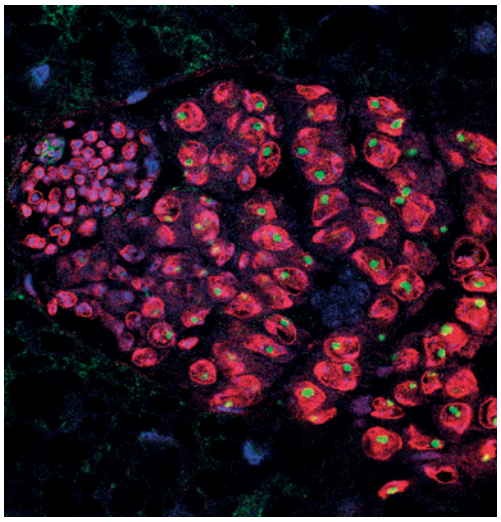
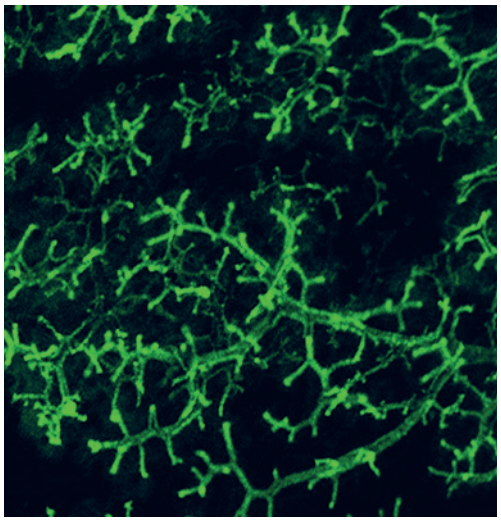
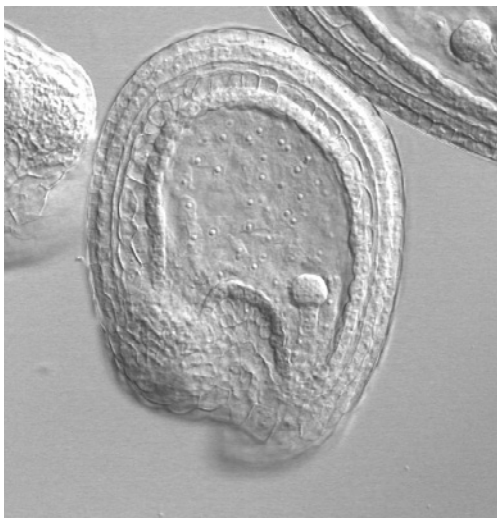
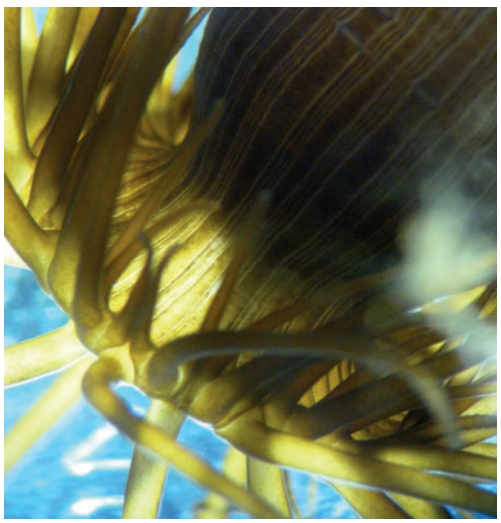
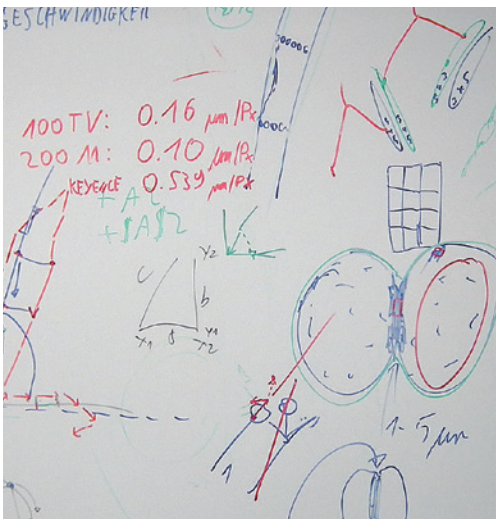
Centre for Organismal Studies Heidelberg

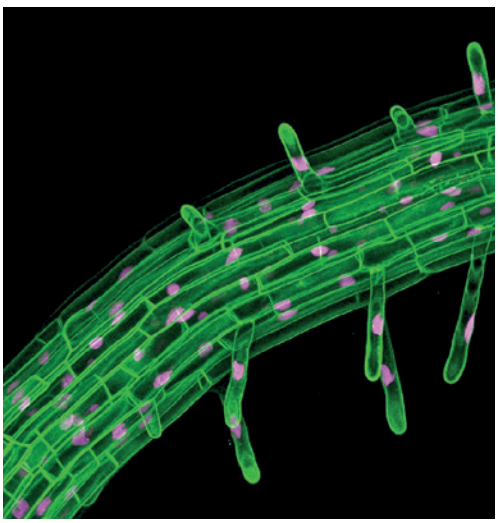
UNIVERSITÄT HEIDELBERG
ZUKUNFT SEIT 1386

REPORT 2009-2013





CONTENT

1 INTRODUCTION **4**
1.1 Preface 5
1.2 Background, development and structure of COS 6
Management and administration 8
1.3 Teaching at COS 9
1.4 Research at COS 10
1.5 Integration in the life sciences on campus 12
1.6 Future perspectives and challenges 14

2 RESEARCH GROUPS **16**
2.1 Animal Evolution, Prof. Dr. Detlev Arendt 17
2.2 Independent Research Group: Dr. Jan Felix Evers 23
2.3 Circadian Clock Biology, Prof. Dr. Nicholas S. Foulkes 29
2.4 Animal Molecular Physiology, Prof. Dr. Stephan Frings 35
Project Leader: Dr. Frank Möhrlen 38
2.5 Independent Research Group: Dr. Emmanuel Gaquerel 41
2.6 Independent Research Group: Dr. Guido Grossmann 47
2.7 Independent Research Group: Dr. Annika Guse 53
2.8 Plant Molecular Biology, Prof. Dr. Rüdiger Hell 59
Project Leader: Dr. Markus Wirtz 64
2.9 Molecular Evolution and Genomics, Prof. Dr. Thomas W. Holstein 67
Project Leader: Dr. Ulrike Engel 72
Project Leader: PD Dr. Suat Özbek 74
2.10 Independent Research Group: Dr. Amal J. Johnston 77
2.11 Biodiversity and Plant Systematics, Prof. Dr. Marcus A. Koch 83
Project Leader: apl. Prof. Dr. Claudia Erbar 88
Project Leader: Dr. Nicolai M. Nürk 90
2.12 Modelling of Biological Processes, Prof. Dr. Ursula Kummer 93
2.13 Independent Research Group: Dr. Steffen Lemke 99
2.14 Developmental Biology, Prof. Dr. Ingrid Lohmann 105
2.15 Stem Cell Biology, Prof. Dr. Jan Lohmann 111
2.16 Independent Research Group: Dr. Alexis Maizel 117
2.17 Developmental Neurobiology, Prof. Dr. Gabriele Elisabeth Pollerberg 123
2.18 Independent Research Group: Dr. Gislene Pereira 129
2.19 Plant Molecular Physiology, Prof. Dr. Thomas Rausch 133
Project Leader: Dr. Steffen Greiner 138
2.20 Plant Developmental Biology, Prof. Dr. Karin Schumacher 141
Project Leader: Dr. Stefan Hillmer 146
2.21 Cell Chemistry, Prof. Dr. Sabine Strahl 149
Project Leader: Dr. habil. Michael Büttner 154
Project Leader: Dr. Mark Lommel 156
2.22 Developmental Biology/Physiology, Prof. Dr. Joachim Wittbrodt 159
Project Leader: Dr. Lucia Poggi 164
Project Leader: Dr. Lazaro Centanin 166
Project Leader: apl. Prof. Dr. Thomas Braunbeck 168
2.23 Independent Research Group: Dr. Sebastian Wolf 171

3 FACILITIES **176**
3.1 Nikon Imaging Center 177
3.2 Deep Sequencing Core Facilitiy 183
3.3 Metabolomics Core Technology Platform 187
3.4 Botanical Garden 191
3.5 Zoological Collection 197

APPENDIX **200**
A.1 COS Funding 201
A.2 COS Teaching 202
A.3 COS Events 205

INTRO-DUCTION

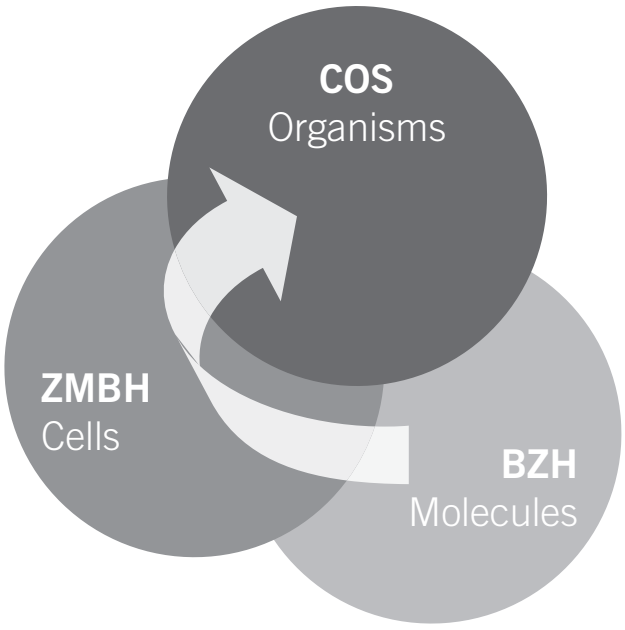
1

1.1 PREFACE

Biology as the science of the living things aspires to understand organismal function in the context of the native habitat. While classically organismal biology was limited to descriptive approaches, modern technologies have opened avenues to decipher the molecular details of biological systems in the context of their environment. Consequently, molecular biology, initially regarded as an independent subject has developed into a tool applied by cell and developmental biologists as much as by physiologists, or ecologists and evolutionary biologists. Along with the revolution provided by live imaging and omix approaches, these leaps now allow us to tackle complex questions at multiple scales across the traditional organismal boundaries. This multiscale, cross-kingdom idea of biology laid the foundation for the establishment of the Centre for Organismal Studies (COS) Heidelberg in 2010. COS was founded with the aim to bridge the gap between molecules and living systems in the context of their environment. Scientists at COS now study highly integrated systems, be these cells, organs, or whole organisms in their native environments – with molecular resolution aiming at a mechanistic understanding, reflecting our understanding of »organismal biology«.

The combination of molecular biology and genomic tools to visualize genome activity and function has proven extremely fruitful. A new frontier lies at the interface between imaging and genomics in the realm of metabolomics. Rather than concentrating solely on the primary products of the genome in terms of messenger RNAs and proteins, we are broadening our focus to encompass molecules that are metabolized under the control of the genome. The portfolio of expertise present at COS and the activities developed therein place us in an ideal position to explore these new directions. In terms of technology we aim to combine genomics, metabolomics, and imaging. Of course our repertoire of technology and expertise is not complete but recruiting outstanding junior groups with both, a strong scientific and technology profile, has and will help to fill the gaps. Driving integration at the level of technology development although not being a viable long term institutional motivation, does provide an opportunity for rapid progress. People who have been working separately on different topics can immediately start talking to each other and interacting, with respect to conceptual integration. On the other hand, conceptual points of convergences driven by fundamental biological topics, do have the potential to federate research efforts on the long run across COS.

The foundation of COS entailed profound changes in the pre-existing structures; such shifts in focus within an existing system are not trivial and require a lot of determination and dedication of all involved. The success of COS in the first five years is a reflection of this determination and team spirit that allowed us to create a new centre without any additional central resources. We have been extraordinarily successful in attracting third party funding and in parallel, junior colleagues (with their own, independent funding) who were inspired by our vision and now share and contribute to the pioneering spirit within COS.



The challenge for the coming years will be to consolidate and foster that spirit and to further develop COS into a driving force in Heidelberg life science landscape. Given that a fair, performance-guided distribution of resources within the University is granted, we are optimistic that COS will live up to the expectations.

1.2 BACKGROUND, DEVELOPMENT AND STRUCTURE OF COS

Centres, in contrast to institutes, are defined according to research interests and have members across faculty- and even institutional-boundaries. With three centres in the life sciences already established (ZMBH, BZH, IZN) COS was the culmination of a process that converted a classical faculty of institutes into a faculty of centres. The merger of the Heidelberg institute for Plant Science (HIP) and the Heidelberg Institute for Zoology (HIZ) was the result of a long-term discussion, leading to the idea to form COS as the fourth centre in the Heidelberg life sciences. The founding of COS in 2010 thus was an important step towards ensuring a dynamic development of research and education within the Heidelberg life sciences. With members from the EMBL (D. Arendt) and the Karlsruhe Institute of Technology KIT (N.S. Foulkes) COS crossed institutional boundaries from the beginning and with Rasmus Schröder (Medical Faculty) as a recent member, COS can now be considered a bona fide centre. A newly established »2012 professorship«, allocated by the state of Baden-Württemberg to cope with the increasing number of students, allowed COS to recruit the developmental biologist Ingrid Lohman to complement the senior faculty of now 15 professorships within COS. Each professor (director) heads a department (»Abteilung«) that can consist of several research groups. All but one department that is currently being filled (Developmental Biology of Plants), are fully operational and COS thus has almost 400 employees including the Core facilities, the Botanical Garden and the independent junior groups.

Independent junior groups that can take advantage of a fully established and supportive environment have not been part of the classical concept of University institutes. Indeed, there were no such groups when COS was founded. In the past four years, ten new groups have been attracted by and contributed to shaping COS and its dynamic spirit. Their research is enriching COS with new lines of technology (metabolomics, imaging, micro-fluidics), covering complementary topics in plants and animals (morphogenesis) and building bridges between various research fields (symbiosis of algae and corals, defense of insects by plants). They have been selected by the prestigious Emmy Noether Program of the DFG (Amal Johnston, Annika Guse, Steffen Lemke, Sebastian Wolf), the Chica and Heinz Schaller foundation (Alexis Maizel) or the Excellence Initiative (Emmanuel Gaquerel, Guido Grossmann). In collaboration with the ZMBH and DKFZ, the group of Gislene Pereira has recently joined COS and, last but not least, Thomas Greb from the Gregor Mendel Institute in Vienna is joining COS later this year as a DFG-Heisenberg Fellow.

The independent groups represent one of the key exploration and innovation platforms of COS. Since they are fully embedded into the COS environment, they are being directed and at the same time directing research. With their dynamic and interactive attitude they actively contribute to an atmosphere of stimulating and friendly competition not just among the independent group leaders but throughout COS.

We are planning to maintain this centre piece of dynamic activity and expect that extra support by the University will allow these groups to come to fruition in the COS environment. In the future we are aiming at a »steady state« of ten independent groups at COS, in balance with the central resources and teaching duties. The independent groups have only limited teaching duties, which allows them to get access to students without being overburdened. This is recognized and well accepted as a great opportunity and the independent groups enrich and stimulate the teaching tremendously. With their originality they challenge established concepts and enhance the range of topics as well as the overall quality of teaching. Their dynamic contribution is one of the most important elements in our concept of overcoming and preventing stagnation, at the level of research and teaching.

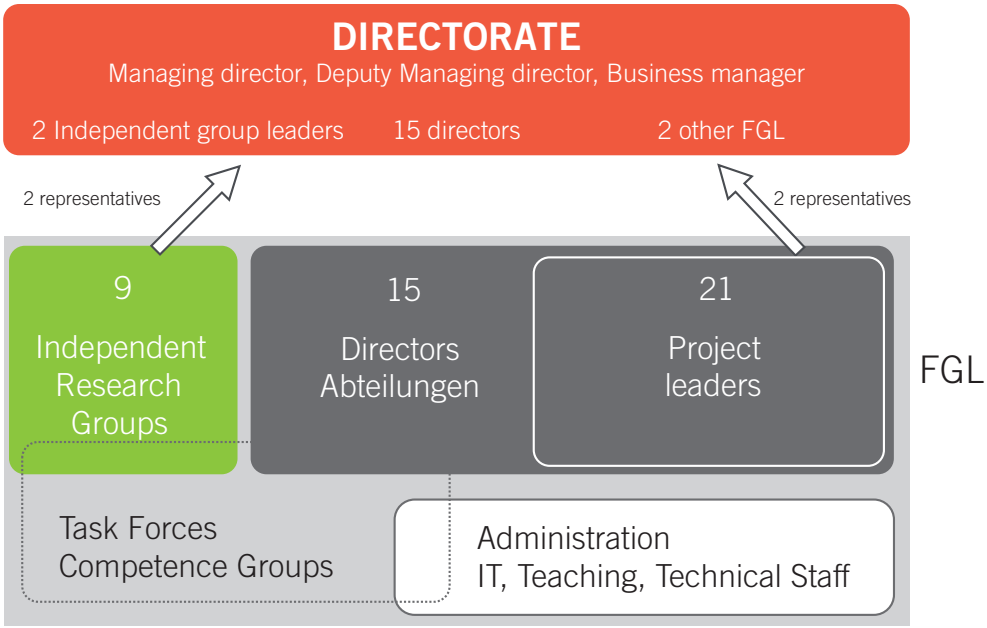

Coral Symbiosis
A. Guse
Evolution of Morphogenesis
S. Lemke
Centrosomes and Cilia
G. Pereira
Neural Network Development
J. F. Evers
Molecular Physiology
S. Frings
Circadian Clock Biology
N. Foulkes
Molecular Evolution and Genomics
T. Holstein
Developmental Biology
I. Lohmann
Developmental Biology/ Physiology
J. Wittbrodt
Evolution
D. Arendt
Nikon Imaging Center
Deep Sequencing Core
Developmental Neurobiology
G. E. Pollerberg
Cell Chemistry
S. Strahl
Metabolomics Core
Electron Microscopy Core
Biodiversity and Sytematics
M. Koch
Molecular Biology
R. Hell
Stem Cell Biology
J. Lohmann
Cell Biology
K. Schumacher
Molecular Physiology
T. Rausch
Developmental Biology
N.N.
Modelling of Biological Processes
U. Kummer
Plant Defense Metabolism
E. Gaquerel
Cell Wall Signalling
S. Wolf
Developmental Plasticity
A. Maizel
Germline Biology
A. Johnston
Cellular Membranes
G. Grossmann

MANAGEMENT AND ADMINISTRATION

The professors, representatives of the PIs (two representatives of the independent PIs and two representatives of the project leaders as observers) together with the business manager establish the COS directorate with monthly meetings deciding on scientific and administrative topics. The directorate is led by the managing director together with the deputy and COS business manager. Structure and format of the directorate follow the »Verfahrensordnung« of Heidelberg University and the »VBO« (Verwaltungs- und Benutzerordnung) of COS. Protocols of the directorate meetings are communicated to all COS PIs.

The assembly of all COS PIs (FGL: consisting of directors, independent group leaders, project leaders) meets on a regular basis (four times per year) to discuss and coordinate teaching and structural issues. Subgroups within the FGL form and dissolve according to needs and interest. The FGL directly participates in the monthly directorate meetings via four elected representatives.

The locally dispersed structure of COS residing in seven different buildings requires measures to ensure efficient communication. This has been addressed by regular meetings on different levels. In particular meetings at the level of the administration have proven to be very fruitful in connecting and integrating. Due to dispersal and very limited numbers of positions (often full positions are shared) it is difficult to enhance synergies at the administration level. The regular joint admin meetings with participants from administration, IT support and staff scientist with administrative duties have successfully cross-connected COS and provide a basis of understanding at that very important level. The monthly admin meetings have facilitated networking and the connection of different and complementary expertise and experience. This has been successful in several ways: we could establish a functional communication hub that allowed connecting and balancing different cultures. This was instrumental to speed up the development of creative admin solutions by combined expertise. The formalized exchange at that level also provides a highly efficient sensor to detect changes in policies of the University administration, allowing immediate feedback to the directorate. Similar activities have been initiated at the level of the technical and research assistants to improve communication, to exchange functional protocols and, last but not least, to coordinate purchases to get more competitive offers by the suppliers.



1.3 TEACHING AT COS

Although COS only represents a quarter of the Bioscience faculty, we deliver the vast majority of teaching (>70 %, see Appendix COS-Teaching). This is true both at the BSc and the MSc level. 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). This apparent imbalance in the distribution of basic and advanced teaching across the life science campus Heidelberg is the consequence of the historical assignment of teaching duties. With its highly appreciated and evaluated teaching, the entire faculty of COS is actively contributing to the international recognition of life sciences in Heidelberg. It would be much easier to cope with the imbalance in teaching distribution across the life science campus, if the teaching delivered by the COS faculty would not only be recognized, but would proportionally impact on the core financial support of the active groups and the centre. We aim at addressing this apparent imbalance in the coming years and are confident that we will find a solution in close discussion with the rectorate, the faculty of Biosciences and the other centres of the life science campus Heidelberg. Since there are no personal grants for teaching, it will be crucial for the Universities to establish incentives to maintain and further improve the high standard in student education on any level. While excellence in research is rewarded by the University, excellence in teaching is not as highly appreciated. We are planning to implement teaching awards aiming at triggering a University wide development in that direction.

