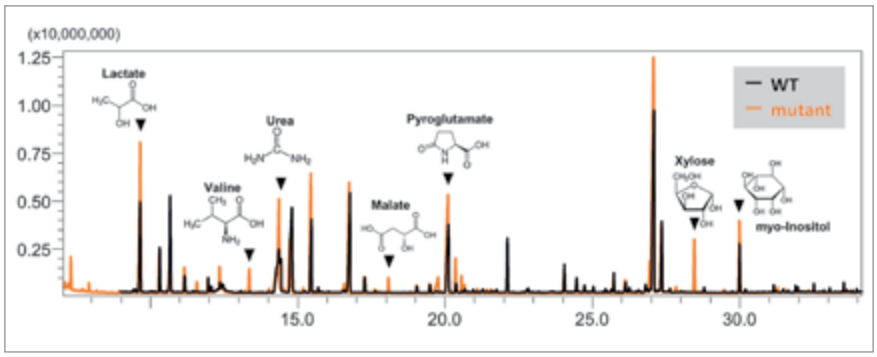

Figure 4
Metabolite Profiling by GC/MS-Analysis. Comparison of wild-type and mutant plant profiles reveals genotype-specific differences in certain metabolites.

The continuous challenge of MCTP by new requests of clients is the major driver of innovation and guarantees the scientific advance of the facility. One major aim is to establish flux analyses that are especially useful to determine mass distribution of metabolites. For instance, carbon labeling is highly requested in tumor research where unexpected specific alterations of the Warburg effect based on branched chain amino acids may be suitable targets for new drugs. In addition, plant sciences are in high demand for the analysis of metabolite fluxes combined with high resolution.

The technical portfolio of MCTP needs to be completed by one more instrument for targeted metabolite analyses. Particularly regulatory metabolites are often of low abundance or stability and therefore difficult to assess by mass spectrometry. The problem is often exacerbated by small sample amounts, particularly when it comes to high resolution at the cell type level following FACS. To address this challenge a Triple Quadrupole mass spectrometer (MS/MS) is an essential requirement otherwise this important research application cannot be handled at MCTP and not be provided as service at the Heidelberg campus.

Selected publications since 2013
Number of peer-reviewed articles 2013-2017: 23

Wallner, E.-S., López-Salmerón, V., Belevich, I., Poschet, G., Jung, I., Grünwald, K., Sevilem, I., Jokitalo, E., Hell, R., Helariutta, Y., Agustí, J., Lebovka, I., and Greb, T. (2017). Strigolactone- and karrikin-independent SMXL proteins are central regulators of phloem formation. Curr. Biol. 27, 1-7.

Lüddecke, J., Francois, L., Spät, P., Watzer, B., Chilczuk, T., Poschet, G., Hell, R., Radlwimmer, B., and Forchhammer, K. (2017). PII Protein-Derived FRET Sensors for Quantification and Live-Cell Imaging of 2-Oxoglutarate. Scientific Reports 7: 1437.

Weger, B.D., Weger, M., Gorling, B., Schink, A., Gobet, C., Keime, C., Poschet, G., Jost, B., Krone, N., Hell, R., et al. (2016). Extensive regulation of diurnal transcription and metabolism by glucocorticoids. PLoS Genet 12, e1006512.

Yang, Y., Pollard, A. M., Höfler, C., Poschet, G., Wirtz, M., Hell, R. and Sourjik, V. (2015). Relation between chemotaxis and consumption of amino acids in bacteria. Mol. Microbiol. 96, 1272-1282.

Demetriades, C., Doumpas, N., and Teleman, A.A. (2014). Regulation of TORC1 in response to amino acid starvation via lysosomal recruitment of TSC2. Cell 156, 786–799.


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CAS:75-05-8
PLASTIPAK

3.5 BOTANIC GARDEN AND HERBARIUM

History

The Botanic Garden was established in the vicinity of Heidelberg's famous castle as a garden of medicinal plants in 1593 and is among the world's oldest Botanical Gardens. After several relocations, the Garden was reopened in 1915 at its present site. During World War II the entire greenhouse collection was lost to combat activities. The collections were greatly enlarged under the directorate of Werner Rauh from 1960 to 1982. These historic collections still form the basis of the Garden's specimens. The affiliated Herbarium HEID encompasses at least 50,000 species, represented by ca. 350,000 specimens with a particular focus on South American taxa, especially from the Andes, and African taxa, mainly from Madagascar and Kenya. The »old herbarium« collections originate from the early 19th century. Significant parts of the »new herbarium« contains 50,000 specimens, especially cacti, bromeliads, orchids and tropical ferns, collected by Werner Rauh and colleagues. Approximately 30,000 vouchers can be attributed to the research activities of its current director, Marcus Koch. HEID encompasses ca. 2,300 vouchers representing type material of nearly 1,500 taxa. However, it is very likely that many more type specimens can be identified in future. A major research and curatorial focus is on the Brassicaceae family with its 4000 species and which is encompassing various important crops and several of the most important plant model systems. For the entire family a species check list has been released, a knowledge database system (BrassiBase) has been launched, and the collections serve the community with reference material, germ plasm and research data.

Mission, Objectives & Vision

The Mission of the Heidelberg Botanic Garden is the conservation and development of its collections and promoting the discovery, understanding, responsible use and enjoyment of plant biodiversity. The living collection with ca 13,000 accessions and the 350,000 specimens in the Herbarium are among the most important plant biodiversity archives in Germany, actively used in internationally recognized scientific research programs.
Being one of the University's leading visitor attractions, the Garden is also dedicated to making biology as accessible as possible to the wider public. It does so by means of its exhibitions as well as teaching and outreach programs. The Garden's vision is to be widely acknowledged as an outstanding plant collection in Germany, valued by stakeholders as a major scientific research facility and a as a centre for innovative public engagement with plant science via its collections and expertise. Likewise, the Herbarium HEID is an active research facility, regularly visited by international scientists to support their research activities and loaning specimens for external research programs. Moreover, supporting loan programs from other international Herbaria via HEID ensures that Heidelberg remains a centre of evolutionary and biodiversity research.

General Collection Management & Development Policy – Living Collection

The collections – kept in greenhouses, outdoor gardens, and germplasm archives – meet the full spectrum of research, educational, cultural, and conservation needs and can be divided into specialized and non-specialized collections, in total representing nearly 5,500 species. Our specialized collections are of a size and significance that merits national and international recognition, ideal suited to research: tropical orchids, bromeliads, succulent plants, and Brassicaceae. Smaller non-specialized collections contribute to the diversity of the collections in general and are primarily used for teaching and display purposes (e.g. insectivorous plants, the arboretum). The main acquisition methods of the Garden are plant or seed exchanges with other Gardens, and field collections. New plant material should generally be from a collection in the wild or, if cultivated, from a known wild origin. Provenances of newly included specimens must be known and must respect the Convention on Biological Diversity (CBD) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and other laws. The documentation of all accessions is recorded in the database »Gartenbank« (currently ca. 13,000 accessions) which is freely accessible. The collection is continuously monitored for specimens that are

in conflict with the collection's criteria and for unnecessary duplicates. Plants of garden or unknown origin – especially in the core collection – are replaced with specimens of known wild origin, preferably of direct wild origin. These processes are under permanent internal evaluation and supervised by Andreas Franzke, curator of the living collections.

Major Achievements & Activities 2013–2017

Living Collection Development
The core collections orchids, bromeliads, and succulents are currently under the special auspices of the Adminstrative District Gouvernmental Department at Karlsruhe, because most of them are regulated by law but with uncertain status in official collections (Koch & Kahl 2014).

The core collections (orchids, bromeliads, succulents) are currently under the special auspices of the Adminstrative District Gouvernmental Department at Karlsruhe. Consolidation of these historic collections (plus modern additions) was an important part of our work over the last years and is still ongoing. This process involved the inventory clearance of approximately 1,000 accessions. From a scientific curation perspective around 300 new determinations or verifications, often by world leading experts of certain taxa, were made. The documentation of over 1000 historic accessions *(provenance research)* was improved significantly. Following long-term commitments in research and *ex-situ/in-situ* conservation to contribute to the national and Federal State Baden-Württemberg strategy to protect and improve the state of Europe's biodiversity over the next decade, key species such as Cheddar Pink and Hardy Water Lily are under cultivation, accompanied by conservation genetic and fitness screening studies for subsequent reintroduction and population backing. The eco-geobotanical sections of our outdoor collections are under reconstruction and at first the Inland dune and heath vegetation was conceptually revised and re-established.