In line with the organismal perspective of COS, we have been shifting the teaching focus accordingly. While the depth of detail is constantly extending on the one hand, we expect our students to put those details into the bigger, organismal context, a difficult, challenging task. To prepare our students for that challenge, we have developed an integrated, research oriented teaching concept. We provide clear conceptual scaffolds to anchor the molecular and mechanistic details with the necessary perspective across kingdom boundaries. This will train our students from the very beginning to combine learning with addressing and solving questions. The benefit of that approach is obvious since it will educate »problem spotters« and »problem solvers« and is thus not restricted to careers in science, but equally applicable in higher administration or the economy, since the increasing complexity in all fields demands the ability to handle complexity across all scales, from the precise details to the »big picture«.

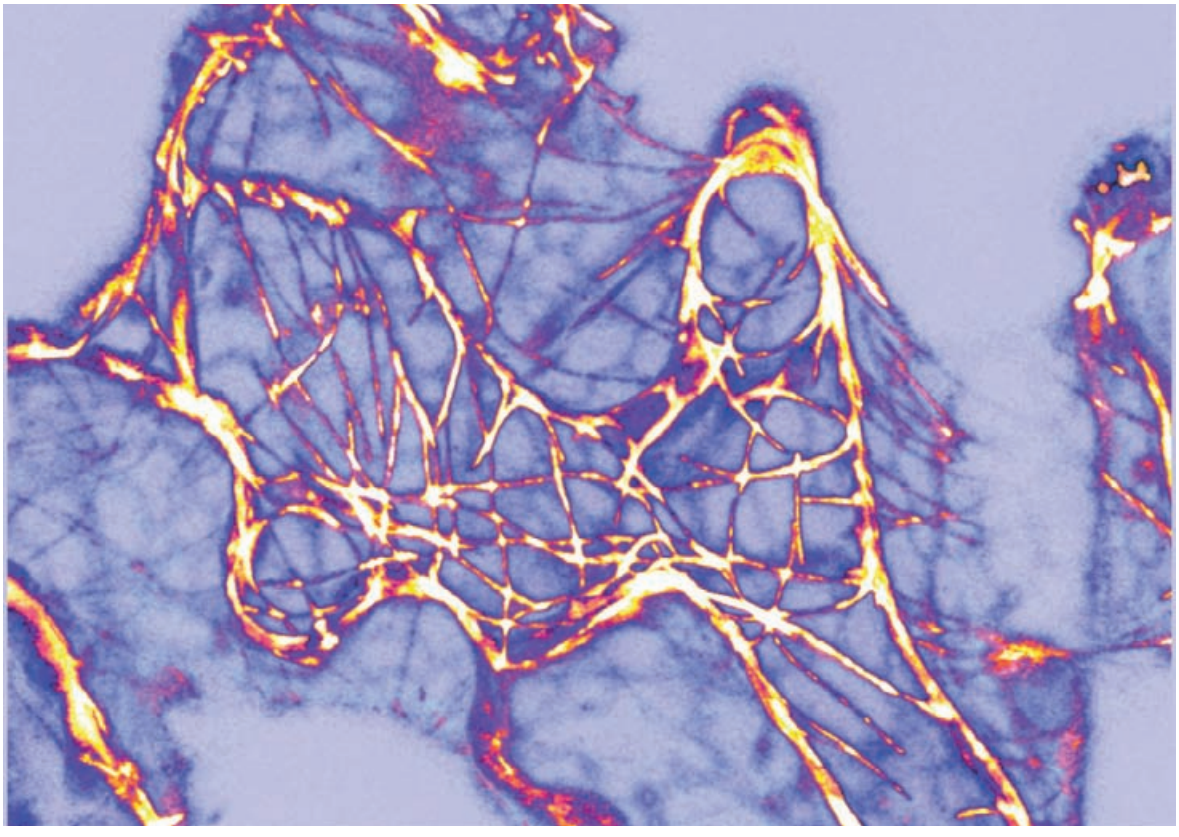
In the absence of adequate research opportunities, teaching at German high schools is struggling to implement comparable concepts although the problems have been spotted. In close collaboration with more than 40 high schools in the Rhein-Neckar area and supported by the »Klaus Tschira Stiftung« we are providing first insight into the basic concepts of up to date, cutting edge research for high school students in Bertalanffy lectures twice a year. A world wide leader in an exciting research area is presenting his/her research and the long way to success to the students. In a full afternoon with a keynote presentation, tutorials and plenary discussions interested high school students (up to 400) 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 developed and actively pursued at COS.

1.4 RESEARCH AT COS

With its almost 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, Developmental Cell, Genes & Development, Nature, Plant Cell, PLOS Biology, PNAS, Science). Three COS faculty members were successful in competing for ERC grants (Detlev Arendt, Jan Lohmann, Joachim Wittbrodt) and several highly recognized prizes were received by COS members (e. g. the Otto Mangold Award, the HMLS research prize or the Manfred Lautenschläger Award). Thus not only the promising talent of the junior faculty has been highly recognized, but also the performance of the more established COS members.

Publications, grants and prizes most of the time reflects the performance of individuals as described in the research aims presented by the individual PIs. Here, existing and emerging collaborative activities that have been particularly stimulated by the founding of COS are highlighted.

1. Physiology was and remains one of the key connecting topics within COS. Physiological studies that integrate genomics, proteomics and metabolomics can provide the complete picture of how the organism deals with the given environmental conditions. While genomics and proteomics provide a comparatively static scaffold of organismal function, metabolite analysis provides the basis for an understanding of the dynamics that drives development and adaptation. 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 second nucleation point together with a metabolomics junior group. The approach is highly successful and highly »contagious«. A new SFB-initiative with the running title »metabolism and development«, broadly anchored within and beyond COS is taking shape. This initiative has the potential to ultimately bridge between both areas: Imaging and metabolomics to facilitate the analysis of highly dynamic processes in vivo, in the organism in its environment.



2. An important topic that serves as a scientific anchor for a diverse range of COS PIs are stem cells. Although this may seem a rather generic topic focus within COS is on evolution, adaptation and ecology rather than regenerative medicine. PIs at COS work on stem cell related questions from plants to animals, 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« coordinated by J. Lohmann was recently appraised by the reviewers and extended for a second funding period. Seven of the seventeen projects are directed by COS PIs.

3. Advanced imaging as represented in the Nikon Imaging Center as well as in a number of individual research groups has gathered PIs with shared interest to address the molecular and cellular mechanisms of tissue and organ morphogenesis in vivo. Here, in particular young PIs were attracted by the technology and expertise in place and have in the meantime considerably extended the critical mass. A COS organized international summer school entitled »Getting in shape: Visualization and Manipulation of Organismal Morphogenesis« last year in Santiago de Chile (Centanin, Evers, Lemke, Maizel, Schumacher, Wittbrodt) was the nucleation point for several COS driven initiatives. One of them, a research unit on quantitative morphodynamics has a strong scientific basis at COS but aims to attract additional expertise throughout Germany. Another is a joint international graduate program together with our partners in Santiago de Chile. These are excellent examples for the success of our core facilities in initially attracting outstanding junior faculty, and now in fostering new initiatives by advanced technology.

Even though there are traditionally strong research topics within COS and emerging collaborative areas there was and is no intention to streamline the individual foci of research. This is reflected in the fact that COS PIs were and are also involved additional SFBs based in Heidelberg (SFB488, SFB 638, SFB1036) or Tübingen (SFB1101) and participate in DFG-Research Units (FOR 1036, FOR 964, FOR 1061) and Priority Programs (SPP 1529, SPP 1710).

As stated above, our independent groups are vital for the successful development of COS. Unfortunately however, most of the currently established collaborative funding schemes (DFG, BMBF, EU) require a rather lengthy application procedure which, a priori, precludes most group leaders with non-permanent positions from participating. To at least partially overcome that problem we are currently following two lines. On the one hand we actively promote applications of small networks to funding schemes of the Excellence Initiative (CellNetworks EcTops, Frontiers Program) or the State (LGFG graduate programs). 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 expect further successful applications in the future. In parallel we are pursuing applications with international partners (Universidad de Chile, Santiago; Nanjang Technical University Singapore) for joint international graduate programs.

Here, a balanced mix of established and independent groups is applying for fellowships in international consortia with complementary research interests. The collaboration with Santiago de Chile currently fosters a collaborative network of animal and plant biologists, where the successful integration at COS is internationally recognized as a role model. This collaborative project is based on a long term collaboration with a regular exchange of students and more advanced scientists. A joint summer school in 2013 (Getting in shape: Visualization and manipulation of organismal morphogenesis) supported by Santander Universidades through Heidelberg University brought all potential partners together for two weeks and was the basis for the application currently in progress.

These are a few examples of COS driven activities aiming to connect COS groups along major topic lines, integrating the non-permanent PIs as well as international collaborators. Many of these activities are just at the beginning and we expect them to be up and running by the time of the next review.

1.5 INTEGRATION IN THE LIFE SCIENCES ON CAMPUS

The examples in the previous sections already indicate the high degree of integration within the Heidelberg life science campus. The integration process on campus was massively fostered during the two rounds of the German Excellence Initiative in 2007 and 2012. In this context, two structural elements across the Heidelberg life science campus emerged, the excellence cluster CellNetworks as well as the Graduate School HBIGS. Already in the application phase, COS played an active role in both activities (as members of the writing team and steering committees) and COS PIs are still driving the development of these campus wide activities as vice cluster coordinator of CellNetworks and members of the respective steering committees.

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 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). The 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. The Nikon Imaging Center (more details below) is sustained by two main sources, COS with personnel and running costs and Nikon (Instruments, part of personnel).

As a core facility it fostered numerous campus-wide collaborations and interactions and is the Heidelberg role model for a successful core facility. By analogy, new core facilities were established all aiming to generate a technological hub, to bridge between different sites of the campus, 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.

This model was a great success and in addition, COS is now providing equipment and 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.

Even though these facilities are supported by individual COS PIs providing positions for technical personnel and staff scientists without immediate benefit, they can be accessed by any scientist on the Heidelberg life science campus. 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.

COS is not only very well embedded in and connected throughout the Heidelberg molecular life science campus, but has also excellent links to the scientific infrastructure at the Karlsruhe Institute of Technology (KIT). One director of COS is appointed by KIT (N. S. Foulkes) and another had initially been co-appointed to Heidelberg and Karlsruhe (J. Wittbrodt). These are only the pioneering bridges. A program established in the context of the excellence initiative and now maintained independent of it is HEIKA, the Heidelberg Karlsruhe research partnership. Four COS PIs are involved in running joint projects and two (T. Holstein, R. Schröder) are the Heidelberg bridgeheads (coordinators) of two of the five HEIKA research bridges. That way, new, technological interfaces are explored adding to the portfolio of COS in basic research on the Heidelberg campus.

As briefly mentioned above and detailed below, COS is the key figure in educating biologists in Heidelberg. Thus it was natural to connect undergraduate education with the structured education of PhD students in life sciences in Heidelberg.

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). The COS PhD program has been developed as one of the integral pillars of HBIGS, which as a school provides a »roof« to cover smaller programs. Most of the COS PhD students are members of HBIGS and part of the COS PhD program 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.

1.6 FUTURE PERSPECTIVES AND CHALLENGES

Where do we want to be in ten years and what will be the key contribution of COS to new developments?

What we will clearly do is to continue providing a highly permissive and stimulating environment. The success model of independent PIs shall be consolidated to maintain a steady state of ten independent research groups. In addition we need 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.

Our students are a highly valued resource and if we educate them well, our science will flourish and spread. The first successes of our activities are already visible and the structures implemented should maintain dynamics: friendly competition for the best students that is based on creativity and originality of the teachers and their concepts rather than on the technological setup of the teaching facilities. Learning by doing, in particular on how to find and ask the right questions is one of the keys to success, not only in science. We are in the process of implementing research oriented teaching starting with high school students and in the BSc program and the new concepts we develop here provide an interesting challenge and promise at the same time. Another key aspect along the same lines is to provide MSc and PhD students more time for their research. Here we will start initiatives together with the graduate school HBIGS to start connecting MSc and PhD programs. This will free time for the students to gain more direct research experience. There is a lot of room for further development in these areas and COS is ideally positioned to take actions here.

As stated repeatedly, our core facilities have been a crucial motor for success in the past. To keep that engine running in times of limited resources we will critically review their performance and adapt them to the needs the 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. Mergers of core facilities from other institutions might be an alternative to retain the advantages of a local facility and at an affordable price. A clear commitment of the University and the Federal State of Baden-Württemberg would provide the necessary perspective and allow us to maintain the spirit of the excellence initiative.

In the absence of influence on our (by international standards low) core funding the only way to attract support for the centre are coordinated research programs. New initiatives for coordinated research programs are currently initiated at and driven by COS as described above to further increase attractiveness for future colleagues. The apparent imbalance of core support and external support must be balanced in the intermediate term to allow the management of the resources. Efficiency seems the key. In light of this it is mandatory to physically unite COS research in one COS centre as published in our VBO: The research groups in animal and plant research working with an organismal focus shall be combined organizationally and spatially in one building together with their central scientific, technical and administrative support [»Die organismisch ausgerichteten Arbeitsgruppen der Tier- und Pflanzenwissenschaften sollen organisatorisch und räumlich zusammengeführt und ihre zentralen wissenschaftlichen, technischen und administrativen Dienstleistungen in einem Gebäude zusammengefasst werden (Universität Heidelberg, Mitteilungsblatt 23/10).«].

This step will immediately enhance a lot of synergies and the COS central building is thus on all levels the way to proceed.

Where is our science developing? The merger of plant and animal sciences has provided an interface that has stimulated and developed COS immensely in unexpected new directions. Animals and plants live in a common environment, but not in isolation. Adaptation and evolution occurs in a close interplay with the environment and new interfaces with geology and environmental physics will ultimately allow us to address organism – environment interactions (in both directions). On the way to exploring these interfaces, novel imaging tools developed on the basis of metabolomics studies will facilitate the visualization of key environmental impacts *in vivo.*

Technological interfaces constantly trigger new developments that, however, can only be pursued if the scientists involved have found a common language. This closes the loop and brings us back to our teaching where the future biologist speaks not only biology but is also fluent in mathematics, physics and chemistry (and vice versa).

RESEARCH GROUPS

2

2.1 ANIMAL EVOLUTION
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Fields of Interest
Marine developmental biology, animal evolution, neurobiology, evo-devo, central nervous system evolution, eye evolution, transcriptional networks

Brief summary of work since 2009
Our current main interest is the evolution of nervous systems. We are working with the marine annelid *Platynereis dumerilii*, for whom we have developed a number of molecular and functional techniques including gene knock-down and knock-out as well as transgenesis. Major breakthrough from our lab has been the development of the PrImR (Profiling by Image Registration) technique and resource, which allows cell type-specific expression profiling via registration of wholemount in situ hybridization patterns of an unlimited number of genes. This has allowed us to compare the *Platynereis* mushroom body tissue, a putative higher brain centre in annelids, to that of the vertebrate cortex and unravel similarities and dissimilarities in cell type identities. These data suggest that the vertebrate cortex has evolved from a higher brain centre that was already present in the bilaterian ancestors and gave rise to mushroom bodies in the protostome lineage. We have then started to extend the PrImR resource to more genes and several stages and to combine expression profiling with the study of the connectome and of neuronal morphologies. We have obtained an ERC Advanced Grant »BrainEvoDevo« for this project, which started in 2012. First outcome has been a detailed map and cell type characterization of the *Platynereis* larval brain, the apical organ, which plays a role in larval settlement and metamorphosis control. Our results indicate that the larval brain (together with the primary larval stage) is conserved across animals. Finally, we have molecularly characterized a dorsal brain region harbouring ciliary photoreceptors with a vertebrate-type phototransduction cascade that synthesize and release melatonin, the hormone of darkness. Using optogenetics and electrophysiological recordings, we have found that melatonin induces a sleep-like behavioural state switch that reduces ciliary beating and thus controls diel vertical migration of the swimming plankton larva in the water column. Diel vertical migration is a widespread ecological phenomenon and represents the biggest transport of biomass on Earth.

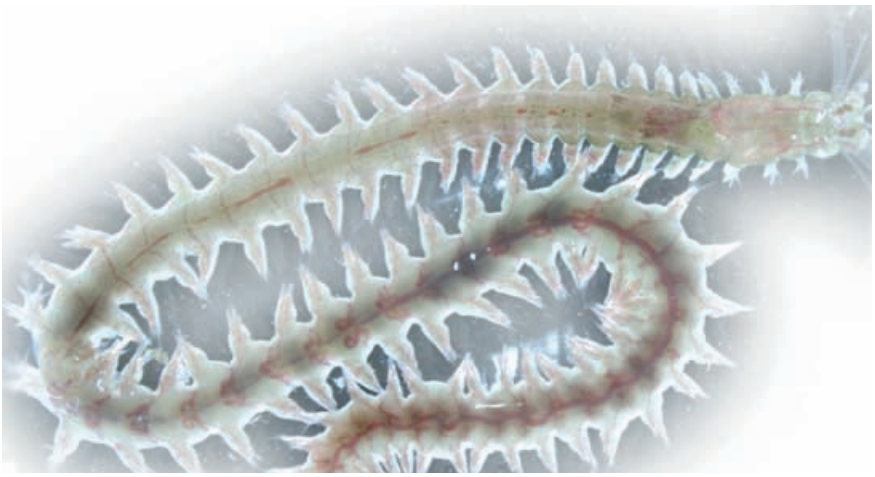


Figure 1
The marine annelid
Platynereis dumerilii

Major contributions

The aim of our lab is to understand the evolution of brains at the level of neuron types and neural circuits. How did the first neurons assemble into circuits? How did additional circuits emerge and connect to pre-existing ones? Can we trace, step by step, the enormous rise in complexity that accompanied the evolution of the vertebrate brain? We have recently put forward the idea that the diversity of neuron types that exists in extant brains has evolved by cell type diversification, and that this process can be tracked based on the comparison of differential expression profiling data.

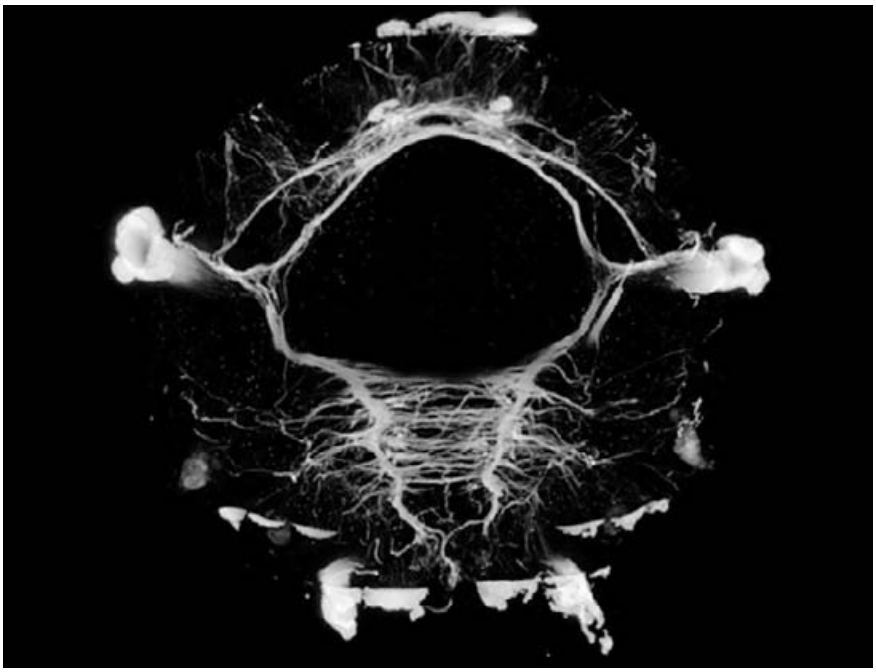


Figure 2
The developing nerve cord in
Platynereis 48hpf larvae

One major problem that we have addressed in last five years's period is one of the remaining grand questions of animal brain evolution, the origin of the vertebrate cortex, or pallium, the site of sensory integration and association. So far, pallium evolution was impossible to track further than early vertebrates. It was unclear whether any invertebrate counterpart or precursor form of the pallium existed. We have traced back the vertebrate pallium to chemosensory integrative brain centres in the protostome-deuterostome ancestor. We have shown that these centres also gave rise to the mushroom bodies found in today's invertebrate groups such as annelids and insects, likewise implicated in sensory integration.

Our study built on a newly developed technique, Profiling by Image Registration (PrImR) that allows *in silico* alignment of multiple brain expression patterns and thus the simultaneous co-expression analysis of an unlimited number of genes. We have applied this protocol to explore the molecular topography of brain development in the slow-evolving marine annelid *Platynereis dumerilii*. We then compared the expression profile of subregions of the developing annelid mushroom bodies to those of the vertebrate pallium and discovered detailed similarities that can only be explained by evolutionary conservation.

As another contribution to the evolution of bilaterian brains, we have unravelled the molecular signature of the *Platynereis* apical organ – the larval brain – which is shared with other marine phyla including cnidarians. We have characterized the cell types that form part of the apical organ by PrImR. Besides the *hox*-expressing apical tuft cells this reveals putative light- and mechanosensory as well as multiple peptidergic cell types that we compare to apical organ cell types of other animal phyla. We have also found that the repression of Wnt signals from posterior body regions is required for apical brain specification. These results strongly indicate homology of larval brains across Bilateria.

Finally, we have recently submitted our research on the evolution of melatonin signalling. 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. Not much is known, however, about the role of melatonin outside vertebrates. We have studied the role of melatonin signaling in the *Platynereis* trochophora larva. Our work has revealed that in the *Platynereis* zooplankton larva, melatonin signaling controls »diel vertical migration«, a circadian behavior that is common to the vast majority of organisms populating the global oceans – swimming upwards at dusk and downwards at dawn. Melatonin acts as a prototypical neuromodulator: by changing neuronal excitability, it shapes neural circuits and induces a »behavioral state switch«. This study is now accepted in Cell. In a related study, we are comparing the melatonin-producing dorsal brain in *Platynereis* to the vertebrate pineal and retina at the cellular level using PrImR.

Planned research and new directions

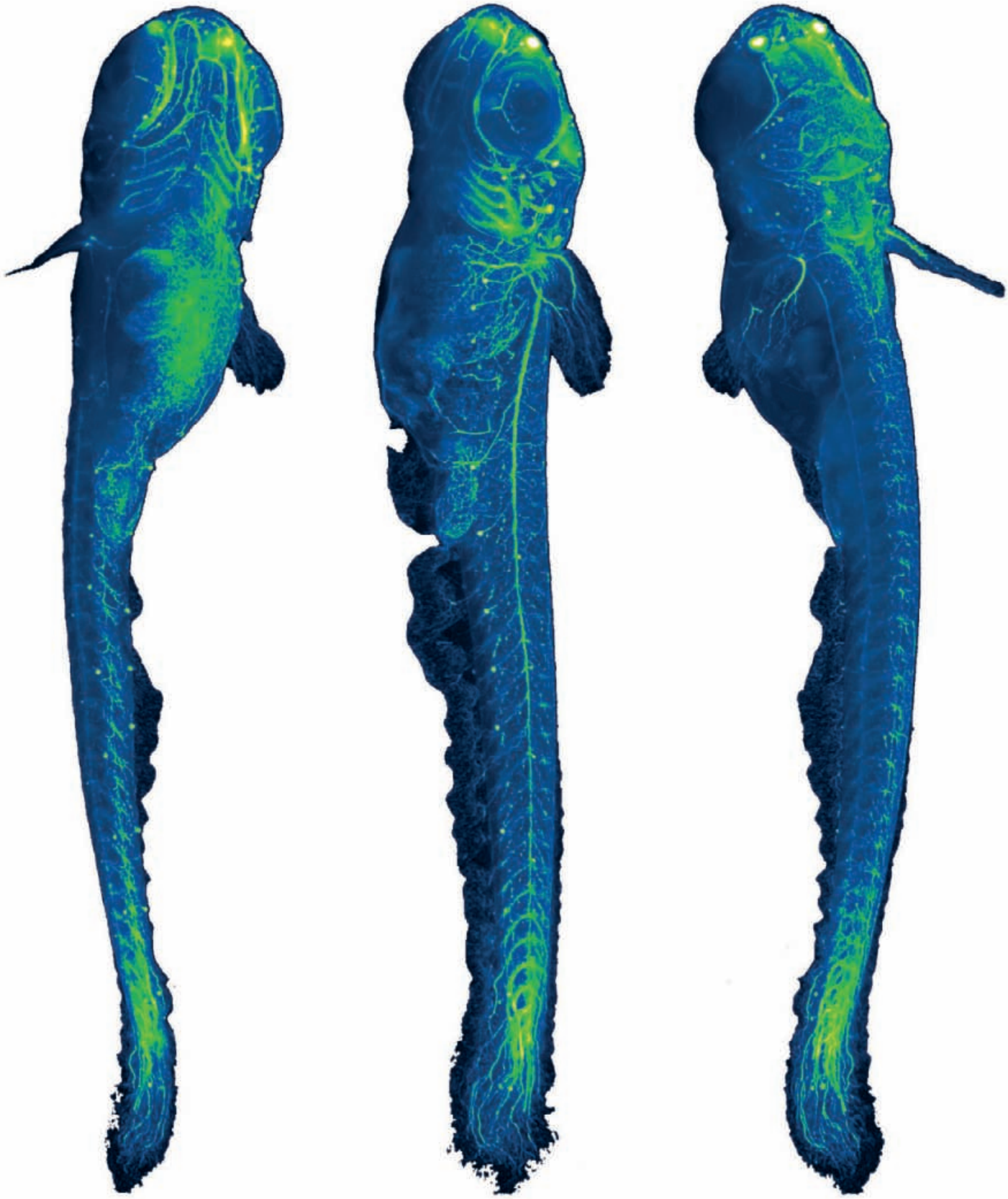
Building on our previous knowledge on cell types in the *Platynereis* brain and nerve cord, we have, started an ERC-funded project called BrainEvoDevo, which aims at generating a neuron-type atlas of the annelid larval brain and trunk nervous system. This atlas will combine neuronal morphologies, axonal projections and cellular expression profiling for an entire bilaterian brain. In the brain, focus will be on the cell types of the mushroom bodies, to understand their molecular anatomy in more detail and to acquire a first understanding of their wiring and connectivity. This will be coupled to behavioural experiments that will allow inferring the sensory modalities that feed into the mushroom bodies. We will use optogenetics to monitor activity of specific cell types in response to specific stimuli and knock out cell type-specific specification and differentiation genes in order to infer their role in specific behaviors. From this we expect new insight into brain evolution in bilaterians.

In the trunk, we will focus on the description of the neuron types involved in the locomotor neural circuits in *Platynereis*. We will systematically investigate and obtain cellular resolution for a huge collection of candidates known to be indispensable for the neural tube development and differentiation in the vertebrates. Adding to this, we will map the connections between neurons to elucidate their wiring. This will be accomplished by block-face scanning electron microscopy, at a resolution that allows tracking of single neurites and most of the synapses. This project will be done in collaboration with Christel Genoud at the FMI Basel. The hardwiring and functional characterization of the components will be essential to decipher the »rules« by which neural circuits operate.

Going beyond these plans, and taking advantage of the PrImR resource, we have started another project in order to generate the first cellular resolution expression atlas for a whole animal nervous system, involving early developmental as well as differentiation stages. We are working in collaboration with the Marioni lab at the EBI in Hinxton and with the Keller lab at the Janelia Farm Research Centre, as well as with the Tomancak lab at the MPI for Molecular Cell Biology and Genetics. We are combining a full tracking of the developmental lineage for all cells at several developmental stages, with cellular resolution expression profiling by image registration and, most important, single-cell sequencing using the Fluidigm C1 system. Building on the Atlas, we will dissect *Platynereis* forebrain circuits, by laser ablation of GFP-labelled single neurons, gene knockout studies and behavioural assays based on microfluidics to explore duplication, divergence and expansion of neural circuits in central nervous system development and evolution.

Selected publications since 2009
Number of peer-reviewed articles 2009-2013: 28, number of citations 2009-2013: 390, h-index (2009-2013): 11 (according to Thomson Reuters). total h-index 29

Arendt, D., Purschke, G. and Hausen, H. (2009). The »division of labour« model of eye evolution. Philos Trans R Soc Lond B Biol Sci 364, 2809-2817
Christodoulou, F., Raible, F., Tomer, R., Simakov, O., Trachana, K., Klaus, S., Snyman, H., Hannon, G. J., Bork, P. and Arendt, D. (2010). Ancient animal microRNAs and the evolution of tissue identity. Nature 463, 1084-88.
Tomer, R., Denes, A. S., Tessmar-Raible, K. and Arendt, D. (2010). Profiling by Image Registration reveals common origin of annelid mushroom bodies and vertebrate pallium. Cell 142(5):800-9
Fischer, A. H., Henrich, T., Arendt, D. (2010) The normal development of *Platynereis dumerilii* (Nereididae, Annelida). Front Zool 7(1):31
Marlow, H., Tosches, M. A., Tomer, R., Steinmetz, P. R., Lauri, A., Larsson, T., Arendt, D. (2014) Larval body patterning and apical organs are conserved in animal evolution *BMC Biol.* 12(1):7



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

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Fields of Interest
Molecular and developmental genetics, motor circuits, development and differentiation, neuronal networks, optics and microscope engineering, computational image analysis

Brief summary of work since 2009
Every individual is unique. Using the locomotor system of the fruit fly *Drosophila melanogaster*, our focus lies on investigating the degree to which nervous systems vary between individuals, and uncover the molecular mechanisms that – despite these differences – allow adequate function to emerge during embryonic life.

An integral part of our research is to develop the molecular and microscopy techniques that allow us to directly visualize neuronal birth, structural growth and synaptogenesis between identified synaptic partners in the central nervous system of intact *Drosophila* embryos and larvae; and software to quantify structural growth and protein interaction such that experimental hypotheses can be rigorously tested by statistical analysis.

Using these techniques, we demonstrated that functional maturation of motor circuits is dependent on endogenous patterns of neural activity, and that postsynaptic rather than presynaptic terminals are limiting the number of synapses made during development. Our data suggests that 1) the postsynaptic cell might be key in regulating the levels and patterns of input in order to adjust connectivity according to functional requirement; 2) formation of motor circuits is the result of homeostatic plasticity, such that specific connectivity that supports behavioral requirement might not be genetically fully specified, but is negotiated during development and therefore variable between individuals.

Major contributions since 2009

Using custom developed imaging techniques, evaluation software and molecular tools, we found that in *Drosophila*, synapses form before motor networks drive muscle contractions. When immature networks first become active, network output is uncoordinated, but coordination gradually emerges over the next 1.5h of development. We demonstrated that this tuning of network properties requires endogenous patterns of neuronal activity, and experimentally altering or disrupting activity during this time disrupts the normal development of functional circuits (Crisp, Evers, & Bate, 2011). At the cellular level, motorneuron dendrites and the amount and distribution of presynaptic sites are variable when networks become first functional, suggesting that there is not a hard-wired program of connectivity development, but rather that it is plastic and acquires functional patterns of connectivity in a homeostatically regulated manner (Tripodi, Evers, Mauss, Bate, & Landgraf, PLoS Biology 2008).

In my independent position at Cambridge and my first year at Heidelberg, we established 1) the genetic tools to target pairs of pre- and postsynaptic neurons independently and 2) the microscopy techniques to visualise endogenous protein levels in intact *Drosophila* embryos (using custom developed Spinning Disk Confocal and Fluorescence Light Sheet Microscopes). We have since shown that larval growth of motoneuron dendrites is regulated cell-autonomously by a steroid hormone receptor (Ecdysone Receptor – B2, EcR-B2), whose functions were previously unknown. As these dendrites enlarge, they form more connections with presynaptic partners, leading to greater levels of neuronal activity. When blocking EcR-B2 signalling, neurons remain developmentally younger and maintain circuit connectivity that is characteristic of an early larval stage (Zwart et al, PNAS, 2013). Interestingly, when looking at the level of synaptic connections made, we find that there is a substantial variability in quantity and quality of synapses made between individual pairs of inter- and motorneurons, and that this variability remains during larval life (Couton L., Diegelmann, S., Mauss, A., Landgraf M. and Evers J.F., in preparation). Together, these data suggest that dendrites rather than axon might decide on the amount and quality of synaptic connections made during development.

Interestingly, also the placement of synaptic sites onto the dendritic tree of motorneuron 5 in the *Manduca sexta* appears to be tuned according to stage-dependent functional requirements. Using theoretical modeling, we found that synapses are differentially localised to either support tightly synchronized generation of action potentials (adult flight motorneuron), as opposed to tonic firing riding on top of sustained depolarization (larval crawling motorneuron) (Meseke et al., 2009).

Taken together, our research suggests that the development of motor circuits in insects might be exploratory, and specific solutions emerge through homeostatic plasticity in the growth and connectivity of the postsynaptic dendrites. To explore the boundaries to this variability set by the requirement of adequate behavioural output, we conducted computational simulations. We generated a robust modeling framework that supports coordinated propagation of network activity (to drive peristaltic movement) and network synchronization on both left and right side of a nervous system, using segmentally repeated units of excitatory and inhibitory neuronal populations coupled with immediate neighboring segments. A single network with symmetric coupling between neighboring segments succeeded in generating both forward and backward propagation of activity (Gjorgjieva et al., 2013). We are now using this model to explore self-tuning mechanisms that might regulate the formation of synaptic input and strength.

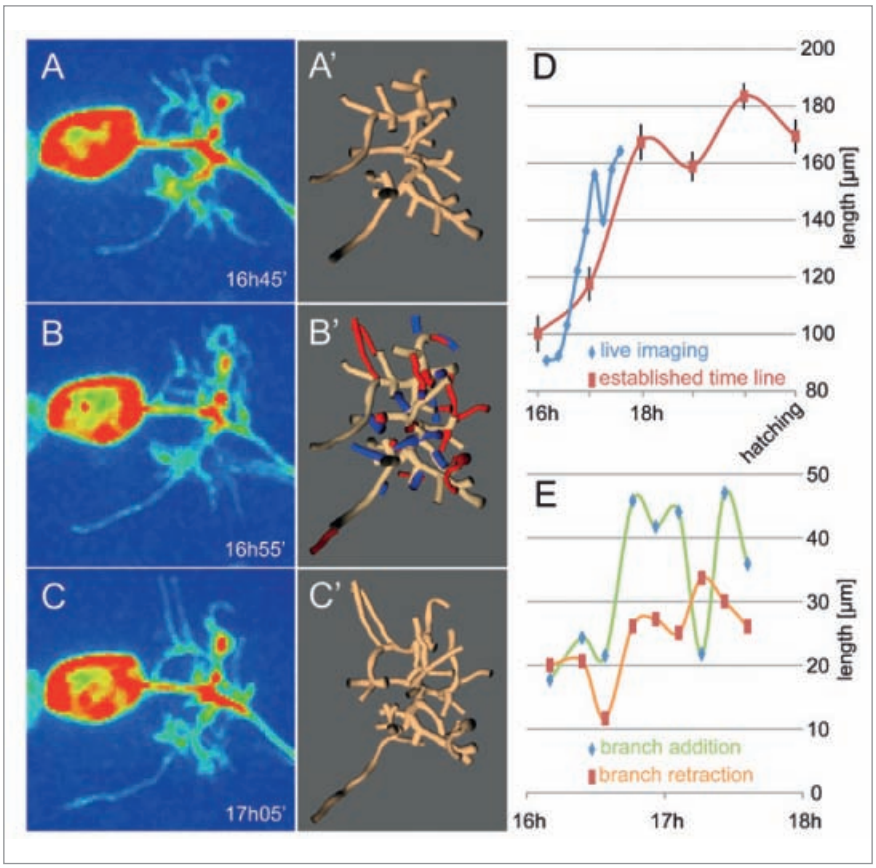

Figure 1
Time-lapse recording of motor neuron (RP2) dendritic growth in an intact, living *Drosophila* embryo. (A-C) Raw image data from a 2h long imaging session, with acquisitions at 10min. intervals. (A'-C') Digital 3-dimensional reconstructions of dendrites without cell body or axon. B' exemplifies that we can track automatically the identity of branches over time. Red indicates branches that will grow in the transition to C', and blue those that retracted since the previous time point, A'. (D) Curve plots of dendritic length as measured from live imaging (blue) with reference to those from acutely dissected nervous systems (red). There is no major difference between both. (E) Automatic analysis of the summed lengths of branch addition (green) and retraction (orange).

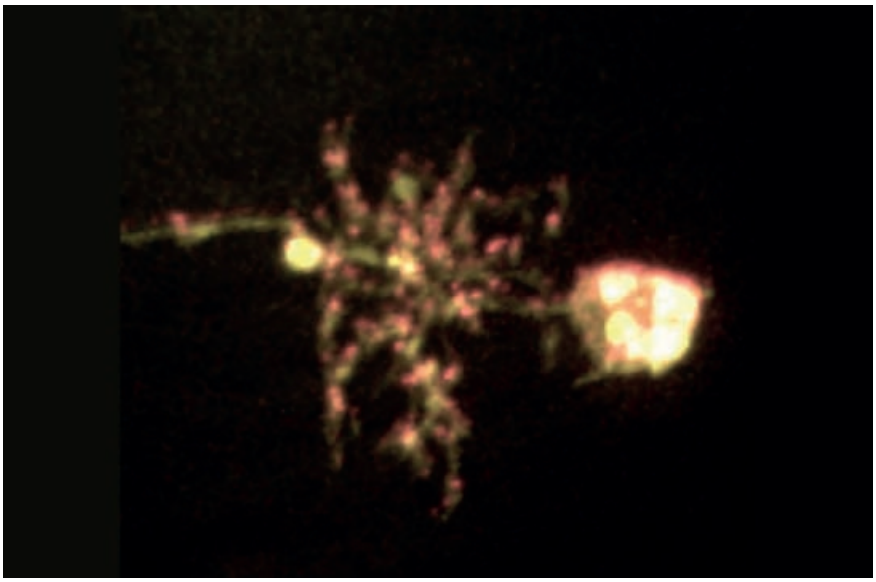

Figure 2
Snapshot of a *Drosophila* motor neuron (RP2, yellow) expressing a marker for postsynaptic specializations (UAS-Drep2, magenta) at larval hatching.

Planned research and new directions
It is widely believed that the insect nervous system is highly stereotypic, and each neuron can be individually identified. To the contrary, we find considerable amount of variability in the functional connectivity patterns of the larval motor system. Data of others suggest that also the numbers of neurons born from identified neuronal stem cells vary in *Drosophila*, and that this variability is maintained even when programmed cell death is genetically abrogated (Rogulja-Ortmann et al., Development 2007).

We will therefore record the dynamic development of neuronal lineages in individual *Drosophila* embryos using light sheet and spinning disk confocal microscopy, and ask: 1) What are the complete lineage of cells born from identified neuronal stem cells, particularly during late neuroblast divisions; 2) Are these lineages variable between neighboring segments, and across individuals; 3) Is neuro-transmitter specification fixed, dependent on neuronal lineage and birth order? 4) Do neurons that are variable between individuals make functional connections in the CNS?

On the level of individual neurons, we will investigate the embryonic development of locomotor circuits at the synapse level. We will ask whether there is a consistent patterning of 1) surface contacts and 2) synaptic connections between individual identified inter- and motorneurons before network activity commences. We will record how these initial contacts resolve into functional patterns of synaptic connections, evaluating synapse life time, synaptic protein scaffold assembly and turn over. We will test the role of neuronal activity in generating these functional patterns of connectivity by experimentally altering activity patterns between inter- and motorneurons and recording network formation using endogenously expressed synaptic proteins tagged with an inducible marker. These data will reveal whether there are exploratory synaptic connections forming, which – dependent on validation – are maintained or retracted in order for functional circuits to emerge.

Selected publications since 2009
Number of peer-reviewed articles 2009-2013: 8, number of citations 2009-2013: 35, h-index (2009-2013): 3, total h-index: 9 (according to Thomson Reuters).

M Zwart, O Randlett, JF Evers*,[1] & 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. doi:10.1073/pnas.1311711110
J Gjorgjieva, J Berni, JF Evers, & SJ Eglen (2013). Neural circuits for peristaltic wave propagation in crawling *Drosophila* larvae: analysis and modeling. *Frontiers in Computational Neuroscience, 7,* 24. doi:10.3389/fncom.2013.00024
S Crisp*,[1], JF Evers*,[1], M Bate. Endogenous patterns of activity are required for the maturation of a motor network. *JNeurosci (2011) vol. 31 (29) pp. 10445-50*
M Meseke, JF Evers, & C Duch (2009). Developmental changes in dendritic shape and synapse location tune single-neuron computations to changing behavioral functions. Journal of Neurophysiology, 102(1), 41–58. doi:10.1152/jn.90899.2008

* equal contribution, [1] corresponding author

2.3 CIRCADIAN CLOCK BIOLOGY
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Fields of Interest
Molecular genetics, transcriptional networks, Signal transduction, Circadian clocks, Behaviour, Evolution of regulatory mechanisms

Brief summary of work since 2009
The circadian clock is a key biological timing mechanism which temporally coordinates most aspects of plant and animal biology, as an adaptation to the day-night cycle. Central to its function is its daily regulation by environmental time indicators, so called »zeitgebers«, such as light and temperature. This ensures that the endogenous clock time remains tightly synchronised with the external day-night cycle. Furthermore, 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 this mechanism in vertebrates. We have revealed the key photoreceptors, signalling pathways, transcription factors and enhancer elements, that relay lighting information to regulate clock gene expression in fish cells and tissues. Furthermore, we have shown that light exposure not only regulates clock gene expression, but also controls the transcription of non-clock related genes in fish cells and tissues using the same light responsive signalling cascades. Exploiting unique properties of the circadian timing system in blind cavefish, we are now embarking on a detailed study of the poorly understood food entrainable clock. In addition, we have also explored the role played by the clock in timing the process of tissue regeneration upon injury.