BrassiBase: Reference Material, Germplasm Collection & Knowledge Database
Since 2011 the Botanic Garden contributed to reference material and germplasm of Brassicaceae (mustards) within the framework of DFG priority research program SPP 1529 (Evolutionary plant solutions to ecological challenges) (Kiefer et al 2014). This material serves not only as research material for more than ten DFG projects but serves also as reference material for high quality reference genome sequences (Jiao et al. 2017) and comparative genomic analyses (Novikova et al. 2016); and is encompassing several hundreds of species (https://brassibase.cos.uni-heidelberg.de/).

Herbarium Collection Development
Currently one third of the 350,000 vouchers are recorded in the online database *»Gartenbank«*. During the last three years approximately 3.000 vouchers were added to the collection and nearly 2000 specimens were digitized including over 100 type specimens. Over 320 specimens were newly identified as types in addition to nearly 100 specimens that represent likely type candidates. Some randomized inspections of the »old herbarium« indicate that there are many hundreds of additional type material vouchers not identified yet awaiting recording during the next years (e.g. Schröder et al. 2014). Moreover, new species have been described at COS Heidelberg and are documented with our collections (e.g., Dönmez et al. 2017).

Werner Rauh Heritage Project
The Werner Rauh Heritage Project – a leading-edge digitization and deep indexing project of its kind – was funded by the Klaus Tschira Stiftung from late 2009 until to mid of 2016. This ongoing project is based upon an online relational database storing heterogenous information found in Rauh's field books and diaries with the aim of retrospectively documenting the Rauh-collection data (25,000 herbarium specimens as well as 10,000 accessions of living plants). Since 2013 the indexing focus has been on *the field diaries*. Over 100,000 external page views during the last three years are testament to the significance of this database for the scientific community.

Material Transfer for Scientific Purposes & Support of Local Research Programms
As a member of the International Plant Exchange Network (IPEN) the Garden supplies material for international research programs conforming the Convention on Biological Diversity (CBD). In this period, material from over 400 accessions was transferred. The Botanic Garden supports Heidelberg-based research programs with the provision of plant material, test areas and horticultural expertise. In the period 2014 to 2017, the Garden's *Scientific Plant Cultivation Service* (SPCS) cultivated between 2,000 and 7,000 individual plants annually from a great variety of wild species, rarely cultivated elsewhere. This also led to a substantial number of SPCS-based publications.

Academic Teaching, Public Education Program, Visitors & Public Events
The Botanic Garden plays an important role in the academic teaching of COS Heidelberg: The Garden provided plant material for ca. 80 course days with ca. 400 students, and the Garden's collections hosted academic courses with ca. 700 students on ca. 50 days each year. The Herbarium is also integrated into education programs. The *Student's Herbarium* of plants, collected during field excursions during the last ten years, incorporate more than 10,000 fully digitalized specimens. The Garden provided plant material or test areas for a variety of academic works. Garden- related, Heidelberg-based theses submitted from 2014 to 2016 include 4 PhD theses, 3 master theses, 5 bachelor theses, and 5 state examination theses.

The »Green School« of the Botanic Garden represents a comprehensive outreach program that has reached about 4,500 children and adults every year. Activities for children and teenagers are part the Heidelberg Young University educational program. New types of events in the last three years included citizen science workshops and a series of popular science lectures.

The Botanic Garden (gardens and greenhouses) is free and open to the public. The gardens are accessible at all times and the 2,000 m^2 greenhouses are open six days per week. Each year more than 50,000 visitors enjoy all the Heidelberg Botanic Garden has to offer. In addition, the Garden hosts its annual *GardenFest* – including outreach activities of COS scientists – which alone attracts 1.000 visitors. Besides the permanent exhibitions, two special exhibitions developed by the Association of Botanic Gardens were presented (»Water for All of Us« and »The Last of its Kind – Threatened Plant Species in Botanic Gardens«).

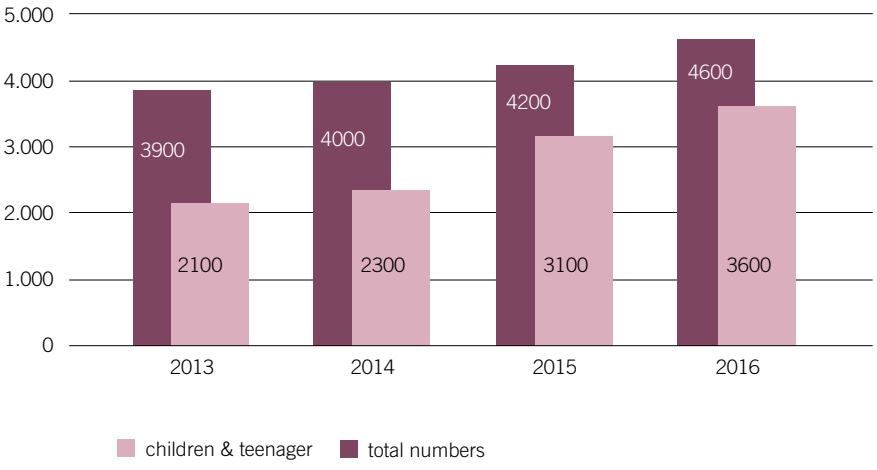

Figure 1
Participants Public Education Programs

A Garden for the Future – General Garden Refurbishment Plan
In 2015/2016 the Botanic Garden celebrated its 100th anniversary on Campus and the several dislocations since 1593. The historical buildings and glasshouses, but also technical installation outdoor are awaiting urgent and fundamental refurbishment. A new concept has been developed for the next 100 years to cope with the need for future challenges. This concept carefully considers the past history and refurbish original building structures while demolishing satellite greenhouses, but *vice-versa* concentrating greenhouse facilities within a new and modern complex. As a first step towards the modern structure a new building uniting administrational, scientific, teaching and social aspects has been opened in 2016.

The concept has been approved by university and Federal State and is awaiting its realization in near future.

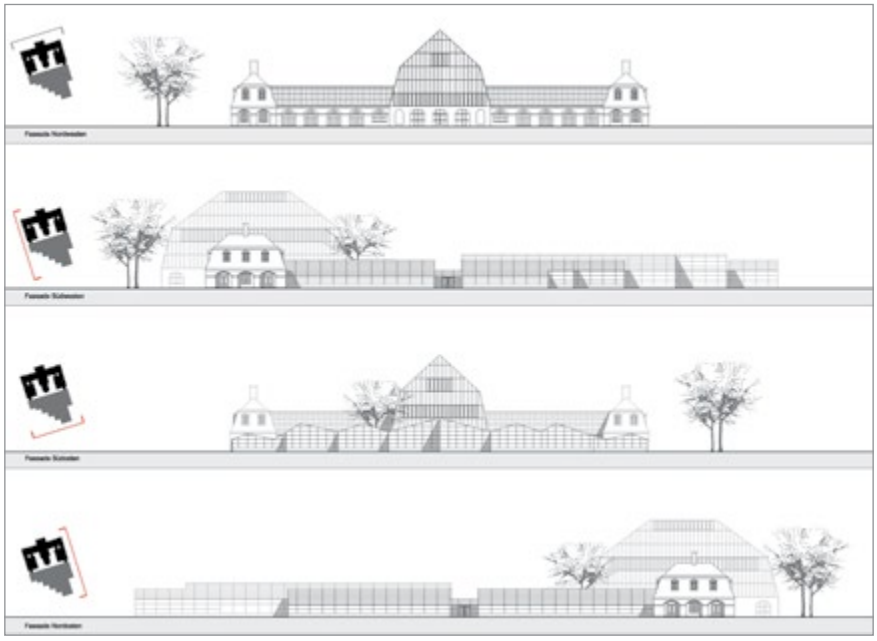

Figure 2
Future Garden Refurbishment Plans, Draft by Haas Architekten BDA

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Jiao W.-B., Accinelli G.G., Hartwig B., Kiefer C., Baker D., Severing E., Willing E.-M., Piednoel M., Woetzel S., Madrid-Herrero E., Huettel B., Hümann U., Reinhard R., Koch M.A., Swan D., Clavijo B., Coupland G., Schneeberger K. (2017) Improving and correcting the contiguity of long-read genome assemblies of three plant species using optical mapping and chromosome conformation capture data. Genome Res., doi: 10.1101/gr.213652.116.