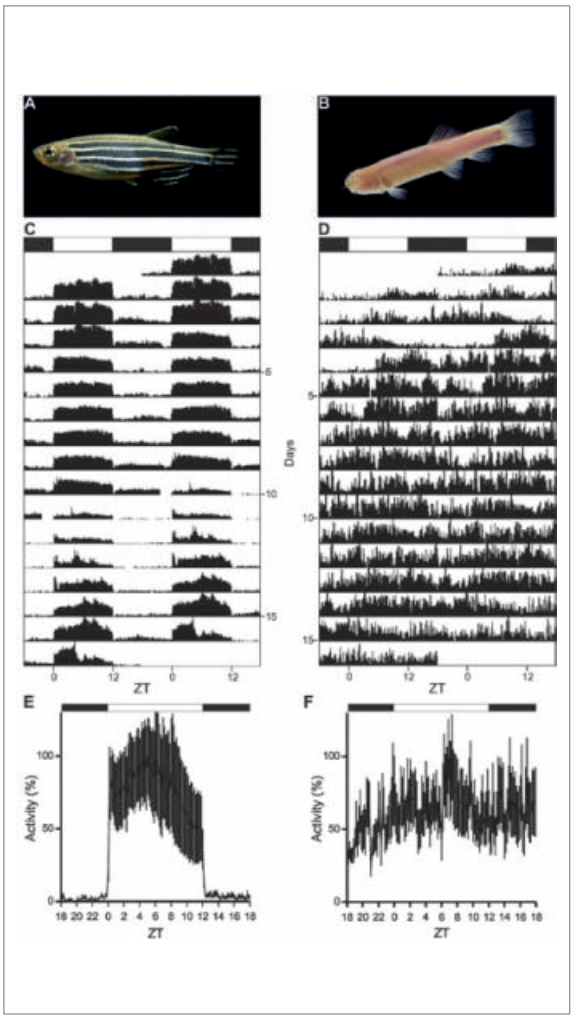

Figure 1
Actograms of zebrafish (A) and Somalian cavefish (B) maintained under LD conditions and fed randomly. (C and D) Records are double plotted on a 48 h time scale; the y-axis progresses in single days with each day being plotted twice (day 1 on the right side is repeated on day 2 on the left side). The height of each point representing the number of interruptions of the infrared light beam. Mean waveforms of zebrafish (E) and cavefish (F) are represented. The cavefish possess blind clocks which are not entrainable by exposure to light dark cycles.

Major contributions since 2009

Fish models offer unique advantages for studying the circadian timing mechanism. Specifically, fish cells and tissues are directly light sensitive and so direct exposure of organs and even cell lines to light triggers resetting of the clock. This contrasts with the situation in mammals where light information is relayed indirectly from the retina to the peripheral clocks via the central clock in the hypothalamic suprachiasmatic nucleus and a myriad of systemic signals. By detailed promoter analysis of a subset of light-regulated clock genes in zebrafish cell lines, we have pinpointed the D-box enhancer as the principal promoter target of light signalling (Vatine et al., 2009; Mracek et al., 2012). This enhancer is regulated by a family of twelve PAR bZip / E4BP4 transcription factors which serve as both activators and repressors. These factors are nuclear targets of a range of light activated signalling pathways including MEK/ERK and ROS (Mracek et al., 2013). To identify the photoreceptor elements which lie upstream of these signalling pathways, we have studied Somalian blind cavefish (Cavallari et al., 2011). In addition to eye loss, these strange animals also exhibit a blind circadian clock (Figure 1). The presence of multiple loss-of-function mutations in cryptochrome and opsin genes in these fish have lead us to a clearer picture of a range of circadian clock photoreceptors which serve to detect the full visible light spectrum.

Unique aspects of the biology of blind cavefish have provided us with additional insight into circadian clock function. Cavefish exhibit an impressive tolerance of long periods of starvation. It is tempting to speculate that this may represent an adaptation to life in a nutrient-poor, perpetually dark environment. In addition, these animals have robust feeding regulated clocks (Cavallari et al., 2011). Specifically, a single meal delivered each 24, 48, or even 72 hours, at the same time of day is sufficient to establish robust 24 hours

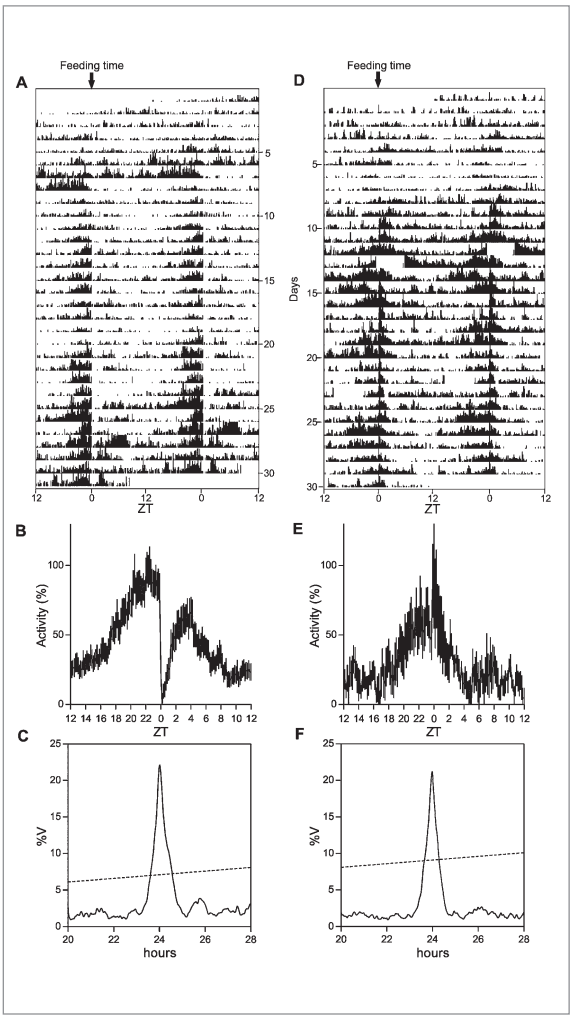

Figure 2
Actograms of zebrafish (A) and cavefish (D) maintained under constant darkness and fed once a day at a fixed time (ZT = 0). Feeding time is indicated by the black arrow at the top of each actogram. (B,E) Mean waveforms of zebrafish (B) and cavefish (E) are represented. (C,F) $\chi 2$ periodogram analysis (confidence level, 95%) for zebrafish (C) and cavefish (F) actograms. The sloped dotted lines represent the threshold of significance, set at $p = 0.05$. Periodogram analysis showed the presence of behavioral activity rhythms synchronized to the 24 h feeding cycles.

rhythms of increased locomotor activity which anticipates the feeding time (Figure 2). These rhythms persist for several weeks when the animals are returned to starvation conditions. However, a paradox exists since cavefish cell culture clocks which can be artificially synchronised by serum treatment, cycle with an infradian period of 47 hours. In addition, these infradian clocks are poorly temperature compensated, with temperature changes in the physiological range resulting in significant changes in clock period length. In contrast the feeding regulated clocks in the fish appear to be temperature compensated. These results point to the existence of two genetically distinct clock mechanisms in fish, one regulated by feeding time and the other light regulated. Interestingly this is consistent with the conclusions of certain studies using clock mutant mice, however the inability of mice to tolerate long periods of starvation severely limits their utility for studying feeding regulated clocks. Thus, we are developing the cavefish as a model to study feeding regulated clocks. This involves the preparation of transcriptome and genome sequences as well as the generation of additional cell culture models. Our ultimate goal is to build a complete picture of the evolution of the cavefish genome that underlies its fascinating phenotype.

The ability of fish tissues to regenerate efficiently following serious injury has made the zebrafish ideally suited to studying the mechanisms of tissue regeneration. The ability of fish to regenerate amputated fins has proven to be a particularly accessible model. We have demonstrated that the clock plays a major role in timing the massive cell proliferation in skin stem cells that follows fin amputation (Idda et al., 2012). The consequence of this regulation is that the time of day that amputation occurs has a considerable effect on the delay between the injury and the onset of cell proliferation. In contrast, cell proliferation in the blastema which accompanies the growth of new fin tissue, is not clock regulated. These results point to the clock playing a key role in the early events following tissue injury.

Planned research and new directions

Our planned future research centres on three main areas:
We aim to explore the molecular mechanisms whereby D-box binding transcription factors are regulated by light-driven signalling cascades such as ROS signalling. Given the current limits of fish for detailed biochemical analysis, we plan to model hop and use mouse models. As well as providing a broad repertoire of tools for biochemical analysis such as extensive panels of antibodies and ChIP methodology, this will also allow us to use genetic tools such as knockout mice strains. Knowledge gained in the mouse system will then be retested in fish models. Another key goal will be to explore precisely how opsin and cryptochrome photoreceptors are coupled with signal transduction pathways, in particular ROS signalling. An improved detailed knowledge of natural photoreceptor function will also benefit the development of optogenetic tools and strategies.

A major goal will be a comparative functional approach using our blind cavefish and zebrafish to study links between clocks and metabolism, in particular the nature of feeding regulated clocks. This will benefit from our current transcriptome and genome sequence analysis of the Somalian blind cavefish as well as cell lines established from cavefish and zebrafish livers. Of particular note, we aim to develop a trans-disciplinary approach in collaboration with Burkhard Luy at KIT, to use NMR to study circadian changes in the liver metabolome in fish as well as documenting the metabolic basis for starvation tolerance of cavefish.

One major new direction will be a study of the genetic and cellular basis of diurnality and nocturnality. Despite detailed knowledge of the core clock machinery, we have practically no insight into the mechanisms which confer diurnal behaviour in some species and nocturnal behaviour in others. To tackle this basic question we will exploit closely related medaka species which we have shown exhibit nocturnal and diurnal behaviour. These species are sufficiently closely related that inter species crosses yield fertile offspring. Combining genetics and cell transplantation studies we will study the genetics of diurnal and nocturnal behaviour and link this with the identification of specific critical regions of the central nervous system dictating the phase of behavioural activity.

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 16, number of citations 2009-2013: 164, h-index (2009-2013): 7, total h-index: 36 (according to Thomson Reuters).

Vatine G, Vallone D, Appelbaum L, Mracek P, Ben-Moshe Z, Lahiri K, Gothilf Y, Foulkes NS. Light directs zebrafish period2 expression via conserved D and E boxes. PLoS Biol. (2009) 7:e1000223.
Cavallari N., Frigato E., Vallone D., Fröhlich N., Lopez Olmeda JF., Foà A., Berti R., Sánchez Vázquez FJ., Bertolucci C., Foulkes NS. A Blind Circadian Clock in Cavefish Reveals that Opsins Mediate Peripheral Clock Photoreception. PLoS Biol. (2011). 9: e1001142.
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Mracek P, Santoriello C, Idda ML, Pagano C, Ben-Moshe Z, Gothilf Y, Vallone D, Foulkes, NS. Regulation of per and cry genes reveals a central role for the D-box enhancer in light-dependent gene expression. PLoS One. (2012) 7: e51278.
Mracek P, Pagano C, Fröhlich N, Idda ML, Cuesta IH, Lopez-Olmeda JF, Sánchez-Vázquez FJ, Vallone D, Foulkes, NS. ERK signaling regulates light-induced gene expression via D-box enhancers in a differential, wavelength-dependent manner. PLoS One. (2013) 8: e67858.


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2.4 ANIMAL MOLECULAR PHYSIOLOGY
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Fields of Interest
Sensory physiology, signal processing in the mammalian olfactory system, trigeminal nociceptive system

Brief summary of work since 2009
We examine the mammalian olfactory system at the levels of receptor neurons in the nose and the first stage of signal processing, the olfactory bulb. In rodent receptor neurons, we have studied the regulation of the transduction channels that serve to convert chemical stimuli into electrical signals. In the past 5 years, we have mainly looked into the biophysical properties of these channels and characterized cAMP-gated cation channels and calcium-gated chloride channels using patch-clamp techniques. Our main approach was site-directed mutagenesis. We identified various functional domains in the channel proteins and studied allosteric effects originating from the assembly of different channel subunits. Currently, our main interest is the interaction of the olfactory system with the trigeminal system. Both systems mediate chemosensory detection, and most chemical agents affect both systems, albeit at different concentrations. Moreover, trigeminal neuropeptides modulate the olfactory system both in the olfactory epithelium and in the olfactory bulb. This cross-talk between the two senses is presently the focus of our research, as it determines chemosensory information processing.

Major contributions since 2009
The olfactory signal transduction cascade combines low ligand selectivity with strong signal amplification. The low selectivity is necessary to accommodate a wide spectrum of different odors – encompassing one trillion odorants according to a recent estimate. The signal amplification is needed to elicit action potentials even when the stimulus consist only of a few odor molecules. Signal transduction and amplification take place in the sensory cilia of olfactory receptor neurons. Although the main players of this process are known, most principal questions about the key steps in transduction are unresolved. These include the time course, the amplification gain, and the adaption of the primary olfactory signal.

We have been working on these questions since 30 years, and have recently contributed a detailed allosteric model for the cAMP-gated cation channel, which is the first channel to open in the cilia upon odor stimulation (Waldeck et al., 2009). The opening of these channels triggers a calcium signal which, in turn, opens chloride channels. We disproved the hypothesis on the molecular nature of the the olfactory chloride channel favored at that time (Klimmeck et al., 2009). We identified the channel protein through a functional screening approach at the same time when three other groups reached the same conclusion in different ways. We cloned these channels (termed TMEM16B or anoctamin 2) and performed an extensive structure-function analysis which leads to the discovery of a calmodulin-dependent inactivation mechanism that controls the channels (Vocke et al., 2013). We also studied the chloride accumulation into olfactory cilia (Hengl et al., 2010).

This was important because, unlike in most other neurons, the calcium-gated chloride channels of olfactory cilia conduct a depolarizing chloride efflux that promotes excitation.

Finally, we found that the same chloride channels are expressed in dorsal root ganglion neurons, in a type of neuron that provides the nociceptive input for pain perception. Before 2009, we had studied chloride homeostasis in these neurons and found evidence that a rise of intracellular chloride may contribute to inflammatory hyperalgesia. The notion that TMEM16/ anoctamin channels may be involved in this process is an exciting prospect for our future research and has since been corroborated by other labs. We started working on the nociceptive system in the frame of the DFG priority program »Integrative analysis of olfaction« and characterized interactions between the trigeminal system and the olfactory system (Daiber et al., 2013). We see multiple parallels between the two sensory systems, and certain aspects of signal transdution and signal amplification appear to be based on the same type of ion channel – the calcium-gated chloride channel.

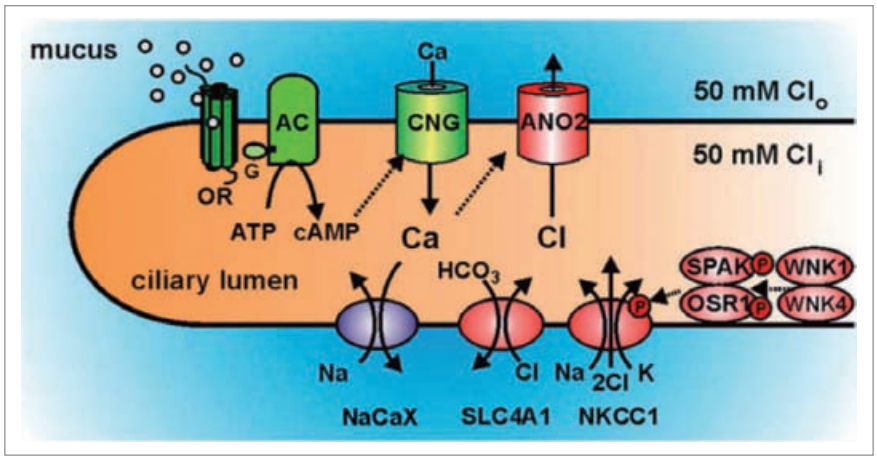

Figure 1
Schematic representation of the molecular components of olfactory signal transduction in a chemosensory cilium. Odorants dissolve in mucus at the surface of the olfactory epithelium and bind to odorant receptors (OR) in the ciliary membrane. The receptors activate the enzyme adenylyl cyclase (AC) to synthesize cAMP and open cyclic nucleotide-gated (CNG) cation channels. Calcium enters the cilium and opens chloride channels of the anoctamin (ANO2) type. Chloride is accumulated in the resting cilium through the chloride transporter NKCC1 which, in turn, is activated by a set of protein kinases (SPAK, OSR, WNK). Calcium is removed from the cilia by a sodium/ calcium exchanger (NaCaX) to terminate the recoetor current. (from Hengl et al., 2010)

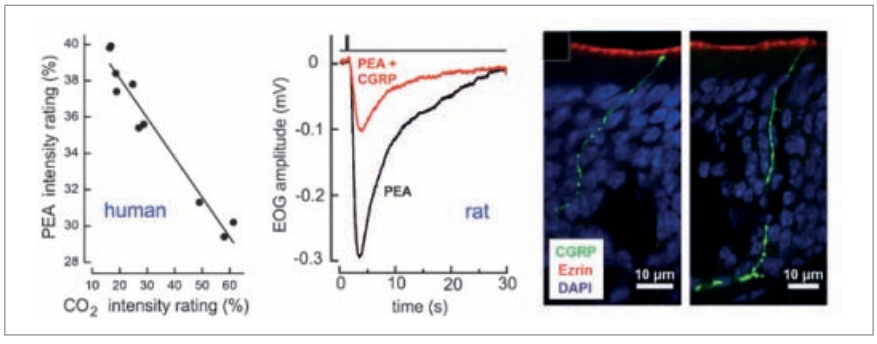

Figure 2
Results from a psychophysical study with co-application of PEA (rose odorant) and CO2 (an irritant) in human subjects (left). The stronger the perceived irritant (chemestesis) the weaker is the odor perception (olfaction). In the rat olfactory epithelium (center), co-application of the trigeminal neuropeptide CGRP with the rose odorant reduced the electrical response of the olfactory epithelium. The images on the right show trigeminal fibers crossing the olfactory epithelium (blue) right up to the tissue surface (red). (from Daiber et al. 2013)

Planned research and new directions
Our interest lies in the role that anoctamin-type calcium-gated chloride channels play in the processing of olfactory and nociceptive signals. To approach this, we have initiated two projects that take us away from the periphery of the sensory systems to the first stages of central processing.

1. Olfactory signal processing
To detect differences of circuit activity that are associated with the chloride channels, we compare wild type with anoctamin 2-knockout animals (collaboration with Thomas Jentsch, Leipniz Institute for Molecular Pharmacology, Berlin). We use vital brain slices of the olfactory bulb, and we record local field potentials and single-unit activity from periglomerular cells, mitral cells, and granular cells. We stimulate the afferent axons with light flashes, using the expression of channelrhodopsin 2 in all mature olfactory receptor neurons (collaboration with Tom Bozza, Northwestern University, USA). The mouse lines and the recording methods are established in our lab, and the experiments are in progress.

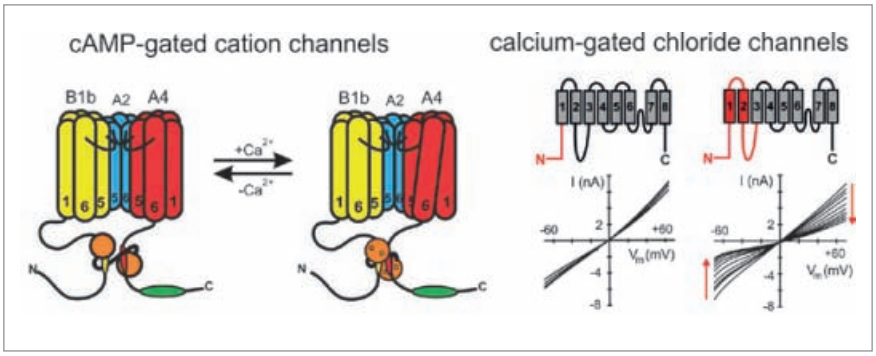

Figure 3
Working models for the two olfactory transduction channels. These models have emerged from functional studies of many channel mutants and provide an explanation for the desensitization that occurs in both channels upon binding of calcium-calmodulin (from Adelman & Herson, 2004 and Vocke et al., 2013).

2. Nociceptive signal processing

To advance our insight into the cross-talk between the olfactory and trigeminal systems, we study the response of the second-order trigeminal neuron to nociceptive and combined nociceptive/olfactory stimulation (collaboration with Richard Carr, Faculty of Medicine, Heidelberg University, Mannheim). Single-unit recordings will be obtained *in vivo* from the spinal trigeminal nucleus during combined nociceptive/non-nociceptive stimulation from the nose, the cornea, the dura, and the facial skin. Co-stimulation from the olfactory system will be delivered through activation of the channelrhodopsin-expressing olfactory receptor neurons. Again, the anoctamin 2-knockout mouse will be examined for a phenotype in the afferent sensory signal integration. We are currently studying the convergence of nociceptive and non-nociceptive fibers on the different parts of the spinal trigeminal nucleus using various GFP-expressing mouse strains. At the same time, we are setting up the *in vivo*-recording (collaboration with Johannes Schenckel, DKFZ, Heidelberg).

Both projects are designed to detect the significance of chloride channel activity and chloride homeostasis for local neuronal circuit function at the primary sensory input to the brain. We hope to gain insight into distinct network aspects including signal amplification, signal filtering, and lateral inhibition through these experiments.

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 13, number of citations 2009-2013: 118, h-index (2009-2013): 6, total h-index: 36 (according to Thomson Reuters).

Daiber, P., Genovese, F., Schriever, V., Hummel, T., Möhrlen, F., Frings, S. (2013) Neuropeptide receptors provide a signalling pathway for trigeminal modulation of olfactory transduction. *European Journal of Neuroscience* 37: 572-582

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: 381-404

Hengl, T., Kaneko, H., Dauner, K., Vocke,K., Frings, S., Möhrlen, F. (2010) Molecular Components of Signal Amplification in Olfactory Sensory Cilia. *Proc Nat Acad Sci USA* 107: 6052-6057

Klimmeck, D., Daiber, P.C., Brühl, A., Baumann, A., Frings, S., Möhrlen, F. (2009) Bestrophin 2 – an anion channel associated with neurogenesis in chemosensory systems. *J. Comp. Neurol.* 515: 585-599.

Waldeck, C., Vocke, K., Ungerer, N., Frings, S., Möhrlen, F. (2009) Activation and desensitization of the olfactory cAMP-gated transduction channel – identification of functional modules. *J. Gen. Physiol.* 34(5):397-408.

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

Brief summary of work since 2009

We are working on several aspects of olfactory sensory transduction and investigate the molecular components involved in the signal transduction process. In collaboration with the German Cancer Research Center we have established the organellar proteome for isolated cilia of rat olfactory sensory neurons. 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. This channel is a member of the recently discovered family of anoctamins. We have examined the tissue specificity and sub-cellular localization of anoctamins in all chemosensory organs of the rodent nose as well as the rodent retina. On the functional level, we have established how anoctamins interact with Ca^{2+}/calmodulin in processes that determine channel gating.

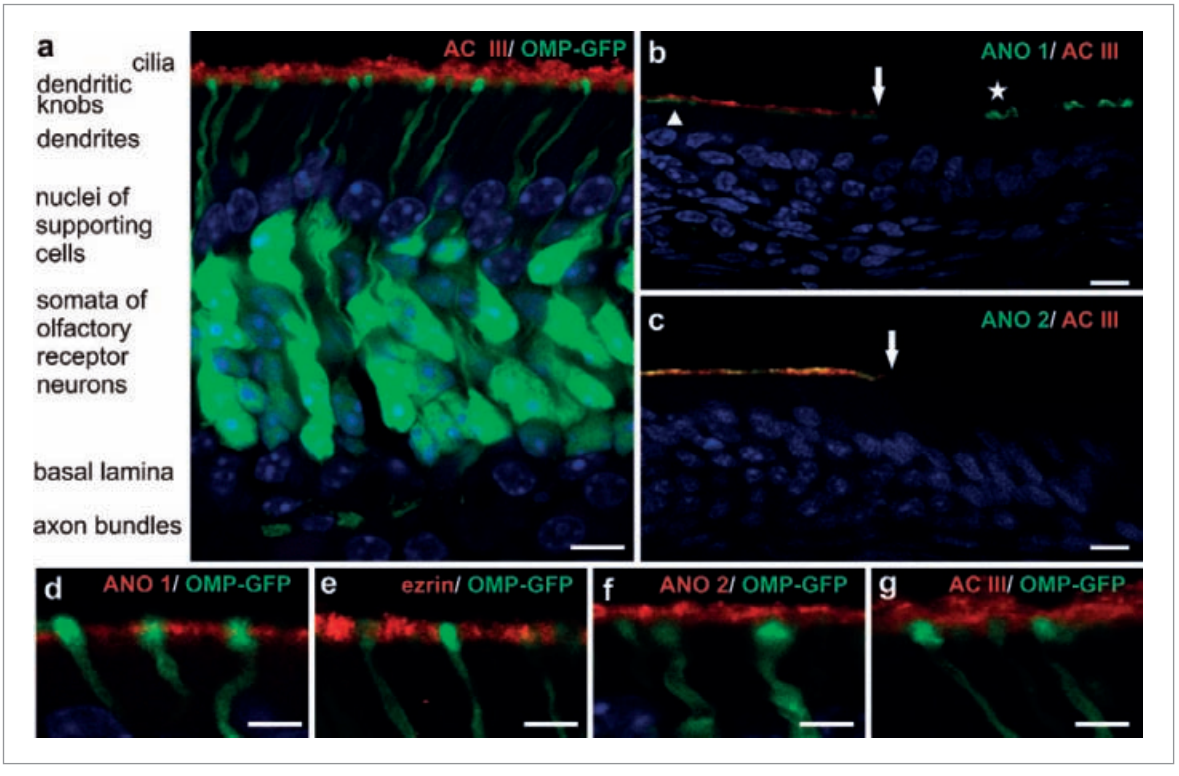

Figure 1
Expression of ANO 1 and ANO 2 in the main olfactory epithelium (MOE) of rats (b, c) and OMP-GFP mice (a, d-g). a Overview of the MOE of an OMP-GFP mouse. Red immunosignals indicate the ciliary marker protein AC III. b Confocal image of the transition region from MOE to respiratory epithelium of the rat (arrow). Double staining of ANO 1 with the ciliary marker protein AC III shows no co-localisation in the apical layer of the MOE (arrowhead). ANO 1 is localized on the surface of the respiratory epithelium in patch-like structures (asterisk). c Confocal image of the transition region between MOE to respiratory epithelium (arrow). The yellow immunosignal illustrates that ANO 2 is co-localized with AC III on the surface of the olfactory epithelium. d-g High-resolution images of single dendritic knobs of ORNs from the OMP-GFP mouse illustrate the expression of ANO 1, ezrin, ANO 2 and AC III, respectively. ANO 1 is expressed in the layer of the supporting cell microvilli, stained by ezrin (d, e). The site of ANO 2 expression is the layer of ORN cilia, stained by AC III (f, g). Scale bar: 15µm (a – c), 5 µm (d – g).

Major contributions since 2009

We started out without knowing the molecular identity of the calcium-activated chloride channels in olfactory receptor neurons. Our solution was to identify every protein that is expressed in the sensory cilia and to apply a functional screening assay for calcium-induced chloride flux. We purified ciliary proteins, applied mass spectrometric analysis and, hence, established the ciliary proteome (Mayer et al., 2009). Our screening assay produced a novel protein which was found to be a calcium-gated chloride channel of the anoctamin family. We studied the expression of anoctamin channels in the nose (Dauner et al., 2012), in the retina (Dauner et al., 2013), and in various other tissues. Moreover, we subjected the anoctamin channels to systematic mutagenesis and discovered key aspects of the channels´ gating mechanism (Vocke et al., 2013). Together with our mutagenesis study of the cAMP-gated cation channel from olfactory cilia (Waldeck et al., 2009; Ungerer et al., 2011), these data provided a concept for the function of both channel types in olfactory signal transduction (Hengl et al., 2010).

For our studies of molecular structure-function relations in ion channels, we combine bioinformatic analyses of domain functions with site-directed mutagenesis of proteins and biophysical studies of peptide properties. Essential protein domains involved in gating, ion permeation and activity regulation can thus be identified. In collaboration with research groups in Heidelberg and Kaiserslautern, 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. The proteomic approach has proved to be successful in the search for unidentified proteins in the sensory cilia. The organelle-specific subset of proteins was identified and helped to advance olfactory research.

Planned research and new directions

The olfactory calcium-gated chloride channel is a highly regulated protein. It forms homo- and heterodimers, it is subject to control by calmodulin and by phosphorylation, and it depends on the presence of yet unknown cytosolic constituents. These regulatory processes will be studied by structure-function analysis, complemented by the investigation of peptides that represent the functional domains of the proteins. Currently, we are screening a peptide library obtained from all known calmodulin-binding proteins for modes of interaction with calcium/calmodulin using peptide chips (collaboration with Ralf Bischoff, PEPperPRINT, Heidelberg). This project is designed to shed light on the aspect calmodulin regulation of anoctamin channels. We can then apply biophysical methods (e. g. fluorescence correlation spectroscopy, analytical ultracentrifugation, isothermal titration calorimetry) to obtain quantitative parameters for this interaction (collaboration with Jörg Langowski, DKFZ, Heidelberg). In this way, we hope to understand, how the anoctamin channels operate on a molecular basis.

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 10, number of citations 2009-2013: 118, h-index (2009-2013): 6, total h-index: 13 (according to Thomson Reuters).

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: 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. Investigative Ophthalmology and Visual Science 54: 3126-3136.

Hengl, T., Kaneko, H., Dauner, K., Vocke, K., Frings, S., Möhrlen, F. (2010). Molecular Components of Signal Amplification in Olfactory Sensory Cilia. Proc Nat Acad Sci USA 107: 6052-6057

Waldeck, C., Vocke, K., Ungerer, N., Frings, S., Möhrlen, F. (2009). Activation and desensitization of the olfactory cAMP-gated transduction channel – identification of functional modules. J. Gen. Physiol. 34(5):397-408.

Mayer, U., Küller, A., Daiber, P.C., Neudorf, I., Warnken, U., Schnölzer, M., Frings, S., Möhrlen, F. (2009). The proteome of rat olfactory sensory cilia. Proteomics 9:322-334.

2.5 PLANT DEFENSE METABOLISM
DR. EMMANUEL GAQUEREL INDEPENDENT RESEARCH GROUP

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Fields of Interest
Plant-insect interactions, Metabolomics, Secondary metabolism, Bioinformatics, transcriptional networks

Brief summary of work since 2009
As sessile organisms, land plants have exploited their metabolic systems to produce an extremely large repertoire of structurally diverse specialized metabolites, some of them conferring chemical shields against insects. Understanding the evolutionary trajectories of these specialized metabolic pathways will impact a fundamental question in biology – how do complex traits evolve? Research in my group aims at combining mass spectrometry (MS)-based metabolomics and genomics resources to explore metabolic pathway adjustments during plants' interaction with insects. While a project group leader at the Max Plank for Chemical Ecology (2010-2013), this research has focused on the gene discovery in the biosynthesis of defensive metabolites that evolved in restricted lineages of the Solanaceae. The gene discovery process in these pathways has been supported by the development of novel bioinformatics methods to elucidate transient tissue-specific gene regulatory networks (GRN) elicited by insect feeding. Novel bioinformatics developments were also applied to the discovery of novel emergent properties of roots' GRNs recruited during leaf herbivory by insects and of floral metabolism regulation. I joined the COS in July 2013 as an independent group leader. My group collaborates with the Metabolomics Core Facility Platform, both being financed as part of the DFG Excellence Initiative II. Ongoing research projects at the COS investigate how restructuring in gene expression that followed an ancient allopolyploidization event in the genus *Nicotiana* (*Nicotiana* section *Repandae*) shaped the evolution of novel metabolic traits. We also plan to explore the role of a *Nicotiana*-specific F-box protein in regulating floral metabolism.

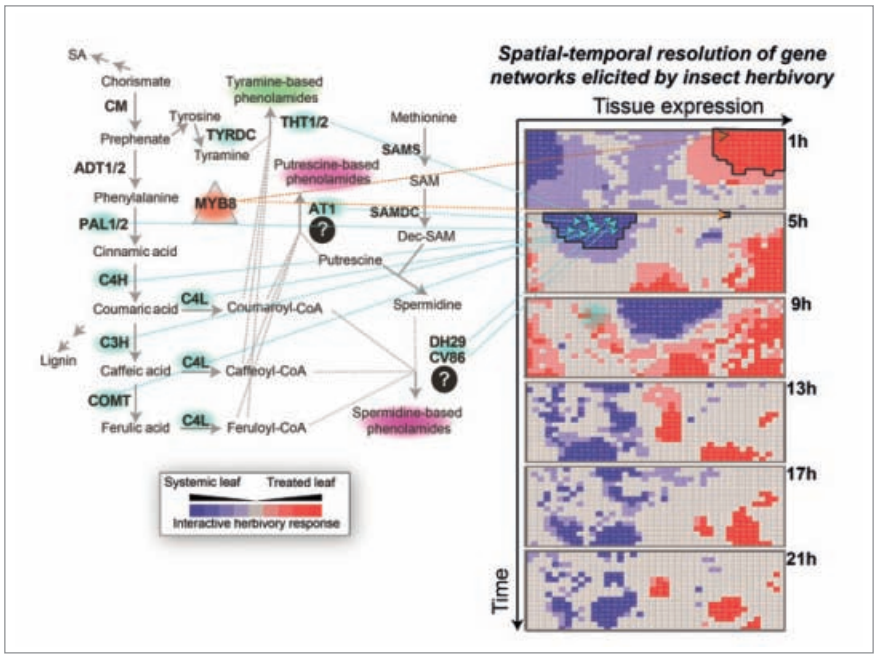

Figure 1
A multifactorial co-expression analysis of systemically induced secondary metabolic pathways (adapted from Gaquerel et al. 2014). Self-organizing maps are used to cluster genes according to their tissue and time behaviors inferred from multifactorial statistics. Bait genes (here from the phenylpropanoid metabolic pathway) are used to identify clusters of interest for stress adaptation.

Major contributions since 2009

Plant cells are capable of producing an incredibly large spectrum of functionally diverse metabolites, of which many secondary metabolites have still unknown functions. Yet, this impressive metabolic complexity likely shaped by the evolutionary pressure of attacking organisms is still hardly exploited, notably since the underlying metabolic pathways have not been investigated. The overarching objectives of my research are to explore, using selected case-study pathways, how plants adjust their metabolism to cope with the attack of insects and how specialized metabolic pathways organize at the molecular level and emerge through natural selection. I articulate below major contributions in the development of metabolomics/bioinformatics methods and their application to the discovery of key innovations in plant metabolism.

Important advances have been made in my group while at the MPI for Chemical Ecology (MPI-CE) in the establishment of MS-metabolomics resources to a non-model organism (*Nicotiana attenuata*) in order to study metabolic adaptations to its main herbivore, *Manduca sexta*. Since appointed at the COS, we implemented these metabolomics methods at the Metabolomics Core Facility Platform as well as a new GC-MS screen for approximately 100 energy-related metabolites. By directly »asking the plant« through metabolic profiling combined with statistical analyses, we discovered and validated the antiherbivore functions of acyclic diterpene glycosides, phenolic-amine derivatives and trichome-based O-acyl sugars, whose biosynthesis is restricted to certain lineages of the Solanaceae. Metabolite identification being still a key bottleneck in metabolomics, we implemented new analytical approaches based on isotope labeling as well as developed, in collaboration with the Bioinformatics group at the Institute for Plant Biochemistry (Halle), a novel network-based informatics pipeline for hypothesis generation for »unknowns« (Gaquerel *et al.*, 2013). Using multifactorial analysis of integrated transcriptomics-metabolomics time series and self-organizing maps, we established the first cartography of transient tissue-specific GRNs and gene-metabolite co-dynamics during insect herbivory (Gulati *et al.*, 2013) (Figure 1). This significant achievement led to a series of discoveries on how *N. attenuata* adjusts its whole-organism physiology to cope with insects. For instance, in a recent article, we discovered via this approach that leaf herbivory induces inversions in roots' semi-diurnal transcript oscillations which facilitate sugars translocation to roots as a tolerance strategy to herbivory (Gulati *et al.*, 2014).

In a series of pathway-specific studies, we demonstrated that combining metabolomics and transcriptomics helps removing much of the guesswork from the process of gene discovery for a given metabolic phenotype. We notably made significant advances in the elucidation of the biosynthesis of phenolic-amine derivatives which are produced by

diversion of the phenylpropanoid flux and conjugation to spermidine and putrescine backbones. In collaboration with Prof. Ivan Galis (Okayama University), we discovered a pathway specific transcription factor and three target N-acyltransferases controlling the conjugation process and accumulation of these metabolites following herbivory as well as a novel flux control at the branching point with the lignin pathway. This work and new tools have been summarized in a recent review article (Gaquerel *et al.*, 2014). We are currently applying the same strategy to *Nicotiana*-specific acyclic diterpene glycosides and already identified three UDP-glucosyltransferases that control the production of the full chemotype.

Reconfigurations of a plant's metabolism are also central in the case of reproductive organs for their advertisement to pollinators by the production of attractive volatiles and rewards (nectar). In the last year and a half, we applied an integration of metabolomics and transcriptomics to the maturation of *N. attenuata* flower organs. By developing new transgenic means of creating jasmonate metabolic sinks (Stitz *et al.*, 2011), we discovered novel floral functions of jasmonate signaling for carbohydrate metabolism with important consequences for flower anthesis. We notably provided the first concrete evidence that jasmonoyl-*L*-isoleucine regulates the unfolding of the limb of sympetalous tobacco flowers (Stitz, Baldwin and Gaquerel accepted in The Plant Cell).

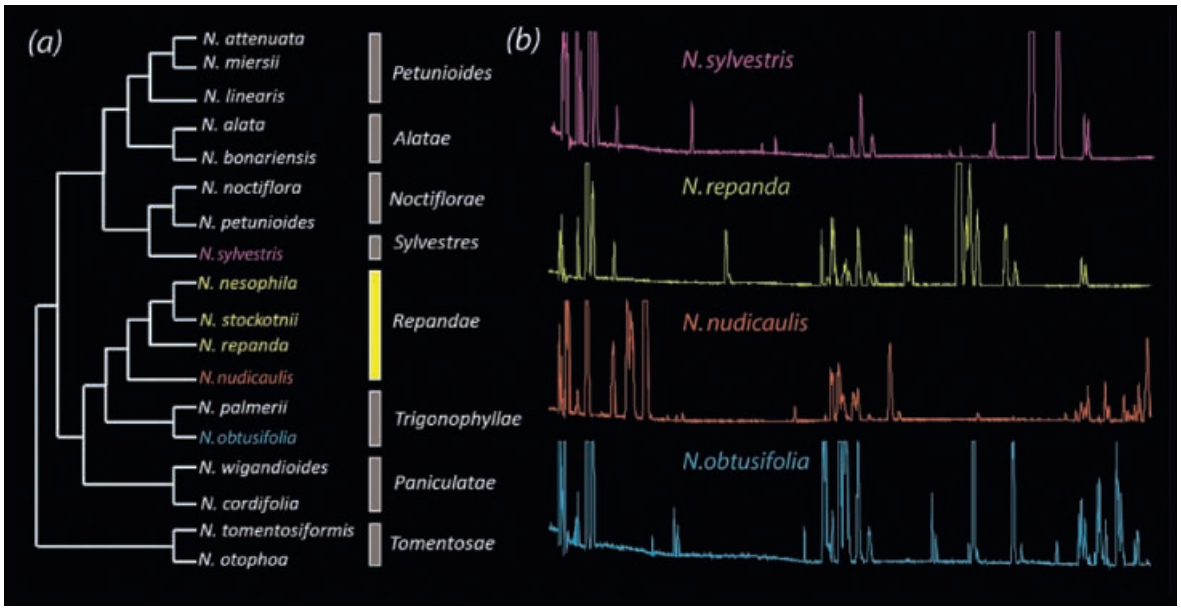

Figure 2
Specialized metabolism diversification in allopolyploids of the sub-section Repandae revealed by MS-based metabolomics. (a) Sectional classification modified from Knapp et al. (2004) of the genus Nicotiana highlighting allopolyploids of the section Repandae. (b) Comparative MS-based metabolomics of allopolyploids and closest ancestral species.

Planned research and new directions

I joined the COS in July 2013 as an independent group leader (»Plant Defense Metabolism« group). My group collaborates with the Metabolomics Core Facility Platform, both being financed as part of the DFG Excellence Initiative II (Metabolomics Platform). Two new research lines have been initiated and aim at a better understanding of the genomic bases of key metabolic innovations.

We are developing *Nicotiana* species from the section *Repandae* as new model systems for testing the rapid diversification of defense metabolic pathways. *N. repanda*, *N. stocktonii*, *N. nesophila* and *N. nudicaulis* which colonize different habitats in the USA and Mexico are derived from a single ancient allopolyploidy event with *N. sylvestris* and *N. obtusifolia* being the closest parental genomes. Our preliminary metabolomics work has shown reconfigurations of the herbivory-induced signaling networks and metabolomes of these species (Figure 2). Importantly, old literature reports supported by our analysis demonstrate that three of these species have evolved a new type of trichome-specific defense metabolites extremely toxic to herbivores called *N*-acyl nornicotines. This provides an ideal phylogenetic framework for testing how (allo)polyploidization contributed to the emergence of gain-of-function defense metabolism traits, something that, to my knowledge, has never been rigorously tested. Importantly, closest parental genomes are

sequenced and we are collaborating to the analysis of *N. obtusifolia* as well as *N. attenuata* genomes. Taking advantage of these resources, we are currently developing a novel approach of combining tissue-specific RNAseq transcriptome, metabolome and bioinformatic analyses with the following objectives:

(1) analyzing the sequence and expression evolution of homeologous genes contributing to divergent herbivory-induced signaling and metabolic phenotypes (Postdoc project Dr. Aura Navarro Quezada),
(2) identifying trichome-expressed *N*-acyltransferases and transporters for *N*-acylnornicotines production and induced exudation,
(3) testing for structure-activity relationships in the defensive function of these metabolites by metabolomics and targeted reverse genetics approaches (Postdoc project Dr. Anne Terhalle).