Novikova P.Y., Hohmann N., Nizhynska V., Tsuchimatsu T., Ali J., Muir G., Guggisberg A., Paape T., Schmid K., Fedorenko O.M., Holm S., Säll T., Schlötterer C., Marhold K., Widmer A., Sese J., Shimizu K.K., Weigel D., Krämer U., Koch M.A., Nordborg M. (2016) Sequencing of the genus *Arabidopsis* identifies a complex history of nonbifurcating speciation and abundant trans-specific polymorphism. Nature Genetics 48, 1077–1082, doi:10.1038/ng.3617

Kiefer M., Schmickl R., German D.A., Mandáková T., Lysak M.A., Al-Shehbaz IA, Franzke A., Mummenhoff K., Stamatakis A., Koch M.A. (2014). *BrassiBase*: Introduction to a Novel Knowledge Database on Brassicaceae Evolution. Plant Cell Physiol. 55 (1): e3, doi: 10.1093/pcp/pct158.

Schröder C.N., Sack P., Koch M.A. (2014) Some notes on original material and locus classicus of *Deuterocohnia meziana* Kuntze ex Mex var. *carmineo-viridiflora* Rauh. Plant Divers. Evol. 131/4, 1–15.

Dönmez A.A., Uğurlu Aydin Z., Koch M.A. (2017) *Aubrieta alshehbazii* (Brassicaceae), a new species from Central Turkey. Phytotaxa 299, 103–110.

CURATORIAL RESEARCH ACTIVITIES IN LIVING COLLECTIONS AND THE HERBARIUM

Curator Living Collection Botanic Garden

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Fields of Interest
Living collection management, evolution of Brassicaceae, evolutionary biology

Brief Summary of Work & Major Contributions Since 2013
Botanic Gardens hold documented collections of living plants for the purposes of scientific research, education, conservation and display. Therefore, curatorial practice includes topical fields of acquisition, documentation, preservation, and use of these collections. As the specimens are living organisms, our collections require a constant and diligent attention. Documenting collections is a facet of Botanical Gardens that thoroughly distinguishes it from other plant collections like parks with very limited reference value. One important part of my curatorial work during the last years was to achieve maximum improvement of documentation for many thousand accessions and to boost the impact of our collections to research and society through an improved online access to this data. So far, only very few German Botanic Garden present their collections online and our Botanic Garden is currently the only one providing full documentary data for its accessions.
In my research, I am focused on Brassicaceae phylogenetics and biogeography. Recently molecular dating analyses played a major role.

Figure 3
Tillandsias hanging in their »summer habitat«

Selected Publications since 2013
Number of peer-reviewed articles 2013-2017: 5, number of citations 2013-2017: 43, h-index (2013-2017): 2, total h-index: 15 (according to Thomson Reuters)

Franzke A., Sharif Samani B-R., Neuffer B., Mummenhoff K., Hurka H. (2017). Molecular evidence in *Diplotaxis* (Brassicaceae) suggests a Quaternary origin of the Capeverdean flora. Plant Syst. Evol. 303, 467–479.

Franzke A., Koch M. A., Mummenhoff K. (2016). Turnip Time Travels: Age Estimates in Brassicaceae. Trends Plant Sci. 21, 554–561.

Salariato D. L., Zuloaga F. O., Franzke A., Mummenhoff K., Al-Shehbaz I. A. (2016). Diversification patterns in the CES clade (Brassicaceae tribes Cremolobeae, Eudemeae, Schizopetaleae) in Andean South America. Bot. J. Linn. Soc. 181, 543–566.

Neuffer B., Hurka H., Friesen N., German D. A., Franzke A. (2014). Evolutionary History of the Genus *Capsella* (Brassicaceae) – *Capsella orientalis*, New for Mongolia. Mong. J. Biol. Sci. 12, 3–18.

Kiefer M., Schmickl R., German D. A., Mandáková T., Lysak M. A., Al-Shehbaz I. A., Franzke A., Mummenhoff K., Stamatakis A., Koch M. A. (2014) *BrassiBase*: Introduction to a Novel Knowledge Database on Brassicaceae Evolution. Plant Cell Physiol. 55 (1): e3, doi: 10.1093/pcp/pct158.

Herbarium Research Associate

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Tel.: 06221 54-5701
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E-Mail: dmitry.german@cos.uni-heidelberg.de

Fields of Interest
Taxonomy, systematics, Brassicaceae, trait evolution

Brief Summary of Work & Major Contributions Since 2013
Herbaria are a major source of biological material for research purposes, but they are also unique archives of biological knowledge. Key to this knowledge are the names of the objects, with its most important »currencies«: species, genera and families. One of my particular interests is the taxonomy of Brassicaceae; and within the *BrassiBase* research framework (DFG SPP 1529) we released recently a new species checklist of the entire family, which is actually being used in collaboration to re-structure large collections such as Zurich (Z+ZT) with nearly 100,000 vouchers from the Brassicaceae. During the last five years we described 2 new species, 2 new genera, published 139 new taxonomical combinations. 35 and 1 synonyms have been established on species and genus level, respectively. And, finally, over 100 lectotypifications have been performed.

Figure 4
Spirit collection in the herbarium

Selected Publications since 2013

Number of peer-reviewed articles 2013-2017: 33, number of citations 2013-2017: 205, h-index (2013-2017): 5, total h-index: 10 (according to Thomson Reuters)

Koch M. A., Karl R., German D. A. (2017) An underexplored biodiversity of Eastern Mediterranean biota: Systematics and evolutionary history of the genus *Aubrieta* (Brassicaceae). Ann. Bot. 119 (1): 39–57.

Friesen N., German D. A., Hurka H., Herden T., Oyuntsetseg B., Neuffer B. (2016) Dated phylogenies and historical biogeography of *Dontostemon* and *Clausia* (Brassicaceae) mirror the palaeogeographic history of the Eurasian steppe. J. Biogeogr. 43: 738–749.

Španiel S., Kempa M., Salmerón-Sánchez E., Fuertes-Aguilar J., Mota J. F., Al-Shehbaz I. A., German D. A., Olšavská K., Šingliarová B., Zozomová-Lihová J., Marhold K. (2015) AlyBase – database of names, chromosome numbers, and ploidy levels of Alysseae, with new generic concept of the tribe. Pl. Syst. Evol. 301: 2463–2491.

Al-Shehbaz I. A., German D. A., Moazzeni H., Mummenhoff K. (2014) Systematics, tribal placements, and synopses of the *Malcolmia* s. l. segregates (Brassicaceae). Harvard Pap. Bot. 19: 53–71.

Koch M. A., German D. A. (2013) Taxonomy and systematics are key to biological information: *Arabidopsis*, *Eutrema (Thellungiella)*, *Noccaea* and *Schrenkiella* (Brassicaceae) as examples. Front. Pl. Sci. 4: e267.

3.6 ZOOLOGICAL COLLECTION

The collection contains specimens allowing insight into zoo-geography, systematics and comparative anatomy. Additional topics covered are domestication, wildlife conservation as well as specimens of extinct species. The large collection of insects pinned and displayed in more than 500 showcases illustrates the collector's spirit of the early times of the collection. A large and systematic collection of sea and snail shells is stored in more than 200 drawers.

A wide range of species is represented in the collection of bird skins stored in the magazines. There is also a number of historic teaching specimens, prepared by the scientists of the former Zoological Institute to highlight blood vessels and body cavities. Some specimens have been reproduced in glass by a glass artist to retain realistic body coloration. A prominent example is the glass representation of a Siphonophore with differentially colored individual polyps.

During the extensive renovation of the building INF 230, the Zoological Collection is currently safely stored. With the completion of the renovation the collection will be made accessible to the public representing a new and timely concept.

The concept: Timeline Evolution of the COS Heidelberg
»Nothing in Biology Makes Sense Except in the Light of Evolution«. This famous phrase of C.T. Dobzhansky (1973) becomes even more meaningful in the post-genomic time where hundreds and in the future even hundred thousands of genomes from different species will be available. Today it is a realistic scenario to reconstruct and trace back the evolution of life on our planet by combining paleontological and molecular data. Although many of the scientifically meaningful specimens of the original collection have been lost when the Zoological Institute moved from its original location in the Sophienstrasse to the Neuenheimer Feld 230, or when they were outsourced to the Senckenberg Research Institute and Natural History Museum in Frankfurt in 2004, there are still some remarkable specimens of the Zoological collection that are of general public interest. These specimens were part of the »Zoologisches Cabinett« founded by Friedrich Tiedemann (1819), a committed fighter against slavery, and it contained specimens collected by Carl Gegenbaur (1826-1903), Otto Bütschli (1848-1920) and others. COS is therefore planning a permanent exhibition with the format of an evolution timeline. This timeline will highlight the important periods during the 4,5 billion years of evolution on planet Earth.