In continuity with the work we carried out on flower metabolism in *N. attenuata*, we recently discovered a *Nicotiana* genus-specific duplication of COI1, the jasmonate receptor. Preliminary work suggests that COI1-like acts a regulatory step for the metabolism of *Nicotiana* flowers. We are currently investigating, in collaboration with Dr. Michael Stitz and Prof. Ian T. Baldwin (MPI-CE) the functional divergence of these two COI1 genes using yeast-two-hybrid protein interaction screening and targeted gene silencing. We believe that this may lead to a paradigm shift in our understanding of jasmonate signaling evolution.

Selected publications since 2009
Number of peer-reviewed articles 2009-2013: 23, number of citations 2009-2013: 544, h-index (2009-2013): 13, total h-index: 13 (according to Thomson Reuters).

Gulati, J., Baldwin, I. T., and Gaquerel, E*. (2014). The roots of plant defenses: integrative multivariate analyses uncover dynamic behaviors of gene and metabolic networks of roots elicited by leaf herbivory. The Plant journal 77, 880-92. (* corresponding author)
Gaquerel, E., Gulati, J., and Baldwin, I. T. (2014). Revealing insect herbivory-induced phenolamide metabolism: from single genes to metabolic network plasticity analysis. The Plant journal In press (* corresponding author)
Gulati, J., Kim, S.-G., Baldwin, I. T., and Gaquerel, E*. (2013). Deciphering herbivory-induced gene-to-metabolite dynamics in *Nicotiana attenuata* tissues using a multifactorial approach. Plant physiology *162*, 1042-59. (* corresponding author)
Gaquerel, E.*, Kuhl, C., and Neumann, S. (2013). Computational annotation of plant metabolomics profiles via a novel network-assisted approach. Metabolomics *9*, 904-918. (* corresponding author)
Stitz, M., Gase, K., Baldwin, I. T., and Gaquerel, E*. (2011). Ectopic Expression of *AtJMT* in *Nicotiana attenuata*: creating a metabolic sink has tissue-specific consequences for the jasmonate metabolic network and silences downstream gene expression. Plant Physiology *157*, 341-354. (* corresponding author)

2.6 MOLECULAR ORGANIZATION OF CELLULAR MEMBRANES
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Fields of Interest
Cell polarity, plasma membrane organization, cytoskeleton, membrane transport, cell-cell communication, plant nutrition, environmental sensing

Brief summary of work since 2009
Cell polarity is an inherent feature of all cells that is responsible for asymmetric distribution of cellular components and functions within the cell and involves lateral organization of the plasma membrane. This asymmetry is the basis for interaction of cells with their environment: cells are exposed to signals that contain directional information such as chemical gradients and they are able to respond to these signals in a targeted manner such as by directed growth, secretion or molecular transport.

To understand how cells perceive and respond to the direction of signals and how they establish asymmetric cell-cell communication and polarized transport, we investigate (I) the formation and stabilization of specialized domains in the plasma membrane and (II) the flux of nutrients, ions or metabolites within tissues.

Using yeast and *Arabidopsis* as model systems we have identified mechanisms of microdomain formation and discovered a role of microtubule-dependent, selective diffusion barriers at the plant plasma membrane in maintaining polar protein distribution.

To follow the flux of molecules in tissues we have developed fluorescent sensors and microfluidic imaging platforms for roots. These tools allow analyzing the dynamics and transport directions of nutrients and signaling components in response to controlled stimulation. Recent projects on calcium signatures have revealed specific distribution patterns at cellular and subcellular resolution.

By combining research on membrane organization with measuring molecular fluxes we aim to identify functional links of cell polarity and transport and provide a better understanding how cells communicate over distances and how nutrients are allocated in plants.

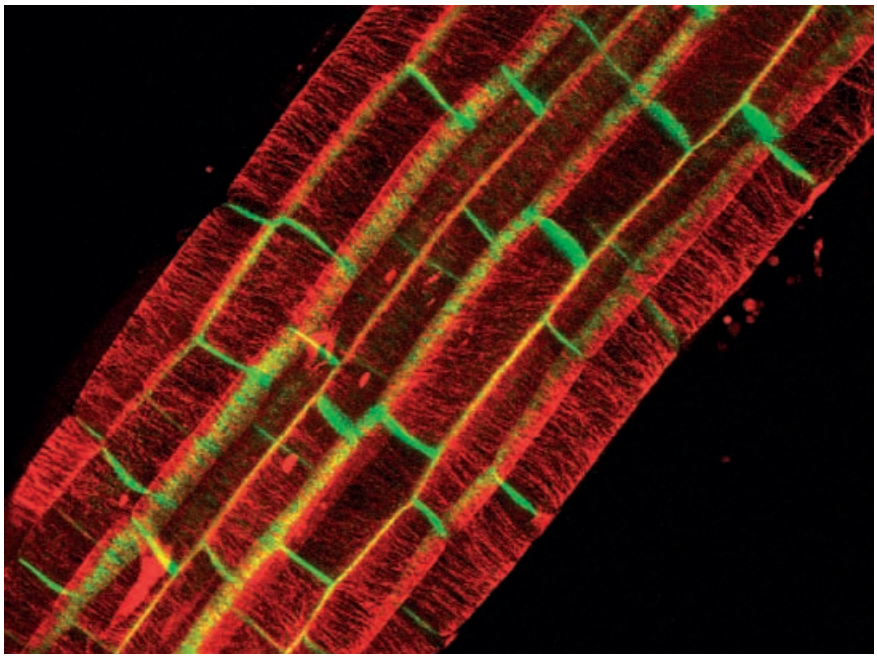

Figure 1
The polarized localization of plasma membrane proteins (green: GFP-labeled flotillins) can be stabilized by microtubule-dependent diffusion barriers (red: mCherry-labeled TuA5).

Major contributions since 2009

Biological membranes are heterogeneous but highly organized. Proteins form clusters, lipids segregate into different phases based on their mutual affinity, and together proteins and lipids can form microdomains of specific composition. On a larger scale, cell polarity results in differential membrane composition of larger membrane areas, dependent on the cell environment, neighboring cells, or the axis of cell expansion. Although there is a general consensus that higher levels of organization exist in membranes of living cells, there is still a significant controversy about the formation, composition and physiological roles of the different types of membrane organization.

To study the fundamental mechanisms of membrane organization and its importance for cells, I joined Widmar Tanner (Regensburg), who was among the first to provide microscopic evidence for a compartmentalization of the plasma membrane of living cells by discovering two stable membrane subcompartments in bakers yeast. During my PhD and a short PostDoc, we investigated mechanisms of membrane organization by studying formation and structure of stable membrane microdomains and identified a function of these domains in turnover regulation of transporters (reviewed in Malinsky et al. 2013).

To pursue my research on membrane organization in the context of multicellularity, I chose *Arabidopsis thaliana* as a model system and joined, as an EMBO fellow, the groups of Wolf Frommer and David Ehrhardt (Carnegie Institution). *Arabidopsis* roots are advantageous for studying membrane protein dynamics, cell polarity and molecular transport, due to their architecture and lower autofluorescence as compared to aerial parts of the plant. Imaging roots was, however, generally hampered by the high sensitivity of roots towards drying and physical contact. In collaboration with the Quake lab at Stanford University, we therefore developed a fully integrated, microfluidic imaging platform, named RootChip, which allows growth, imaging and controlled perfusion of *Arabidopsis* roots in separate observation chambers for several days (Grossmann et al., 2011). This device has significantly improved reproducibility of experimental results and allows automation of measurements, which increases throughput and data quality. A number of collaborations on projects involving root imaging could be initiated since the development of the RootChip.

When examining localization and dynamics of various GFP-labelled plasma membrane proteins, I discovered for a number of these proteins linear domains from which the proteins appeared absent and that acted as barriers for lateral protein diffusion. As underlying structures of these linear barriers I could identify cortical microtubules, indicating a novel role of microtubules in partitioning the plasma membrane (Grossmann et al., in prep.). This function of cortical microtubules could be seen analogous to the function of actin forming a »membrane skeleton« in animal cells.

Asymmetric distribution of a transporter may provide an indication on transport direction of its substrate. It became clear, however, that transporter localization and transport direction could only be functionally linked if one was able to follow the dynamics of transported molecules. To this end I utilized genetically encoded fluorescent biosensors for *in vivo* measurements and participated in their development. These sensors either consist of a single fluorophore that responds with intensity changes to substrate binding or of two fluorophores linked by a substrate-binding domain that report binding by Förster Resonance Energy Transfer (FRET). Their high specificity allows selective and quantitative measuring of absolute concentrations and relative concentration changes in real time. FRET sensors for zinc allowed us to determine steady state levels and uptake kinetics of this transition metal in roots (Lanquar et al. 2013). As a tool for direct visualization of active transport of a nutrient, a single-fluorophore transport sensor for the nitrogen source ammonium was developed, directly reporting the conformational change of the transporter due to transport activity (De Michele et al. 2013). The recent development of the first sensor for detection of real time changes of the plant hormone abscisic acid (Jones et al. 2014a) presents a major step towards understanding the roles of hormone levels and gradients in signalling during development or responses to environmental stresses.

Fluorescent sensors can also provide insights into signalling processes and thereby aid studying the direction of cell-cell communication. Continuous improvement of calcium sensors by several groups has yielded fast responding, highly sensitive sensors. In collaboration with Karin Schumacher and Nana Keinath (Heidelberg) we used a sensitive single-fluorophore calcium sensor to quantify calcium transients in roots upon stimulation with different elicitors (Keinath et al., in prep.). Expressing a troponin-based calcium sensor in cell types of the female gametophyte of *Arabidopsis* we detected, in collaboration with Thomas Dresselhaus (Regensburg), polarized calcium spiking in synergids and specific signatures in egg cell and central cell during double fertilization (Denninger et al., 2014).

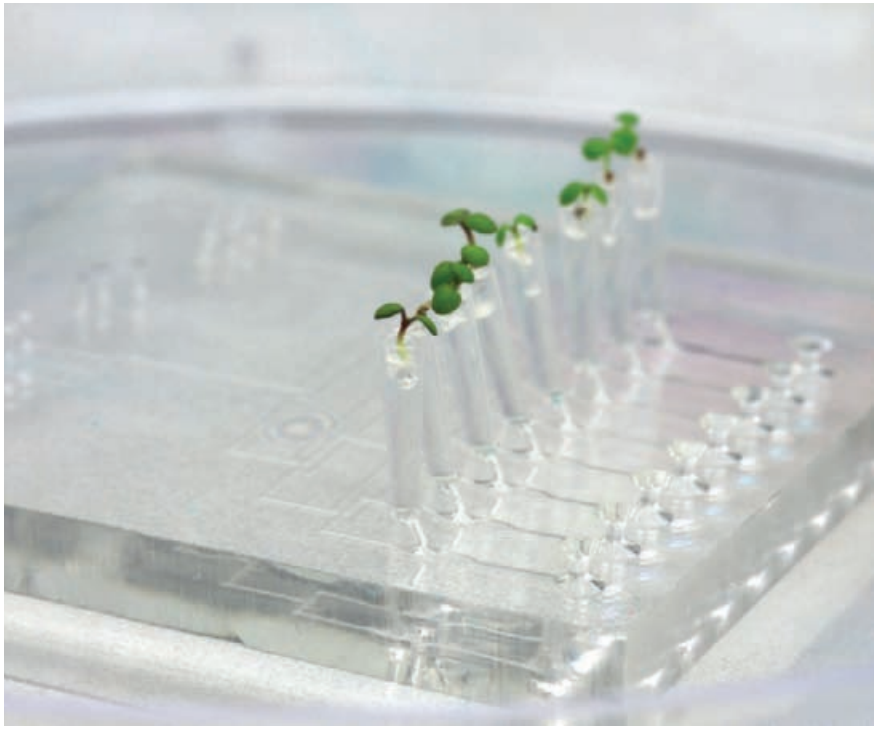

Figure 2
Roots of Arabidopsis seedlings growing into the RootChip, an integrated microfluidic device for root imaging.

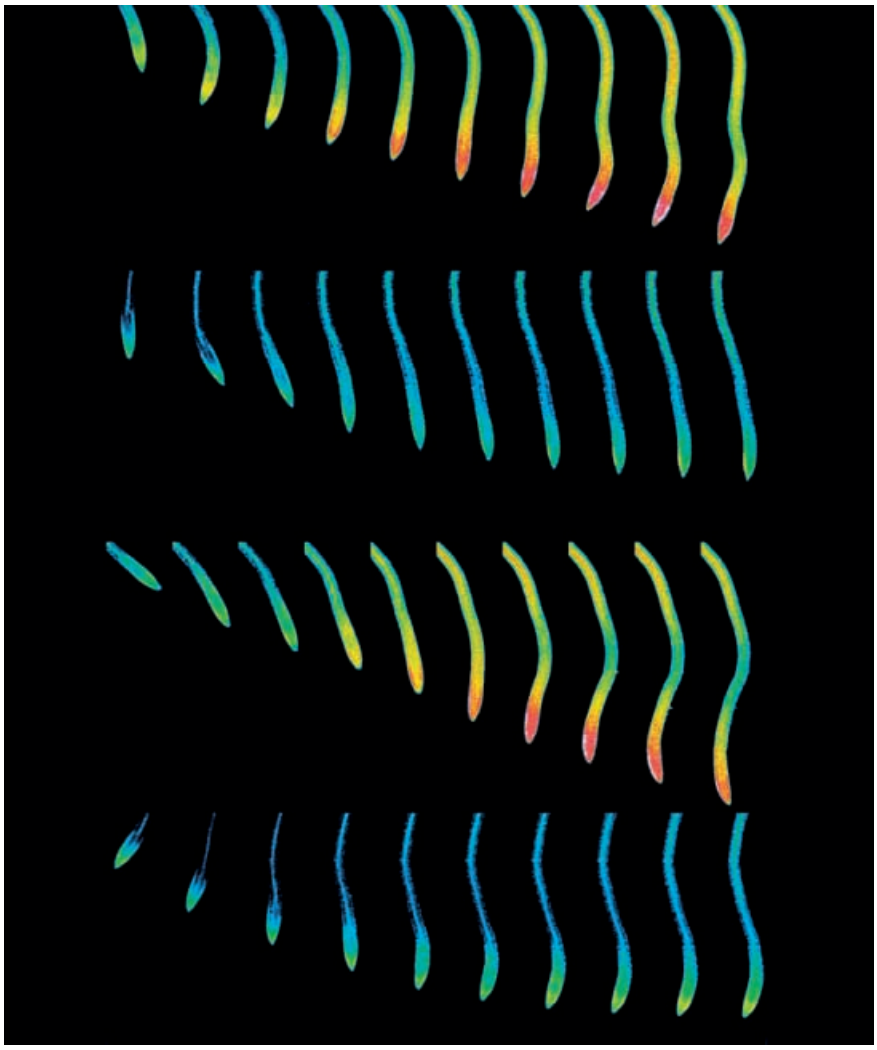

Figure 3
Series of growing Arabidopsis roots expressing genetically encoded fluorescent sensors for sugars.

Planned research and new directions

Cells can perceive, process and respond to the direction of signals and are themselves able to asymmetrically communicate with their environment. Gradients of nutrients, hormones or metabolites can be perceived and transport directions can be set within a tissue, e. g. to distribute nutrients or to propagate signals. It has become clear that asymmetric organization of the plasma membrane into specialized domains plays a central role in the response to and generation of directional information.

In the coming years, our lab will be following three complementary lines of research, in which we aim to elucidate the roles of plasma membrane organization in polar transport and cell-environment interactions.

1. Coordination of plasma membrane remodeling during plant cell polarization: What are the key players that control protein and lipid assembly to locally create specialized membrane compositions? What is the hierarchy of the regulators and how are the individual sorting processes timed?
2. Impact of environmental signals on plant cell polarity establishment and plasma membrane organization: Which environmental stimuli are perceived at the plasma membrane and trigger changes in membrane organization? How are chemical gradients perceived and processed by cells and tissues to determine their axes of polarity?
3. Directed molecular transport in plant tissues for signal propagation and nutrient allocation: How are directions for transport of nutrients and metabolites within and across tissue boundaries set? How are coordinated molecular flux patterns such as calcium signatures established?

To address these questions our lab employs high-resolution confocal imaging. We will take advantage of the aforementioned benefits of the *Arabidopsis* root architecture and its predictable development. Furthermore, a rich information resource of membrane protein interactions has become available (Jones et al., 2014b) that will aid hypothesis generation. We typically follow subcellular membrane sorting processes and development of polarity in live specimen over time. To minimize detrimental phototoxic effects during time-lapse measurement, we mainly apply spinning disk microscopy. The combination with microfluidic devices such as the RootChip and newly developed platforms will allow us to not only precisely control the microenvironment of investigated plant tissues but also to expose tissues to asymmetric environments or localized manipulation. Genetically encoded fluorescent biosensors will aid the detection of molecular flux in tissues and allow quantitative analyses of polarized transport activities.

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 9, number of citations 2009-2013: 120, h-index (2009-2013): 6, total h-index: 10 (according to Thomson Reuters).

Denninger, P, Bleckmann, A, Lausser, A, Vogler, F, Ott, T, Ehrhardt, DW, Frommer, WB, Sprunck, S, Dresselhaus, T, Grossmann, G. (2014) Male–Female Communication Triggers Calcium Signatures During Fertilization in Arabidopsis. Nat Commun, DOI: 10.1038/ncomms5645.

Jones, AM, Danielson, J, Manoj-Kumar, S, Lanquar, V, Grossmann, G, Frommer, WB. (2014a) Abscisic acid dynamics in roots detected with genetically encoded FRET sensors. *eLife*, 3:e01741.

Lanquar, V, Grossmann, G, Vinkenborg, JL, Merkx, M, Thomine, S, and Frommer, WB. (2013) Dynamic imaging of cytosolic zinc in *Arabidopsis* roots combining FRET sensors and RootChip technology. *New Phytol.*, DOI: 10.1111/nph.12652.

DeMichele, R, Ast, C, Loqué, D, Ho, CH, Andrade, SL, Lanquar, V, Grossmann, G, Gehne, S, Kumke, MU, Frommer, WB. (2013) Fluorescent sensors reporting the activity of ammonium transceptors in live cells. *eLife*, 2:e00800.

Grossmann, G, Guo, W-J, Ehrhardt, DW, Frommer, WB, Sit, RV, Quake, SR, and Meier, M. (2011) The RootChip: An Integrated Microfluidic Chip for Plant Science. *Plant Cell*, 23 (12), 4234-4240.


58-130

2.7 MOLECULAR BASIS OF CORAL SYMBIOSIS

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Fields of Interest
Molecular Cell Biology, Biochemistry, Ecology, Cnidaria, Endosymbiosis, Environmental Change

Brief summary of work since 2009

Coral reefs are the world's most diverse marine ecosystems and their existence depends upon a functional symbiosis between dinoflagellate algae (genus *Symbiodinium*) and their coral host. A range of environmental stressors including elevated seawater temperature, acidification, and pollution causes the obligate symbiosis between corals and their intracellular algae to break down. This phenomenon, known as coral »bleaching«, often leads to coral death (Figure 1A, left panel). Our long-term goal is to understand the molecular mechanisms underlying the establishment and maintenance of coral symbiosis.

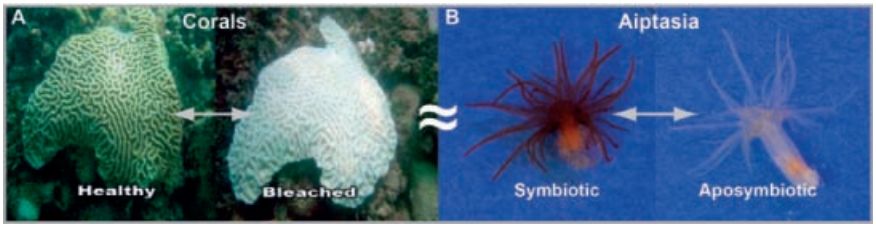


Figure 1
Healthy corals contain intracellular algae and therefore appear colored. Under environmental stress, the corals loose their symbionts and appear white (bleached) before they ultimately die (A). Similar to corals, symbiotic *Aiptasia* with intracellular algae appear brown. *Aiptasia* can be maintained in a bleached (aposymbiotic) but healthy state if fed regularly (B).

To date, progress in this research area has been slow, largely because corals are notoriously difficult to work with in the lab. We use an emerging model system, the marine sea anemone *Aiptasia*, (Figure 1, right panel) to analyze the molecular machinery and the cellular mechanisms responsible for symbiosis establishment and maintenance.

We have established key resources in our lab (e. g. clonal cultures of host and symbionts, inducible gametogenesis, molecular techniques) and generated microscopic assay systems to visualize and quantify symbiosis establishment. Using these tools, we have shown that (1) symbiosis establishment is highly specific in *Aiptasia*; (2) *Aiptasia* machinery to distinguish between compatible and incompatible symbionts becomes active a few days after fertilization and persists through adulthood; (3) younger larvae take up symbionts more efficiently than older larvae and (4) compatible algae proliferate actively within larvae after intracellularization. These tools and findings provide the crucial foundation upon which we continue to build further detailed analyses into the molecular and cellular underpinnings of symbiosis establishment and maintenance.