Figure 1
Tasmanian Wolfe (also called Tasmanian Tiger, *Thylacinus cynocephalus*)

The principal aim of this timeline is to give the visitor an understanding of Darwinian evolution in the context of new findings of molecular and genome biology. Although there are still many open questions, we are beginning to get mechanistic view on the origin of life and how the major clades in tree of life evolved. In this context, systems biology approaches are important, as L.v. Bertalanffy has outlined them for the first in his general system theory. The central theme of this timeline is defined by the evolutionary process itself, which was starting with simple self-replicating biomolecules up to humans and the threat of our planet by mankind. We are planning the following topics: (i) origin of life, (ii) Cambrian explosion, (iii) Origin of biodiversity, (iv) mass extinction caused by astro- and geophysical catastrophes as well global biogenic factors, (v) the origin of humans and finally (vi) patterns and mechanism of the evolutionary process. At the moment an internationally highly renowned designer and expert for collections, exhibitions, and museums is implementing our evolution time-line concept. One highlight will be the radiation of the marsupians, mammals living primarily in Australasia and the New World with the common characteristic of a pouch in which the embryo is carried and protected after birth. Here, our Zoological collection has a number of unique specimens collected by the late Heinz Möller, including the Tasmanian Wolfe (also called Tasmanian Tiger, *Thylacinus cynocephalus*), the largest carnivore marsupian, extinct in 1936 (Figure 1). Another example is the passenger pigeon (*Ectopistes migratorius*), which was one of the most common birds on our globe before it became extinct at the end of the 19th century in North America.

APPENDIX

A

A.1 COS FUNDING

Finances

As a central research institution of Heidelberg University, the Centre for Organismal Studies Heidelberg (COS) receives basic funding by the state of Baden-Württemberg through the budget of the University (internal funding). Research group leaders at COS are very active in acquiring additional funding from several different funding organizations (external funding). While the internal funding increased very moderately during the reporting time, the acquired third party funding increased further and almost doubles the internal income (Figure 1): The ratio between internal and external funding varies between 1,8 (2013) and 2,1 (2016). Internal Funding mostly provides for staff appropriations.

The main funding organization for external grants is the Deutsche Forschungsgemeinschaft (DFG) through several different funding instruments such as Collaborative Research Centers (CRCs), Excellence Initiative (ExIni) and research grants including the Emmy Noether Programme. Other funding bodies are the European Union (grants by the European Research Council (ERC), Career Integration Grant (CIG), International Training Network (ITN) within Framework Programme 7 and Horizon 2020), the Bundesministerium für Bildung und Forschung (BMBF), Foundations (Boehringer Ingelheim Foundation, Klaus Tschirra Stiftung, Baden Württemberg Stiftung, Lautenschläger Stiftung etc.) and others (including industry funding, equipment purchases, scholarships). The strong increase in third party funding can be explained such that more research groups participate in research networks like CRCs and within the Excellence Initiative and that an additional eight independent research groups started at COS during the reporting period. This status group contributes with an average of 25 % to third party funding of COS.

Figure 1
COS global finances: Government sources versus external funding in € p.a. from 2013 till 2016. Government sources without individual financial offers of appointment (Berufungszusagen), funds from open positions (Mittelschöpfung) and project-bound state funding (Zweitmittel). Numbers according to the budget of Heidelberg University and SAP expenses.

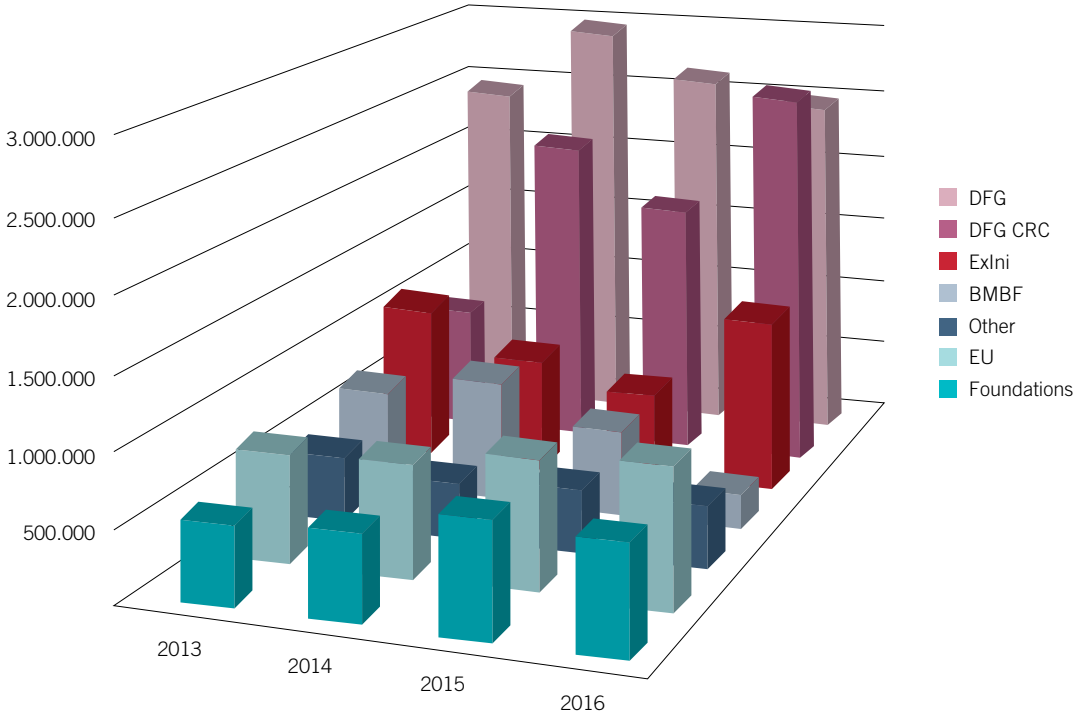
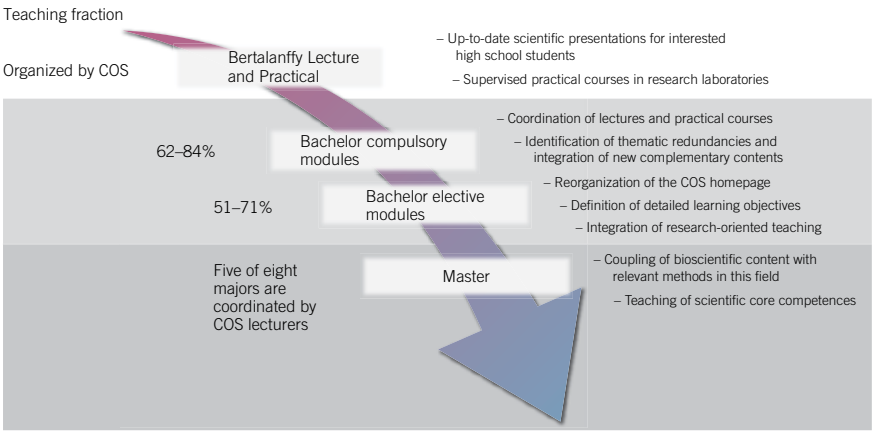


Figure 2
Detailed analysis of external funding 2013 – 2016 based on SAP expenses. Abbreviations: BMBF Bundeministerium für Bildung und Forschung, CRC Collaborative Research Center, DFG Deutsche Forschungsgemeinschaft, EU European Union, ExIni Exzellenzinitiative des Bundes und der Länder.

A.2 COS TEACHING

With 13 full professors and numerous other staff involved in its teaching effort, COS is the biggest contributor to teaching within the faculty of biosciences at Heidelberg University. This presents COS with the responsibility but also the great opportunity to provide excellent teaching encompassing a broad range of topics at all stages of our students' academic career – from Bachelor studies to Master and PhD programs. COS aims to present the same philosophy in its teaching efforts that characterizes its research interests: COS teaching ranges from fundamental topics of biology like molecular biology, biochemistry, and evolution to the central principles of development and physiology all the way to the most complex topics like neurobiology and ecology. At all of these stages, a strong presentation of the idea of organismic biology is emphasized to allow students to view the biological sciences as a complex web of closely interconnected fields that transcends the boundaries of single model organisms or narrow fields of interest. All of the COS-teaching aims at a strong research orientation. This aims to allow students not only to understand the concepts presented, but also to learn how scientific ideas are arrived at, with the ultimate goal to enable them to develop their own ideas, and become active, highly motivated young scientists.

These guiding ideas of COS are incorporated into academic studies as soon as possible, starting with the early bachelor studies. Of these, two programs are currently running at Heidelberg University: The Bachelor Biowissenschaften as a core scientific degree program and the Bachelor Biologie 50 % as a program for future teachers, which is complemented by a second field of study. In these programs COS lecturers provide 77 % and 84 % of compulsory practical courses, respectively. Of the compulsory lectures 62 % are offered by COS lecturers for both programs. Moreover, regarding the compulsory elective modules, COS provides 51 % and 71 % in the Bachelor Biowissenschaften and Bachelor Biologie 50 %, respectively. Consequently, COS is a central player when it comes to the education of our future scientists and teachers, representing an optimal foundation to integrate the institute's teaching concepts to the curricula but also giving COS a great responsibility in providing an overall excellent student education in the Heidelberg life sciences.



In recent years several measures have been taken to further improve COS' contribution to teaching. One crucial project was to optimize the coordination of compulsory lectures and the corresponding practical courses within semester one and three with respect to their content and time flow. This reorganization aims at encouraging an efficient transfer of the theoretical knowledge acquired to the practical experiments. During these reorganizations we were also able to identify thematic redundancies, especially within the compulsory lectures, which created space to integrate new complementary contents. Overall these measures of coordination and streamlining aim at demonstrating the scientific impact of different subjects and facilitates the student's capability for sustainable deep learning.

Another central goal continually developed is to increase the transparency of specific requirements within the curricula as well as in individual teaching modules. This included the reorganization of the teaching pages of the COS homepage, which now present a detailed and clearly arranged overview of the Bachelor curricula Biowissenschaften and Biologie 50 %. The revised web site allows current students as well as those interested in our programs a quick overview of the institute's teaching offers as well as their optimal combination with respect to module content and time.

To further promote an efficient teaching environment we defined detailed learning objectives for the COS coordinated compulsory lectures and practical courses in the first and third semester. These include defined knowledge concerning the theoretical scientific background but also focus on important basic working techniques. To communicate these requirements the general e-learning presence of COS teaching modules was extended to now include teaching documents and learning objectives and therefore enable a directed support of learning processes especially in the early phase of studying. In addition for the basic lectures of semester one and three, two collections of multiple choice questions similar to those presented in the exams were created and included as an interactive module, which allows students to analyze their own learning progress.

The central idea of COS includes the implementation of organismic and integrative working not only in research but also in teaching. This can be seen in the basic practical courses, where the fields of anatomy, organic phyla, physiology, and developmental biology are taught with different model systems, which facilitates a direct extraction of common and unique characteristics. A further measure to integrate this guiding idea into the curriculum was the conception and realization of the modular three-week practical course Red-Green Bridges. Its modular structure allows the science oriented deepening of a self-chosen research topic out of a spectrum of different scientific fields. Moreover, a specific scientific question is investigated to the same extent in animal and plant systems to demonstrate the gains achieved by an approach going beyond the confines of a single clade of model organisms.

A very important stage at which we spread the organismic idea already during school life is the education of future teachers in the Bachelor program Biologie 50 %, which has been established in the winter semester 2015/16 to supersede the former Lehramtsstudium. In addition to providing our well-established teaching modules for this new program we developed a new seminar in cooperation with the Heidelberg School of Education, which combines organismic knowledge with didactics (Vertiefungsseminar: Fachwissenschaft trifft Fachdidaktik). Here students show their scientific knowledge in respective seminar presentations first, and then integrate the theory into practice, while planning as well as holding a school teaching unit.

COS is also a strong contributor to the international Master of Molecular Biosciences program of Heidelberg University. Of the eight majors offered in the master program five are currently coordinated by lecturers from COS (Development and Stem Cell Biology, Evolution and Ecology, Molecular Plant Sciences, Neurosciences, and Systems Biology) and the majors Development and Stem Cell Biology, Evolution and Ecology, and Molecular Plant Sciences are fully organized by COS lecturers. One aim of these majors is, according to the COS philosophy, to break the boundaries between classical fields and encourage the students to gain experience in other related bioscientific areas, e.g. via lab rotations in groups formally listed in another major.

Throughout the major program research-oriented teaching is a major goal, providing the students with the theoretical and technical knowledge and the practical lab experience required for successful scientific work. One contribution to this goal has been a reorganization of the first lecture of the master program, attended by students of all majors, Frontiers of BioScience I. This lecture now presents bioscientific content coupled directly to the methods relevant for establishing the presented knowledge to encourage students to not just memorize facts but rather gain a deeper understanding of the scientific process that leads to the establishment of knowledge. The following lecture Frontiers of BioScience II has also been reorganized and now contains a mandatory module regarding scientific core competences like writing, research ethics, statistics, and image processing. After this module, every student can choose from two biological fields to study in depth. Overall this program allows its attendants to acquire core competences and a broad knowledge base while also specializing on specific fields of interest which can subsequently be expanded on in their laboratory work.

With regard to the master program, another important issue the COS-teaching staff has been working on is the improvement of our internet presence as well as advertising our majors, e.g. via posters. These measures not only aim at increasing our visibility on and beyond the Heidelberg campus and attract the best applicants to our program, but also to inform future master students as well as the interested public on the scope and content of our contributions to the master program.

Beyond its commitment in core university teaching, COS also offers the Bertalanffy program directed towards high schools, allowing interested students to get a first glance at university level biology. The program consists of the Bertalanffy lectures taking place twice a year where distinguished scientists present their work in two lectures for pupils and scientists, respectively. These lectures are regularly attended by about 200 high school students and 30-100 university students and are accompanied by tutorials that allow all participants to address questions regarding the science presented and the life and work at a university. In addition COS offers the two week Bertalanffy practical as a summer school allowing highly motivated participants to gain first lab experience before choosing their study.

To support the lecturers working at COS, to coordinate teaching efforts, and to continuously develop our teaching program we established a team consisting of a Hochschule 2012-professor (Prof. Dr. Ingrid Lohmann) and a teaching coordinator (Dr. Roland Gromes). This team was further reinforced from 2013 to 2017 by a second teaching coordinator (Dr. Natalie Keib up to 2014, Dr. Monika Huber afterward) financed via an Innovations- und Qualitätsfonds (IQF) grant provided by the Ministerium für Wissenschaft, Forschung und Kunst Baden-Württemberg. This team aims to continue working on improving, evaluating, and expanding the COS teaching effort to meet all challenges encountered in the next years.

A.3 COS EVENTS

Events: Seminars, Symposia and Public Outreach Activities
Researchers at COS engage in scientific events such as lectures, seminars and symposia as well as events for the interested public with the aim to provide stimulating scientific discourse for the research community reflecting the diverse research interests of COS Heidelberg, and to present selected topics in an easy to understand manner to a general audience beyond the Heidelberg life science campus.

Lectures at COS
The COS Lecture Series is running since 2014. On a monthly basis, a speaker is invited to talk about a research topic of general interest to the COS scientific community. PhD students and postdocs each have once a year one slot available to invite a speaker of their interest.

COS Lectures 2014–2016

Date	Invited Speaker	Title
21.01.2014	Cyril Zipfel	Regulation of early receptor kinase-mediated innate immune signalling
10.04.2014	Damian Brunner	Mechanisms and principles of tissue gap closure
10.07.2014	Chao Yang, Burkhard Höckendorf	Schmeil-Awards
22.07.2014	Gerd Jürgens	Plant cytokinesis – a tale of membrane traffic and fusion
28.10.2014	David Miller	Stress responses and the apoptotic repertoire of the coral Acropora millepora
08.01.2015	Dagmar Iber	From Networks to Function – Computational Models of Organogenesis
05.02.2015	Magdalena Götz	Neural stem cells in homeostasis and after brain injury
12.02.2015	Christiane Gatz	Functional analysis of plant-specific CC-type glutaredoxins
12.03.2015	Timothy Sharbel	Evolutionary approaches to deciphering the functional switch from sexual to asexual (apomictic) reproduction in natural plant populations
07.04.2015	Panagiotis Tsonis	Dissecting regeneration through the lens
16.04.2015	Eva Benkova	Hormonal regulation of root system architecture
30.04.2015	Hernán Lopez Schier	Building and regenerating a sensorineural circuit underlying polarised mechanoreception
21.05.2015	Francois Parcy	Structural insights into the function and the evolution of a master floral regulator
26.06.2015	Julian Schroeder	Drought-Induced Abscisic Acid Signalling and Atmospheric CO2 Sensing in Plants
09.07.2015	Josephine Adams	Thrombospondins are conserved players in metazoan extracellular matrix organisation
12.08.2015	George W. Bassel	Complex systems analysis of plant development
20.08.2015	Markus Grebe	Cytoskeletal Organization and Lipid Domain Function During Planar Polarity Establishment In Arabidopsis