Major contributions & research highlights

Most reef-building corals produce eggs without symbionts, and therefore they must establish symbiosis anew during planula larval stages; this symbiosis is then maintained through adulthood (Figure 2). Currently, there exists no detailed understanding of the molecular basis underlying symbiosis establishment, including in planula larvae. This lack of information is partially due to the fact that corals only spawn once a year, thereby significantly limiting the available amount of planula larvae.

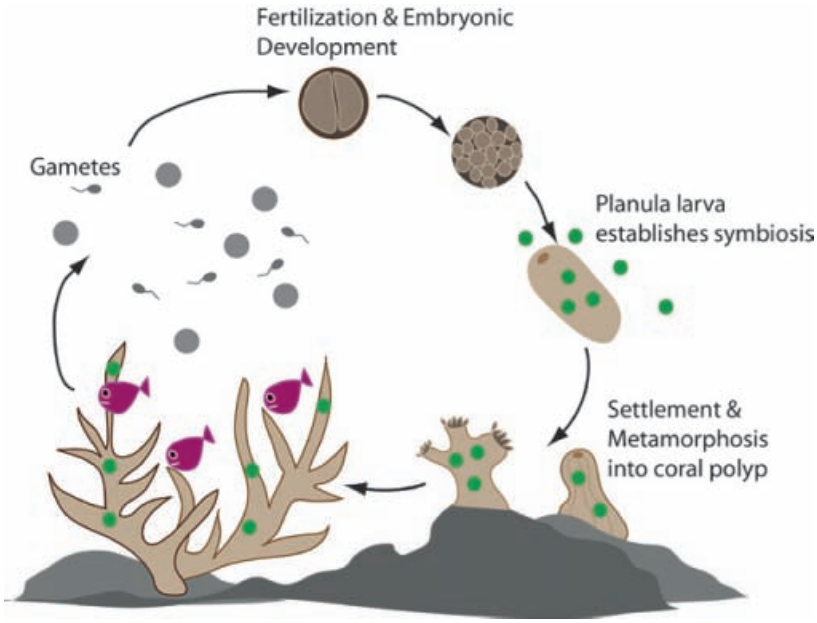


Figure 2
Most reef-building corals release gametes into the surrounding water and fertilization occurs externally. Next, embryos develop into a free-swimming planula larvae that take up symbionts from the environment. After symbiosis is established, the planula larvae search for a suitable substrate to settle down and metamorph in to the primary polyp.

We have now established an assay system using *Aiptasia* larvae (which can be generated regularly in the laboratory and, like corals, are naturally born without symbionts) as well as algae-free *Aiptasia* adult animals to analyze symbiosis establishment and maintenance under controlled conditions. We presented *Aiptasia* to different Symbiodinium algae strains and found that both *Aiptasia* larvae and *Aiptasia* adults take up Symbiodinium into their gastrodermal cells, where the algae persist and contribute to the host´s nutrition by transferring photosynthetic products to the host organism (Figure 3).

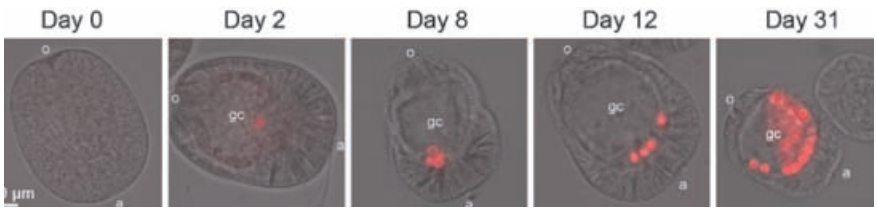


Figure 3
Aposymbiotic *Aiptasia* planula larvae establish symbiosis over time by taking up compatible *Symbiodinium* algae (visualized by the autofluorescence of their chlorophyll content) into their gastrodermal cells from the environment. Algae were added to larvae shortly after fertilization (Day 0) and maintained in seawater for 31 days (Day31). o oral; a aboral; gc gastric cavity (adapted from Hambleton et al., 2014).

The genus *Symbiodinum* is very diverse and it is widely accepted that a variety of factors including the species, the ecological niche and other environmental factors shape the specificity of a particular host-symbiont association. In line with that, we found that *Aiptasia* larvae and adults form a stable symbiosis with two *Symbiodinium* strains, but not with two other strains. This indicates that symbiosis specificity and the molecular machinery responsible for it is maintained between *Aiptasia* larvae and adults.

Symbiosis establishment has been described as a so-called »winnowing« process comprising different stages at which specificity could be achieved. During these steps, symbionts have to be ingested by the larvae and then recognized, intracellularized and subsequently maintained by the host cell. The distinction between compatible and incompatible symbionts may be achieved during each of these steps. To date, we have never found incompatible algae within host cells, suggesting that the distinction between compatible and incompatible algae takes place before intracellularization. However, our assays might also just not have the temporal resolution to detect transient uptake of incompatible algae. In the course of this study, we made two other interesting observations: older larvae (e. g. 12-day old larvae) ingested compatible symbionts less efficiently than younger larvae (e. g. 2-day old larvae); algae, once they are intracellularized into the host cells, proliferate rapidly within *Aiptasia* larvae.

Taken together, these findings provide the basis to develop a novel area of research to elucidate the molecular underpinnings of coral symbiosis using *Aiptasia* as a laboratory model system. Currently, we are developing important methods including microscopy, transcriptomics, and molecular techniques to help answer major remaining questions such as: What is the molecular basis for symbiosis specificity? How is symbiosis establishment and larval development correlated? To what extent are the symbionts integrated into the host cell function and how are they propagated? And what are the molecular key players involved in these processes?

Planned research and new directions

The long-term goal in the lab is to establish a detailed cellular and molecular analysis of cnidarian endosymbiosis using *Aiptasia* as a model system. Moreover, we aim to put our results into an ecological context by analyzing the effects of environmental factors on specific aspects of symbiosis as well as through comparative approaches using coral larvae collected in the field.

To achieve these goals, we are focusing on three distinct areas. First, we plan to use already available assays and techniques to provide a detailed description of *Aiptasia* larval development and the cellular events during symbiosis establishment and maintenance using microscopy. Specifically, we will answer questions including: At what developmental stage are larvae most competent to establish symbiosis? How long does efficient establishment take? How fast do algae proliferate within larvae and how are they distributed? How do larvae distinguish between compatible and incompatible algae and what is the effect of a changing environment on these processes?

Second, we aim to identify a set of symbiosis specific genes and proteins that are key for symbiosis specificity, establishment and maintenance using RNAseq of relevant larval stages with and without algae to identify differentially expressed genes. To identify conserved cnidarian genes, we will undertake similar approaches with coral larvae.

Finally, we seek to analyze the molecular function of identified key players at the cellular and molecular level. To do so, we need to further develop the molecular techniques mentioned above including *in situ* hybridization to analyze gene expression, gain- and loss-of-function techniques for functional analysis as well as advanced microscopy assays to investigate the localization of the symbiotic algae with *Aiptasia* larvae, as well as the subcellular localization of key molecules.

Publications since 2009

Number of peer-reviewed articles (2009-2014): 5, number of citations 2009-2013: 77, h-index (2009-2013): 3, total h-index: 6

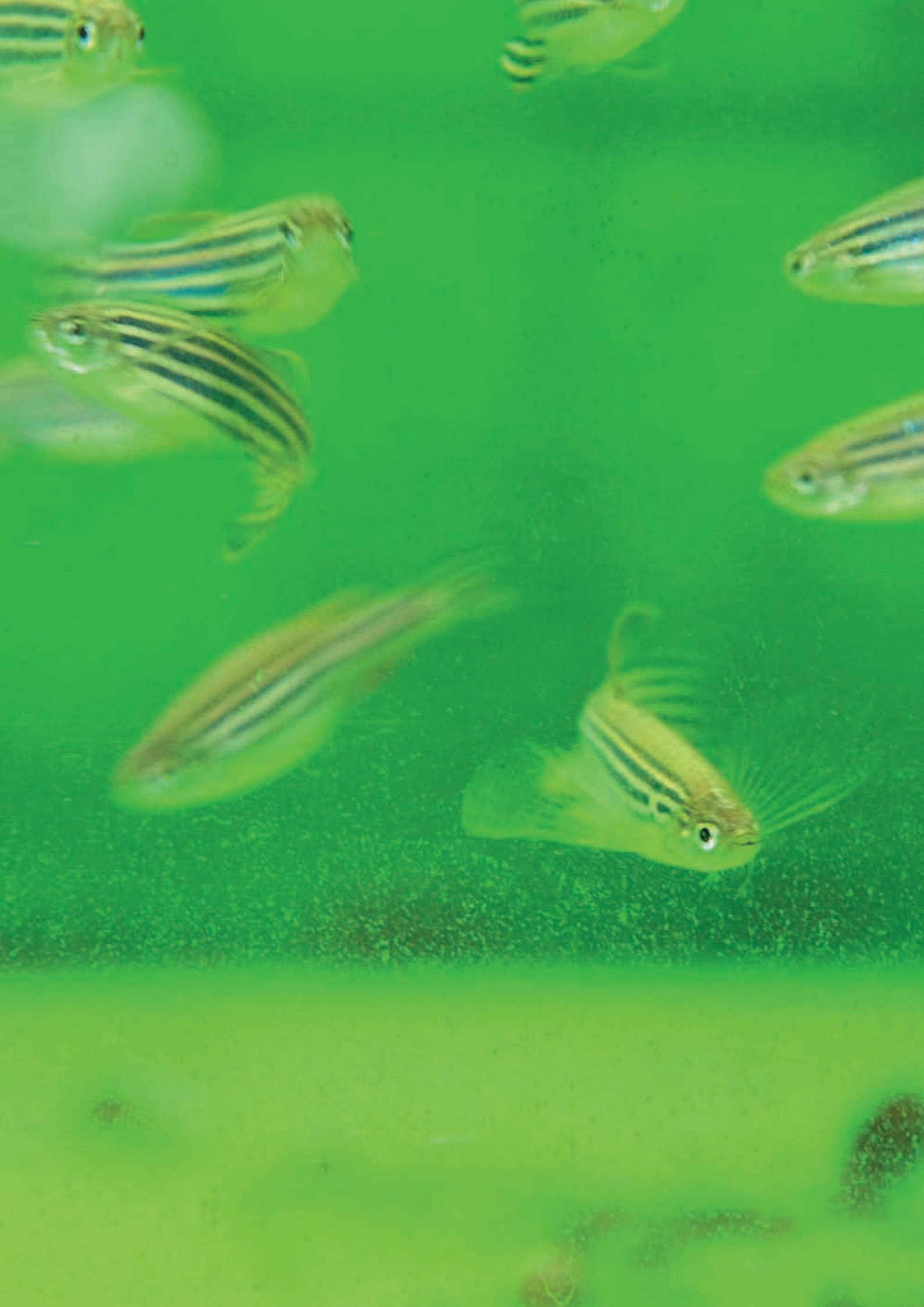
Hambleton EA*, Guse A*, and Pringle JR (2014). Similar specificities of symbiont uptake by adults and larvae in an anemone model system for coral biology. *J Exp Biol* 217, 1613-1619.

Miell MDD, Fuller CJ, Guse A, Barysz HM, Downes A, Owen-Hughes T, Rappsilber J, Straight AF, and Allshire RC (2013). CENP-A confers a reduction in height on octameric nucleosomes. *Nat Struct Mol Biol* 20, 763–765.

Guse A, Fuller CJ, and Straight AF (2012). A cell-free system for functional centromere and kinetochore assembly. *Nat Protoc* 7, 1847–1869.

Guse A, Carroll CW, Moree B, Fuller CJ, and Straight AF (2011). *In vitro* centromere and kinetochore assembly on defined chromatin templates. *Nature* 477, 354–358.

Strebe N*, Guse A*, Schüngel M, Schirrmann T, Hafner M, Jostock T, Hust M, Müller W, and Dübel S (2009). Functional knockdown of VCAM-1 at the posttranslational level with ER retained antibodies. *J Immunol Methods* 341, 30–40.



2.8 PLANT MOLECULAR BIOLOGY
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Fields of Interest
Molecular biology and physiology, plant nutrition, metabolism, metabolite sensing, environmental stress, regulatory mechanisms, protein modification and quality control

Brief summary of work since 2009
The Department of Molecular Biology of Plants uses the pathway of sulfur assimilation as a paradigm to understand the functional links between gene regulation, metabolism, development and environment. Sulfur is one of the most versatile elements in biology and as such allows the integrated study of molecular and organismal processes. Sulfur is taken up by roots in inorganic form, allocated throughout the plant, transported between cellular compartments, reduced at high energy cost, and is connected to carbon and nitrogen assimilation. It contributes to energy metabolism, functions in cell homoeostasis and signal transduction, is connected to biotic and abiotic stress responses and a constituent of many essential nutrient components for human nutrition.

Thus, based on the biology of sulfur in plants, the projects in the department range from plant nutrition, stress physiology, cell biochemistry and cell biology to development and biotechnology. This open concept allows to affiliate and foster young researchers with related scientific concepts in the department. Until 3/2010 Ute Krämer, now Professor at the University of Bochum, investigated the molecular physiology of metals, and until 3/2011 Andreas Meyer, now Professor at the University of Bonn, worked on redox regulation. Since beginning of 2012 the department hosts the Metabolomics Core Technology Platform and since summer 2013 supports the junior research group of Emmanuel Gaquerel. More recently a joint research program was developed with Markus Wirtz, permanent staff researcher in the department, on protein modification and protein quality control. All members of department contribute to teaching at Bachelor, Master and PhD levels and provide support for biochemical analyses throughout COS.

Major contributions since 2009
The fundamental reactions and pathways of metabolism are mostly understood, however the regulatory links between pathways, the sensing of supply and demand, and the functional link to growth and development are largely unclear. The study of sulfur metabolism allows the assessment of the corresponding molecular, cellular and organismal processes at all levels. A family of plasma membrane transporters orchestrates sulfate distribution by differential gene expression in response to sulfate supply and endogenous demand (reviewed in Takahashi et al., 2011). In contrast, most of the subsequent assimilatory steps of the pathway are regulated at the protein and metabolite level. *Arabidopsis* T-DNA mutants with disruption of defined metabolic steps are exploited to dissect of these regulatory processes and their links to other pathways and developmental processes. A knock-down of the gene encoding sulfite reductase (SiR) was particularly

useful to demonstrate the intrinsic roles of primary sulfur metabolism by simulating internal sulfur deficiency (Khan et al., 2010). SiR is localized exclusively in plastids and releases sulfide that is subsequently fixed in the cysteine synthesis process (Figure 1). In *sir1-1* plants with lowered activity of SiR the flux of sulfate reduction is very low and results in strongly retarded vegetative growth and seed development, de-regulated primary carbon and nitrogen metabolism and altered transport processes. Seeds of *sir1-1* plants adapt to this by changes between sulfur-rich and sulfur-poor storage proteins (Figure 2). Such analyses connect the enormous plasticity of metabolic and developmental processes that ensure optimal viability under changing environmental conditions.

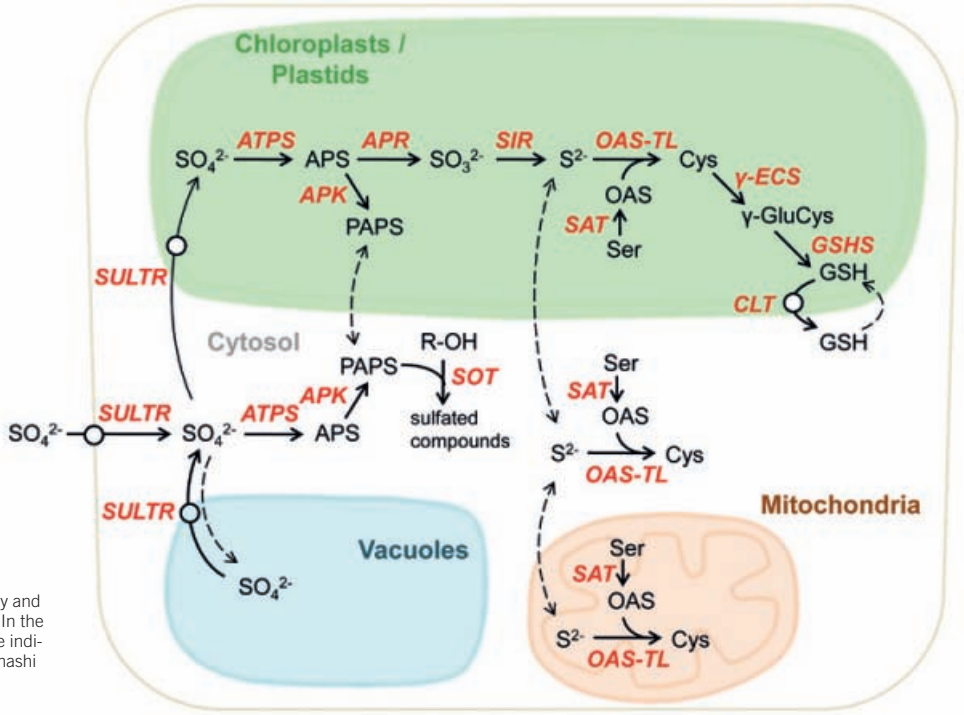

Figure 1
Cellular organization of primary and secondary sulfur metabolism. In the simplified scheme proteins are indicated in red (taken from Takahashi et al., 2011).

The partitioning of flux of reduced sulfur between primary and secondary metabolism was analysed in cooperation with S. Kopriva (then a JIC Norwich). Double mutants of the enzyme adenosine phosphosulfate (APS) kinase that provides PAPS, the activated donor for sulfation of secondary compounds such as glucosinolates, surprisingly show strongly impaired growth. De-sulfated precursors of glucosinolates accumulated and genes encoding glucosinolate biosynthetic genes were up-regulated, suggesting a feedback sensing for the presence of these defense compounds. At the same time, sulfate and thiols accumulated. This altered partitioning of flux defined APS as the branching point between primary and secondary sulfur metabolism (Mugford et al., 2009).

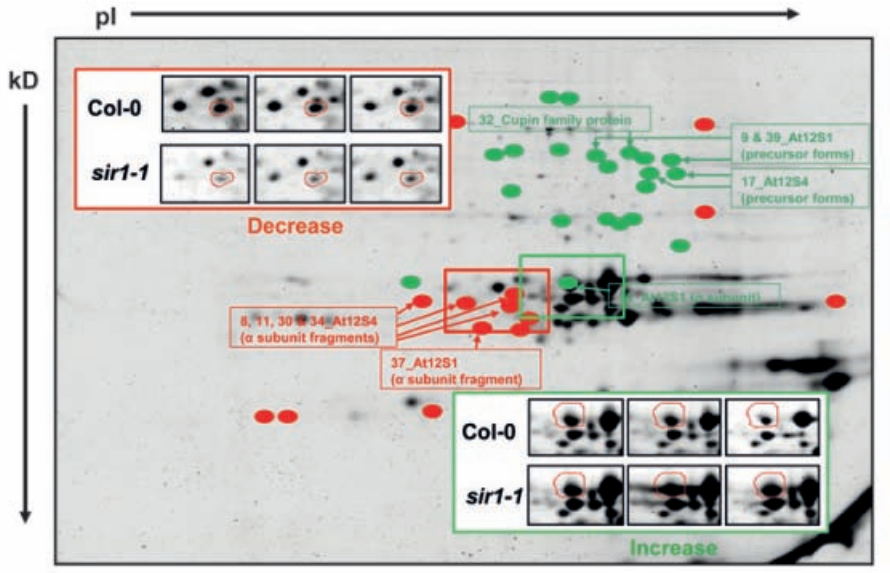

Figure 2
Seed protein composition adapts to availability of reduced sulfur. Two-dimensional gel electrophorenses followed by mass spectrometry of proteins from wildtypes and the sulfite reductase knock-down mutant *sir1-1*.

In contrast to sulfate assimilation the synthesis of cysteine by the enzyme complex between serine acetyltransferase (SAT) and O-acetylserine (thiol) lyase (OAS-TL) takes place in the plastids, mitochondria and the cytoplasm of nearly all eukaryotic phototrophs (Figure 3; Birke et al., 2012a). The systematic analysis of *Arabidopsis* mutants for the three sites of cysteine synthesis revealed that sulfide is generated in plastids, but mitochondria provide most of the intermediate O-acetylserine (OAS) while in the cytoplasm most of the cysteine is synthesized (Heeg et al. 2008). The special role of mitochondria in sulfur metabolism was further shown by efficient detoxification mechanisms for sulfide (Birke et al., 2012b) and efficient uptake and exchange of cysteine at the outer and inner envelope (Lee t al., 2014). Mutants and double mutant combinations of the three major OAS-TL enzymes showed hardly any phenotype in the vegetative stage, indicating redundancy at this stage. However, an OAS-TL triple mutant is lethal. Genotypic combinations with only one functional OAS-TL allele left (e. g. *oastlA-A-B-B-C-C+*) demonstrated that at least one functional allele is required for development of the male gametophyte (Birke et al., 2013).