Vertebrate organ origins
Ludwig von Bertalanffy
DARWIN 2.0
NEW TOOLS TO GO THROUGH TIME


Centre for
Organismal
Studies
Heidelberg

17.09.2015	Yves Gibon	Fruit Systems Biology
28.09.2015	Vsevolod Belousov	Novel molecular tools for redox imaging and metabolic engineering
26.10.2015	Minoru Tanaka	New roles of germ cells – sex determination and fecundity
12.11.2015	Sinichi Sunagawa	Eco-Systems Biology of the Human Gut and the Global Ocean Microbiome
08.10.2015	David Twell	Plant male germline development: a life after meiosis
10.12.2015	Dagmar Iber	From Networks to Function – Computational Models of Organogenesis
21.01.2016	Mario Malagoli	How should we feed the planet?
11.02.2016	George Coupland	Comparative analysis of pathways controlling seasonal flowering in annual and perennial Brassicaceae species
14.04.2016	Wolfgang Busch	Uncovering key genes and networks regulating root growth using systems genetics
27.04.2016	Martin Groth	Metabolic control of DNA methylation in Arabidopsis
09.06.2016	Muriel Perron *	Retinal stem cells and the Hippo/YAP pathway
07.10.2016	Edward Farmer *	Long distance signalling of wounded plants
10.11.2016	Antony Dodd	Circadian and environmental regulation of chloroplasts
12.01.2017	Richard Baines	Setting the Set-point: A challenge for neuronal homeostasis
09.03.2017	Thomas Ott	Dynamics of plant cell surface receptors and partners
06.04.2017	Ildoo Hwang	Epistructural control on RNA G-rich element specifies phloem differentiation
11.05.2017	José Pardo	Molecular connections between salinity stress signaling and the regulation of flowering time in Arabidopsis
22.09.2017	John Allen	Why do chloroplasts and mitochondria contain DNA?
12.10.2017	Robert Patrick Zinzen	The Drosophila embryo at single cell transcriptome resolution
14.11.2017	Peter Brodersen	Insights into the requirement for small RNA methylation in plants
23.11.2017	Isabel Bäurle	Adaptation to environmental stress by a chromatin-based stress memory in Arabidopsis

* Student or postdoc organized COS Lecture
Further information can be found on the COS webpage.

Seminars and Seminar Series at COS

Apart from seminars organised by each research group fostering scientific exchange within COS and on campus, the PhD students and postdocs at COS are organising a seminar series since 2014 solely for the COS community, the »COS PhD/Postdoc Seminar«. This student and postdoc organized series is held on a weekly basis and provides opportunity to present research concepts for PhD students, postdocs and research group leaders.

Symposia at COS
COS Symposia are organized on a biannual basis reflecting a topic selected by the research group leaders of COS. Renown experts from inside and outside COS are invited, short talks selected from abstracts as well as poster sessions provide a platform for PhD students and postdocs to present their projects. COS symposia have received generous financial support by the Klaus Tschira Foundation, HBIGS, GfE, EMBO, Eurofins Genomics, elife and Nikon.

3rd International COS Symposium
»Building Beauty – From Genes to Shape«, June 20 and 21, 2013

Session 1: Cell

Alain Prochiantz, Paris, FR	The simple beauty of homeoprotein transduction
Andrea Brand, Cambridge, UK	Stem cells to synapses: regulation of self-renewal and differentiation in the nervous system
Olivier Pertz, Basel, CH	Building a polarized fibroblast – From cytoskeletal dynamics to shape
Mihaela Zigman, Heidelberg, DE	From individual cell polarization to coherent tissue morphogenesis: building the zebrafish neural tube
Guido Grossmann, Heidelberg, DE	Membrane polarization through selective diffusion barriers and anisotropic protein dynamics
Ulrich Schwarz, Heidelberg, DE	On force and form: what cell and tissue shape can tell us on actomyosin force generation

Session 2: Tissue

Jan Traas, Lyon, FR	From genes to shape: morphodynamics at the shoot apical meristem
Damian Brunner, Zürich, CH	Cytoskeleton architecture, force generation and mechanistic principles of tissue closure
Ingrid Lohmann, Heidelberg, DE	Hox Control of Drosophila Feeding Movements
Khaled Khairy, Heidelberg, DE, and Janelia Farms, US	A spherical harmonics-based approach to developmental mechanics in the fruit fly
Francois Graner, Paris, FR	Fly thorax morphogenesis: from cell dynamics to tissue shape

Session 3: Organisms

Alexis Maizel, Heidelberg, DE	Lateral root morphogenesis in Arabidopsis thaliana
Erez Raz, Münster, DE	Motility and directed migration of primordial germ cells in zebrafish
Venera Altapova, Heidelberg, DE	X-ray phase-contrast imaging for developmental biology
Przemyslaw Prusinkiewicz, Calgary, CA	Biology, Computation, and Art: Understanding the Form of Trees

4th International COS Symposium
Darwin 2.0 – New Tools to Go Through Time, June 17, 2015

Session 1: Ecology & Evolution

Francesca Benzoni, Milan, IT	Upheavals in coral taxonomy: Findings from the TARA Oceans expedition, a modern-day "Voyage of the Beagle"
Emmanuel Gaquerel, Heidelberg, DE	Genomic bases of key metabolic innovations in the genus Nicotiana
José Jiménez-Gómez, Cologne, DE	eQTL analysis using allele-specific expression
John Pannell, Lausanne, CH	Transitions between combined and separate sexes in plants

Session 2: Molecular Evolution

Alexandros Stamatakis, Heidelberg, DE	Computational biology as computational science: Challenges and problems
Nicolas Gompel, Munich, DE	Regulatory evolution and the diversification of pigmentation patterns in Drosophila
Gáspár Jékely, Tübingen, DE	Systems neurobiology of the Platynereis larva
Przemyslaw Prusinkiewicz, Calgary, CA	Biology, Computation, and Art: Understanding the Form of Trees

Session 3: Evolution & Development

Ulrich Technau, Vienna, AU	Insights into evolution of eumetazoan regulatory developmental networks from the sea anemone Nematostella vectensis
Steffen Lemke, Heidelberg, DE	Evolution of cell and tissue coordination during fly gastrulation
Graham Budd, Uppsala, SE	Crossing the line: New perspectives on the Precambrian-Cambrian transition in the fossil record
Przemyslaw Prusinkiewicz, Calgary, CA	Biology, Computation, and Art: Understanding the Form of Trees

5th International COS Symposium
Senses and Sensitivity, June 21 and 22, 2017

Session 1: Sensory machineries

Jonathan Jones, Norwich, UK	EMBO Kenote Lecture: Plant immune receptors: dissection, diversity, and deployment
Lucia Prieto-Godino, Lausanne, CH	Evolution of olfactory circuits in Drosophila: zombie genes and other surprises
Chris Bowler, Paris, F	Epigenetic phenomena in response to environmental signals in plants and marine diatoms
Charalambos Kyriacou, Leicester, UK	Molecular bases of rhythmic behaviors in animals
Erika Tsingos, Heidelberg, DE (Speaker selected from abstacts)	On growth and form in a complex organ: the neural retina drives postembryonic eye morphogenesis in fishT

Session 2: Processing and decoding

Jürgen Gross, Dossenheim, DE	Chemical communication between phytopathogens, their host plants and vector insects – from basic research to applications in plant protection
Anne Pfeiffer, Heidelberg, DE	Influence of light on shoot stem cell regulation in plants
Michael Brecht, Berlin, DE	Social touch – the cortical neurobiology of physical contact
Marieke Essers, Heidelberg, DE	Hematopoietic stem cells and their niche under inflammatory stress

Session 3: Behavior and phenotypic plasticity

Rainer Hedrich, Würzburg, DE	Venus flytrap – a plant on animal diet
Rüdiger Hell, Heidelberg, DE	Schmeil Award Ceremony for the best PhD in organismal biology at the COS
Aurelio Teleman, Heidelberg, DE	Regulation of mitochondrial functions via a dietary lipid
Eva-Sophie Wallner, Heidelberg, DE (Speaker selected form abstracts)	Strigolactone and karrikin-independent SMXLproteins are central regulators of phloem formation
Anke Steppuhn, Berlin, DE	Plants use insect eggs as telltale signals for an upcoming herbivory
Nicholas Foulkes, Karlsruhe/Heidelberg, DE	Food, light and the evolution of the circadian timing system

Bertalanffy Lecture Series
The Bertalanffy Lecture Series was initiated with the aim to provide a better understanding of integrative approaches in systems oriented biology both for high school students and for scientists on campus. In its sixth year and with eleven events having taken place already, the lecture now attracts regularly more than 200 students from high schools in Heidelberg as well as other cities and resonates very well on campus.

In brief, one event is held over two days: Day one is reserved for high school students in their final two or three years. The lecture is followed by a tutor-lead discussion in small groups of 12–15 persons. During this discussion, the invited speaker tours all subgroups to answer questions personally. Students, tutors (recruited from COS research groups) and speaker finally meet for concluding discussion and remarks. This part of the lecture series is coordinated with the »Stützpunktschulen Molekularbiologie«[1] in Baden Württemberg and provides the opportunity for teachers for continuing education. The lecture is also open to the interested public. On day two, the invited speaker will discuss latest research results with scientists from COS and the Heidelberg life science campus both in a formal lecture and in individual meetings.

Since 2014, the Bertalanffy Lecture Series is complemented by a 2-week summer course for high school students, in which they will work on small research projects.

In a further step, the program was expanded in 2016 by the third element »Science goes to school«. COS PhD students apply to visit biology courses at schools and talk about their research project. This way, high school students gain insight and PhD students train their communication and presentation skills.

1 www.stuetzpunktschulen.de

Date	Invited Speaker	Title
31.01.2013	Enrico Coen, John Innes Centre, Norwich, UK	Leaves, Loops and Leonardo: the Generation of Biological Forms
01.02.2013		Hidden signposts of Development: Tissue Cell Poarity and Its Role In Morphogenesis
02.05.2013	Russell Foster, University of Oxford, UK	Do you take sleep and your body clock sersiously?
03.05.2013		Light and Time: A New Look at the Eye
26.09.2013	Hannah Monyer, Heidelberg University Hospital, DE	Brain, Plasticity, Learning and Memory
27.09.2013		Can Studies in Mice help us understand Memory Functions in Humans?
10.04.2014	Benjamin Prud'homme, IBDM, Marseille, F	How Did The Fly Get Its Spot?
11.04.2014		The Regulatory Mechanism of Morphological Pattern Evolution
18.09.2014	Andreas Trumpp, DKFZ Heidelberg, DE	Cancer Stem Cells – The Root of all Evil in Cancer and Metastasis
19.09.2014		Cancer and Metastasis Stem Cells in Hematopoietic and Solid Tumors
07.05.2015	Martin Wikelski, Max Planck Institute for Ornithology, Seewiesen, DE	Animals as our eyes and ears in the world
08.05.2015		Intelligent environmental sensing via the study of global movement patterns of animals
24.09.2015	Alejandro Sánchez Alvarado, Howard Hughes Medical Institute, Chevy Chase, Maryland, USA	Hypothesis- und curiosity-driven inquiry: the importance of discovery research
25.09.2015		The developmental plasticity of planarians and what they teach us about living, dynamic systems
28.04.2016	Ottoline Leyser, University of Cambridge, UK	Thinking without a brain – how plants decide what to do
29.04.2016		Auxin and the self-organization of plant form
08.12.2016	Marcos Gonzalez-Gaitan, University of Geneva, CH	How do stem cells divide? The physics of asymmetric division
09.12.2016		Asymmetric endosomes in asymmetric division
01.06.2017	Henrik Kaessmann, ZMBH, Heidelberg, DE	Dawn of the Mammals – Revealing Molecular Secrets ...
02.06.2017		The molecular foundations of mammalian phenotypic evolution
26.10.2017	Miguel L. Allende, Universidad de Chile, Chile	Looking for fish in the wrong places: from salt pans to dried up ponds
27.10.2017		Exploring the genomes of South American cyprinidontiform fish displaying unique life histories

Sunday Matinée
The Sunday Matinée was a highly successful lecture series running every winter semester from 1980 till 2017. The aim was to present current topics of life science research and related disciplines to the interested public.

Topics in recent years have been:

WS 2013/14	Der Lebensbogen – Werden, Sein, Vergehen
WS 2014/15	Faszination Biologie: Teil 1
WS 2015/16	Faszination Biologie: Teil 2
WS 2016/17	Faszination Biologie: Teil 3

Since 2001, this very well received lecture series was organized by Prof. Dr. Dr. h.c. Volker Storch, »Seniorprofessor« at the Heidelberg University from 2010 to 2017 and formerly professor at the Heidelberg Institute for Zoology. The matinée was financed through the »Verein der Freunde und Förderer des Zoologischen Museums der Universität Heidelberg e. V.«.

Preschool Education
The two visits of preschool classes to COS in 2016 and 2017 to explore science are starting to establish a new avenue in public outreach for COS. Assisted by PhD students and postdocs, these five and six year old children have the opportunity to get to know plants, flies and anemone used in biological research, take a first mini-microscopy course and conduct very small experiments such as mixing and unmixing colors and even extracting DNA from tomato.

In addition to the here mentioned scientific and outreach programs, research groups at COS participate in many other initiatives on campus, notably the »Ferienforscherkurse« of the »Tschira Jugendakademie«[1] and the »International Summer Science School Heidelberg«[2].

1 www.klaus-tschira-stiftung.de/aktivitaeten.php?we_objectID=1096
2 www.ish-heidelberg.de

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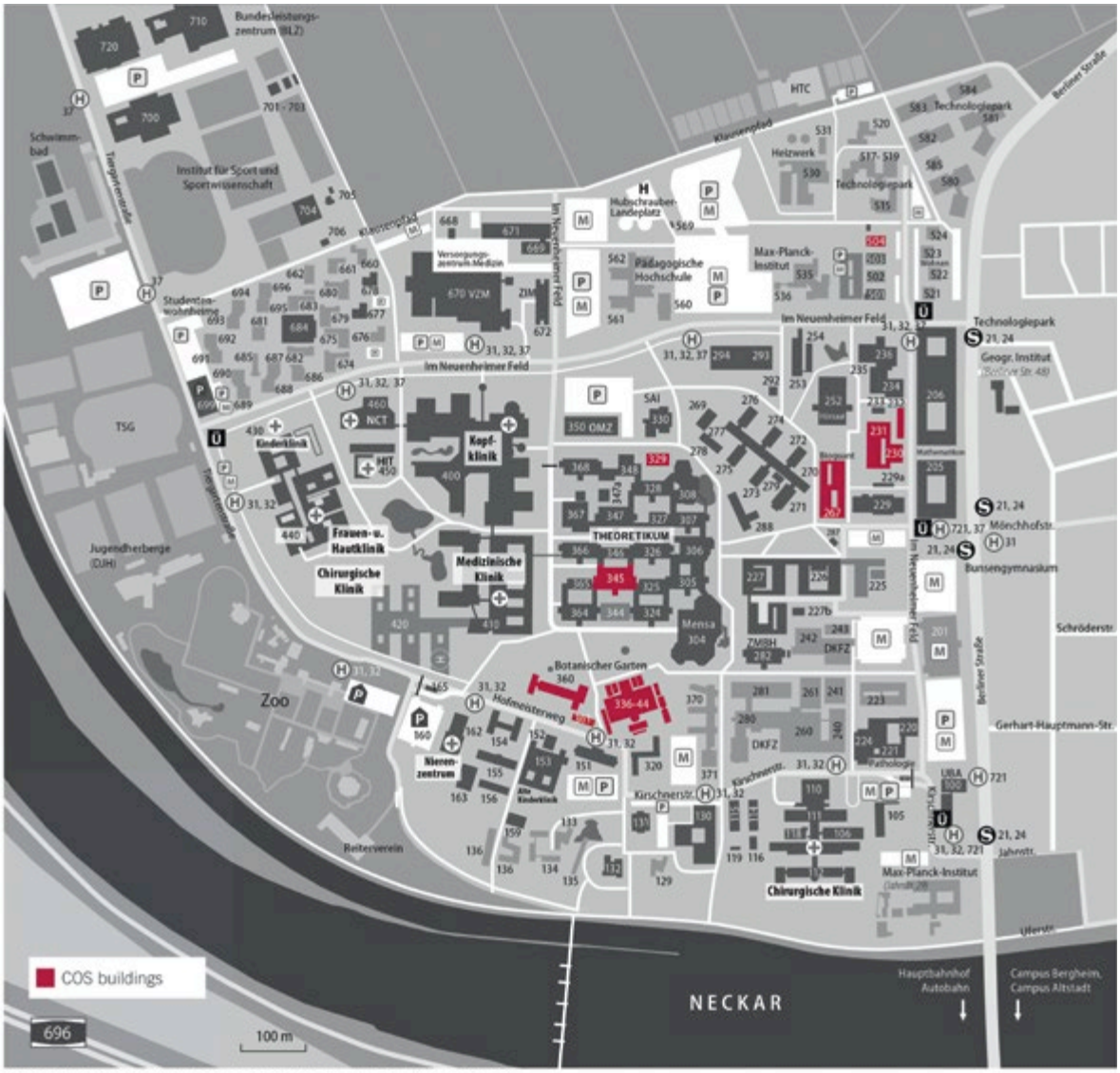

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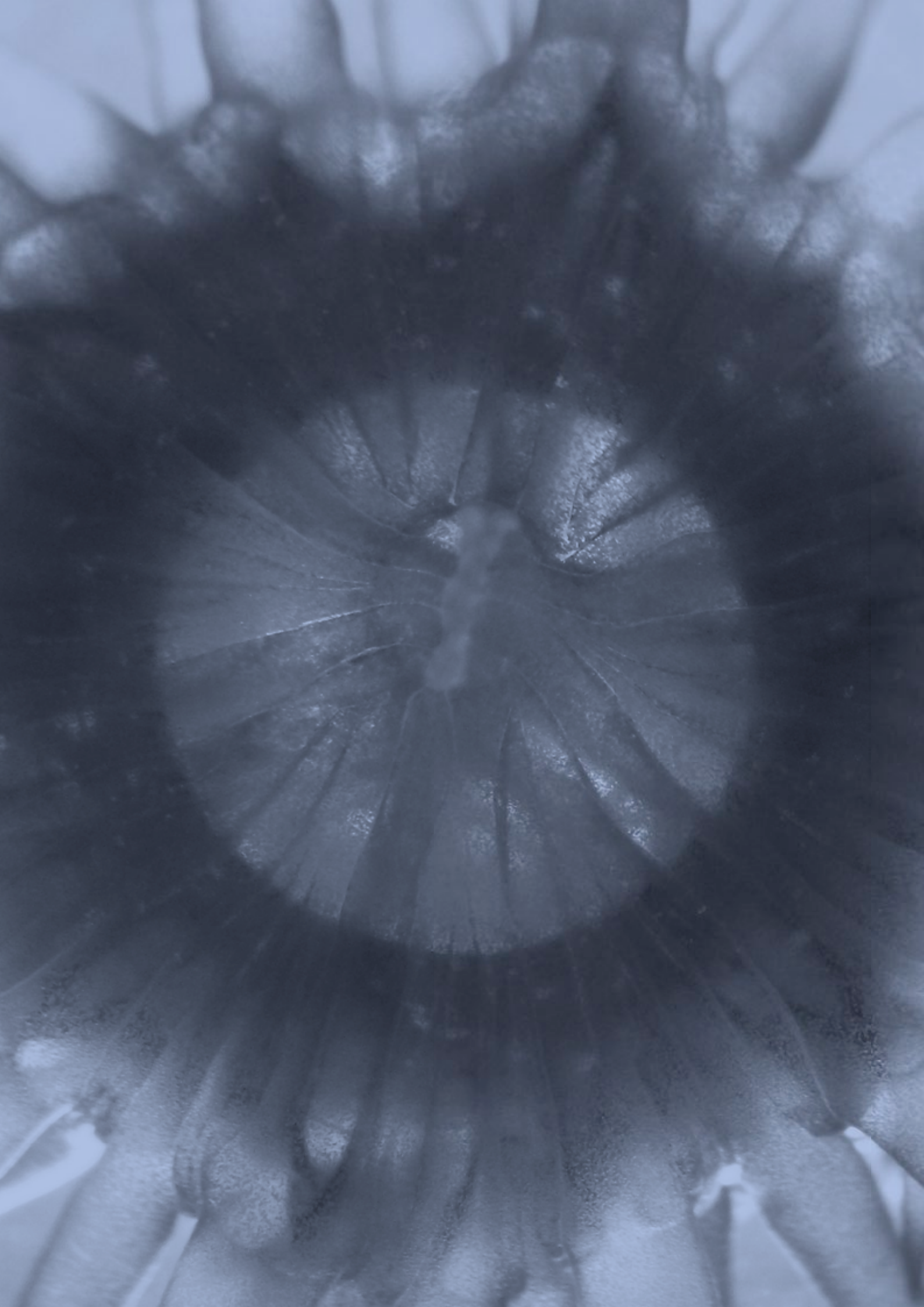

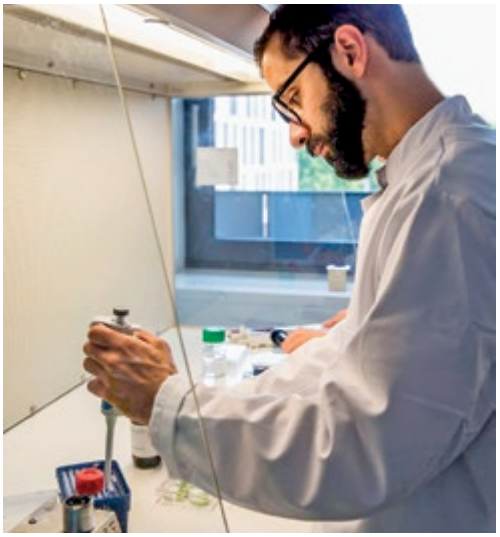

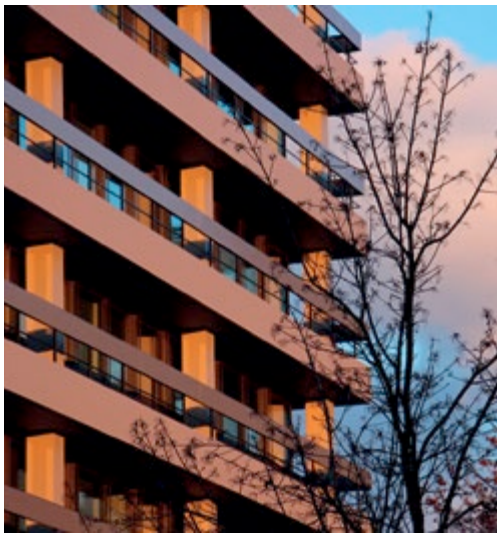

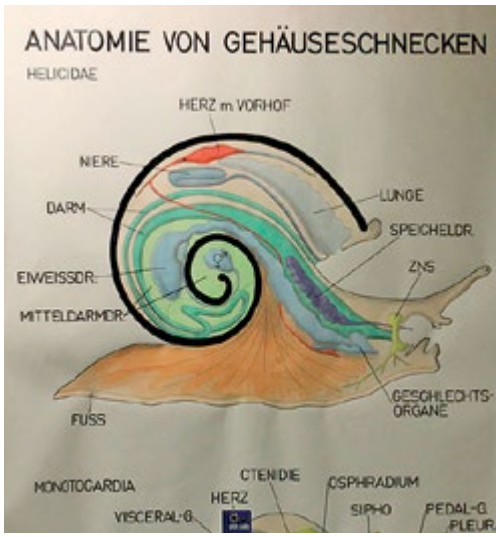
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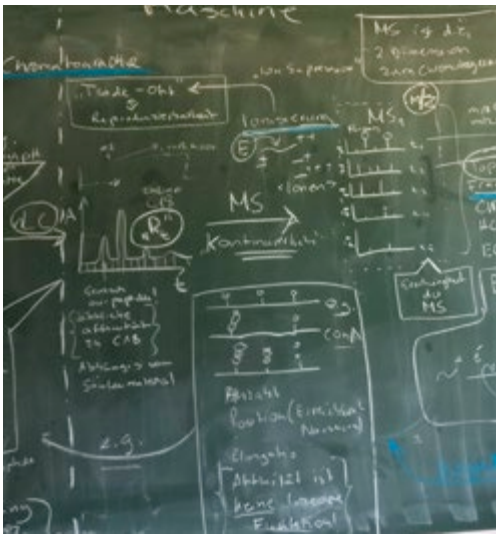
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