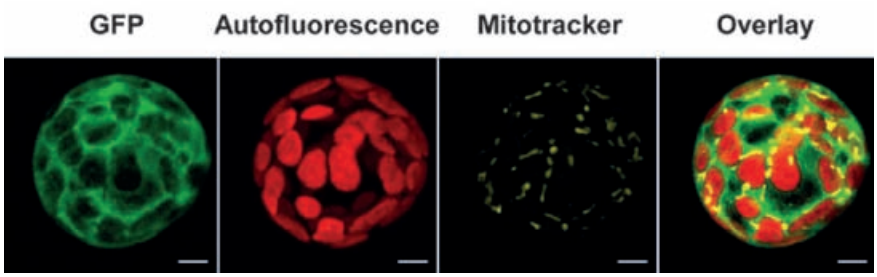

Figure 3
Physcomitrella carries out cysteine synthesis predominantely in plastids. Subcellar localization of OAS-TL: GFP fusion proteins in *Physcomitrella patens* protoplasts. Scale bar 5 µm.

The provision of reduced sulfur is a prerequisite to maintain the redox balance in cells. A major player in this context is glutathione and its redox potential. The role of the compartments for redox balance was probed in mutants lacking cytosolic glutathione reductase (GR)1 or dual targeted GR2. In cooperation with Andreas Meyer a redox-sensitive variant of GFP was expressed in the cytosol of *gr1* mutants and surprisingly showed dynamic reduction of oxidized glutathione during induced oxidative stress. Biochemical and genetic experiments identified the NADPH-dependent thioredoxin system as back-up for *Gr1*. However, lack of both cytosolic systems results in a pollen lethal phenotype (Marty et al., 2009).

The complex of the enzymes of cysteine synthesis has been extensively studied over the years in the department (see Hell and Wirtz, 2011, for review). Numerous unique and unexpected observations have defined the cysteine synthase complex as a cellular sensor for OAS and sulfide that operates via the reversible interaction of its subunits and concomitant changes of activities of the enzymes in free or bound state (Wirtz et al., 2010; Feldman-Salit et al., 2012; Wirtz et al., 2012). These and all other sulfur-related approaches have been carried out jointly with Dr. Markus Wirtz, permanent staff researcher in the department. More information about major contributions to sulfur metabolism and the new topic of N-terminal protein modification are provided in the report of Dr. Wirtz.

Planned research and new directions
The overall theme of future projects is to understand regulatory processes within the metabolism of sulfur, the functional links to developmental adaptations and the targets of N-terminal protein acetylation in cell surveillance under abiotic and biotic stress.

Flux analyses using *Arabidopsis* plants mutated in sulfur metabolism are underway to dissect the partitioning of reduced sulfur between protein biosynthesis (»growth program«) and glutathione synthesis (»stress program«). This includes efforts to identify the connection between metabolic activity and growth regulation by translational control.

Combined *in vitro* and *in vivo* approaches will be used to unravel the redox regulation of glutathione synthesis (in collaboration with T. Rausch). The key enzymes of sulfate reduction and glutathione synthesis have redox switches, suggesting a redox-based feedback signaling. It is hypothesized that the sensing by these switches contributes to decision making between the growth and stress programs that define plant growth.

Sulfur as nutrient has long been investigated in reductionist approaches but not seen in context with other nutrients, although this is the more natural situation. Towards the understanding and hopefully breeding of nutrient efficient plants the interaction between the uptake and metabolism of iron and sulfur is investigated. The fact that most of the iron in a leaf is bound to sulfur in iron-sulfur clusters of the electron chains *predict* cross-regulatory mechanisms to balance sulfur and iron contents for growth and stress responses.

The metabolite sensing system of the cysteine synthase complex has been demonstrated in biochemical and modelling approaches. The next step will be the study of *in vivo* function of the system. *Arabidopsis* molecular genetics, life measurements of protein interaction and redox potential and metabolite profiling will be used to unequivocally prove the flux control function *in vivo*. Downstream signaling to expression of genes related to sulfate transport and metabolization needs then to be unravelled by genetic screens that are based on modification of suitable readouts.

These efforts are indeed connected with the investigation of the role of N-terminal protein acetylation in response to environmental stress. Our group has shown that sulfur metabolism is up-regulated under drought stress and biotic stress and that expression of sulfur-related genes is altered in mutants with changed acetylation patterns. The joint investigation of these unexpectedly related processes promises exciting new discoveries.

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 36, number of citations 2009-2013: 660, h-index (2009-2013): 14, total h-index: 36 (according to Thompson Reuters).

Birke, H., Heeg, C., Wirtz, M., and 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

Wirtz, M., Beard, K.F.M., Lee, C.P., Boltz, A., Schwarzlander, M., Fuchs, C., Meyer, A.J., Heeg, C., Sweetlove, L.J., Ratcliffe, R.G., and Hell, R. (2012) Mitochondrial cysteine synthase complex regulates *O*-acetylserine biosynthesis in plants. J. Biol. Chem. *287*, 27941-27947

Khan, M.S., Haas, F., Allboje Samami, A., Moghaddas Gholami, A., Bauer, A., Fellenberg, K., Reichelt, M., Hänsch, R., Mendel. R.R., Meyer, A.J., Wirtz, M., and Hell, R. (2010). Sulfite reductase defines a new bottleneck for assimilatory sulfate reduction and is essential for growth and development in *Arabidopsis thaliana*. Plant Cell *22*, 1216-1231

Marty, L., Siala, W., Schwarzländer, M., Fricker, M.D., Wirtz, M., Sweetlove, L.J., Meyer, Y., Meyer, A.J., Reichheld, J.-P., and Hell, R. (2009) The NADPH-dependent thioredoxin system constitutes a functional backup for cytosolic glutathione reductase in *Arabidopsis*. PNAS *106*, 9109-9114

Mugford, S.G., Yoshimoto, N., Reichelt, M., Wirtz, M., Hill, L., Mugford, S.T., Nakazato, Y., Noji, M., Takahashi, H., Kramell, R., Gigolashvili, T., Flügge, U.-I., Wasternack, C., Gershenzon, J., Hell, R., Saito, K., and Kopriva, S. (2009) Disruption of adenosine-5'-phosphosulphate kinase in *Arabidopsis* reduces levels of sulphated secondary metabolites. Plant Cell *21*, 910-927

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

Brief summary of work since 2009
In the last five years our research mainly focused on identification and characterization of stress-induced cellular surveillance mechanism that enables higher plants to quickly adapt to environmental changes. We selected sulfate deficiency and drought stress as models to uncover surveillance mechanism for nutrient acquisition and to dissect internal demand from external availability signals. Surprisingly, we revealed a significant co-regulation of the sulfur metabolism with the drought stress response that was more far more elaborate than a simple decrease of soluble nutrient up-take by roots upon water limitation (reviewed in Chan et al., 2013). The research group collaborates closely in several joint projects with Rüdiger Hell in the Department of Molecular Biology of plants.

Major contributions since 2009
As a basis for investigation of sulfate deficiency-induced responses we characterized the *in vivo* signaling function of the cysteine synthase complex (CSC). The CSC consists of serine acetyltransferase (SAT) that synthesizes *O*-acetylserine (OAS) and OAS (thiol)lyase, which incorporates sulfide into OAS to produce cysteine. We demonstrated that two OAS-TL dimers reversibly interact with one central SAT hexamer and that the dissociation kinetic of the CSC is regulated by key signals of sulfur deficiency response, OAS and sulfide (Wirtz et al., 2010a). *In vivo* FRET analysis of the mitochondrial CSC revealed its importance for regulation of net cysteine synthesis in Arabidopsis cells (Wirtz et al., 2012). The role of CSC formation for regulation of cysteine synthesis was addressed in different sub-cellular compartments and revealed a tight co-regulation between exclusively plastid synthesized sulfide (Khan et al., 2010) and mainly mitochondrial generated OAS. This sophisticated sub-cellular compartmentalization requires a tight regulation of cysteine precursor synthesis during abiotic stress responses by retrograde signals that were further characterized in collaboration with Thomas Pfannschmidt (University of Grenoble, France) and Barry Pogson (Australian National University, Australia) (Brautigam et al., 2009; Estavillo et al., 2011). We contributed to the identification of plastid generated PAP, an intermediate of secondary sulfur metabolism (Mugford et al., 2009), as an important ABA-independent regulator of the drought and high light stress response (Estavillo et al., 2011). Very recently, we demonstrated in close collaboration with Prof. Chengbin Xiang (USTC, China) that sulfate availability also regulates ABA-dependent signaling pathway by limitation of ABA3 maturation (Cao et al., 2014).

The identification of N^{α}-terminal protein acetylation (NTA) as the dominant modification of OAS-TLs (Wirtz et al., 2010b) motivated the characterization of the major N-acetyltransferase complex (NatA) in higher plants. Reverse genetics revealed the essentiality of NatA activity during embryogenesis. RNAi mediated depletion of NatA caused retarded growth,

strong down-regulation of sulfate metabolism and significantly enhanced drought tolerance. The drought tolerant phenotype of NatA depleted plants could be explained by constitutive closure of leaf stomata (Fig 1A) and inhibition of lateral root formation. Both processes are triggered by ABA in higher plants. However, ABA levels were not affected in NatA depleted plants. Since NatA did not work up-stream of ABA, we tested if NatA is a new target of ABA in plants and mediates part of the ABA triggered drought stress response. Indeed, we were able to show that ABA application results in down-regulation of NatA activity (Fig 1B). Our results demonstrate that NTA by NatA is a dynamic hormone regulated process that affects approximately 60 % of all cytosolic proteins.

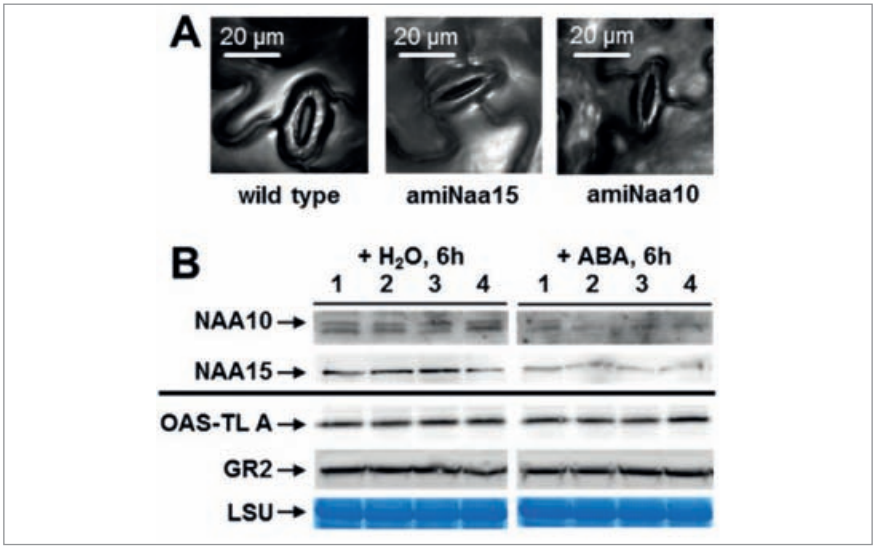

Figure 1
A) Closure of stomata in NatA depleted plants (amiNAA10 and amiNAA15). B) Application of ABA results in a fast and significant decrease of catalytic (NAA10) and auxiliary (NAA15) subunit of NatA, while control proteins (OAS-TL A, GR2) are not affected.

Planned research and new directions

Research will focus on the hormonal control of NatA activity and the impact of NatA and other Nat complexes on stress-induced cellular surveillance mechanisms. This approach will be extended by analysis of biotic stress-factors in collaboration with Xin Li (University of British Columbia, Canada), Emmanuel Gaquerel (COS) and the Metabolomics Core Technology Platform. The relevance of NTA for regulation of sulfate assimilation will be characterized with Rüdiger Hell.

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 34, number of citations 2009-2013: 537, h-index (2009-2013): 12, total h-index: 25 (according to Thompson Reuters).

Brautigam, K., Dietzel, L., Kleine, T., Stroher, E., Wormuth, D., Dietz, K.J., Radke, D., Wirtz, M., Hell, R., Dormann, P., et al. (2009). Dynamic plastid redox signals integrate gene expression and metabolism to induce distinct metabolic states in photosynthetic acclimation in Arabidopsis. Plant Cell 21, 2715-2732.
Chan, K.X., Wirtz, M., Phua, S.Y., Estavillo, G.M., and Pogson, B.J. (2013). Balancing metabolites in drought: the sulfur assimilation conundrum. Trends Plant Sci 18, 18-29.
Estavillo, G.M., Crisp, P.A., Pornsiriwong, W., Wirtz, M., Collinge, D., Carrie, C., Giraud, E., Whelan, J., David, P., Javot, H., et al. (2011). Evidence for a SAL1-PAP Chloroplast Retrograde Pathway That Functions in Drought and High Light Signaling in Arabidopsis. Plant Cell 23, 3992-4012.
Khan, M.S., Haas, F.H., Allboje Samami, A., Moghaddas Gholami, A., Bauer, A., Fellenberg, K., Reichelt, M., Hansch, R., Mendel, R.R., Meyer, A.J., et al. (2010). Sulfite Reductase Defines a Newly Discovered Bottleneck for Assimilatory Sulfate Reduction and Is Essential for Growth and Development in Arabidopsis thaliana. Plant Cell 22, 1216-1231.
Mugford, S.G., Yoshimoto, N., Reichelt, M., Wirtz, M., Hill, L., Mugford, S.T., Nakazato, Y., Noji, M., Takahashi, H., Kramell, R., et al. (2009). Disruption of Adenosine-5'-Phosphosulfate Kinase in Arabidopsis Reduces Levels of Sulfated Secondary Metabolites. Plant Cell 21, 910-927.


Nikon

2.9 MOLECULAR EVOLUTION AND GENOMICS
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Fields of Interest
Molecular evolution of development, axis formation, Wnt and Tgf-β signalling, transcriptional networks; biology of regeneration; cell type evolution; nerve cells, nematocytes, stem cells

Brief summary of work since 2009
To understand the origin and evolution of key regulators in animal development, our lab is analysing cnidarians, simple diploblastic aquatic animals with a > 600 Myr fossil record. Cnidarians exhibit a gastrula-like body plan with a nerve net and stem cell systems. Our model systems are the freshwater polyp *Hydra*, which is well known for its almost unlimited life span and regeneration capacity, and the sea anemone *Nematostella vectensis*. On the molecular level, we discovered cnidarian Wnt and Bmp/Tgf-β signalling, two major pathways in bilaterian embryogenesis and stem cell regulation. We were also mainly involved in sequencing the first cnidarian genomes revealing their complex gene repertoire, conserved exon-intron structures and large-scale gene linkages making them more similar to vertebrates than to flies or nematodes. Thus, it is likely that the genetic repertoire, responsible for the formation of the bilaterian body plan, already existed in the common cnidarian/bilaterian ancestor. We are now using combined transcriptome and SILAC proteome approaches to study the function of stem cells in *Hydra* regeneration and pattern formation. Among a number of novel genes we identified a Wnt signalling cascade as major contributors to the regeneration response. Downstream of canonical Wnt/β-Catenin signalling members of the TGF-β superfamily are activated in patterning the newly formed tissue in regenerates or buds. In our current and future research we focus on the evolutionary origin of the nervous system, the molecular mechanism of stem cell recruitment in cnidarian pattern formation, and the specificity of Wnt ligand-receptor interactions.

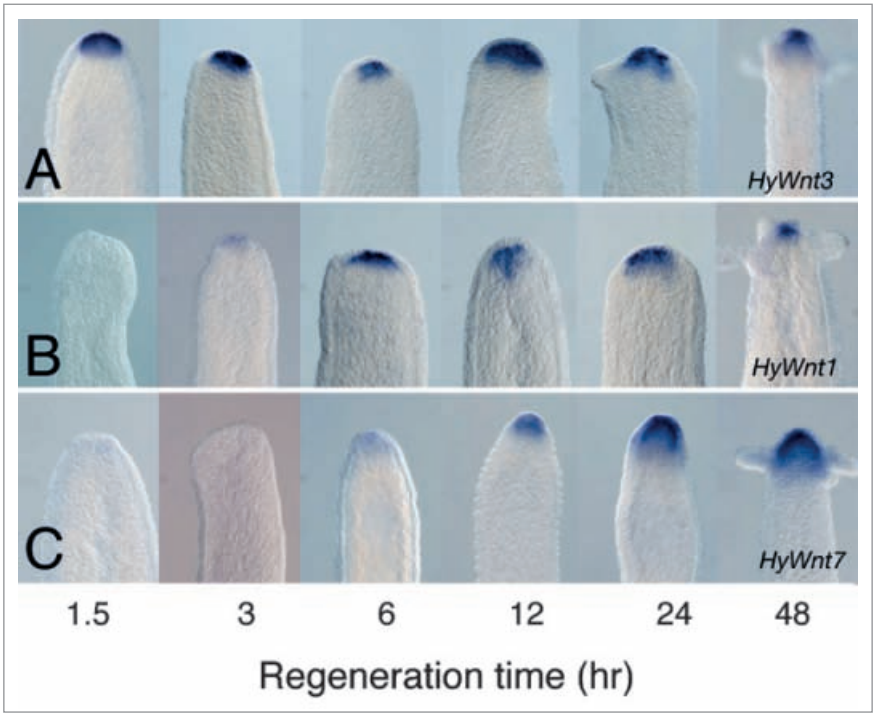

Figure 1
Expression patterns of three Wnt genes (A-C) in Hydra during head regeneration. Animals were bisected below the head (80% BL) at the times indicated (from Lengfeld et al. 2009, Developmental Biology 330, 186-199).

Major contributions since 2009

The freshwater polyp *Hydra* has been the object of study for 300 years, and the experimental work by Abraham Trembley (1736-1744) culminated in the discovery of regeneration in an animal (Chapman et al., 2011). To decipher the *Hydra magnipapillata* genome and its gene repertory we cooperated in an international consortium with two genome research institutes and colleagues in Germany, Austria, Japan and the US. The outcome of our investigations indicates how much the gene repertories of primitive organisms, higher animals and humans have in common. Like vertebrates, *Hydra* already has a set of approximately 20,000 genes. Comparisons of the *Hydra* genome to the genomes of other animals demonstrated that the key molecular switches for the formation of epithelial tissue, muscles, stem cells and nervous and immune systems originated at the level of this simple multicellular organism. They also shed light on the evolution of developmentally regulated transcription factors, the Spemann-Mangold organizer, pluripotency genes, and the neuromuscular junction. Decoding of the *Hydra* genome was a major stage in the understanding of the molecular »toolbox« underlying the evolution of animals and humans. We expect answers on crucial questions in biology: What does the fundamental genetic blueprint for animals look like and how have all the more complex types developed from it?

In order to understand the unparalleled regeneration capacity of *Hydra*, we performed an integrative transcriptome and SILAC proteome study on *Hydra* head regeneration. As major contributors to the regeneration response we identified new regeneration-specific pathways (endocytosis, focal adhesion), novel genes, and Wnt/TGF-β signalling that establish two distinct molecular cascades: an early injury response and a subsequent, signalling driven patterning of the regenerate. A key factor of the injury response is a large net up-regulation of proteins mediated by GSK-3, followed subsequently by a transcriptional activation cascade including Wnt genes constituting the head organiser. The kinetics of Wnt gene expressions during head regeneration suggests a cascade of consecutive Wnt activation accompanies regeneration, with Wnt3 beginning the cascade. Recombinant HyWnt3 protein could rescue regeneration in head regeneration-deficient mutants (reg-16), and by perturbing canonical and non-canonical Wnt signalling during head regeneration we found that non-canonical Wnt signalling acts downstream of canonical Wnt signalling (Lengfeld et al, 2009; Phillip et al 2009; Holstein 2012; Peterson et al, unpublished).

Using transgenesis we analysed the mechanisms governing localized Wnt expression. Two functionally distinct cis-regulatory elements control head organizer-associated HyWnt3. An auto-regulatory element mediates direct inputs of Wnt/β-Catenin signalling and activates HyWnt3 transcription in the head region. A repressor element is necessary and sufficient to restrict the activity of this auto-regulatory element, thereby allowing organizer-specific expression. This combination of auto-regulation and repression is crucial for establishing a Wnt-expressing signalling centre suggesting that the transcriptional control is an evolutionarily old strategy in the formation of Wnt signalling centres (Nakamura et al., 2011).

Three orthogonal body axes and corresponding molecular vectors define the bilaterian body plan, i.e. the AP-, DV-, and LR-axis and Wnt, Bmp2/4, and Nodal, respectively. In pre-bilaterian animals only the oral-aboral body axis is well defined (Wnt) while the origin of the other body axes is largely unclear (Holstein, 2012). We analysed a so far »overlooked« aspect of the cnidarian body plan, i.e. the lateral branching morphology of budding polyps. The genetic basis for this lateral budding was unknown so far, but it could have been a preadaptation for the evolution of bilateral symmetry. We identified the genes and corresponding pathway that is essential for setting up an axial asymmetry along the main body axis. We presume that this pathway became co-opted for various modes of axial patterning, for example lateral branching in cnidarians and LR patterning in bilaterians (Watanabe et al, unpublished).

In a study on the early neurogenesis of *Nematostella* we provide evidence for a function of signalling pathways setting up the oral nervous system of *Nematostella* embryos. Our data give insights how a first step in the evolution of a centralized nervous system may have been accomplished (Watanabe et al, unpublished).

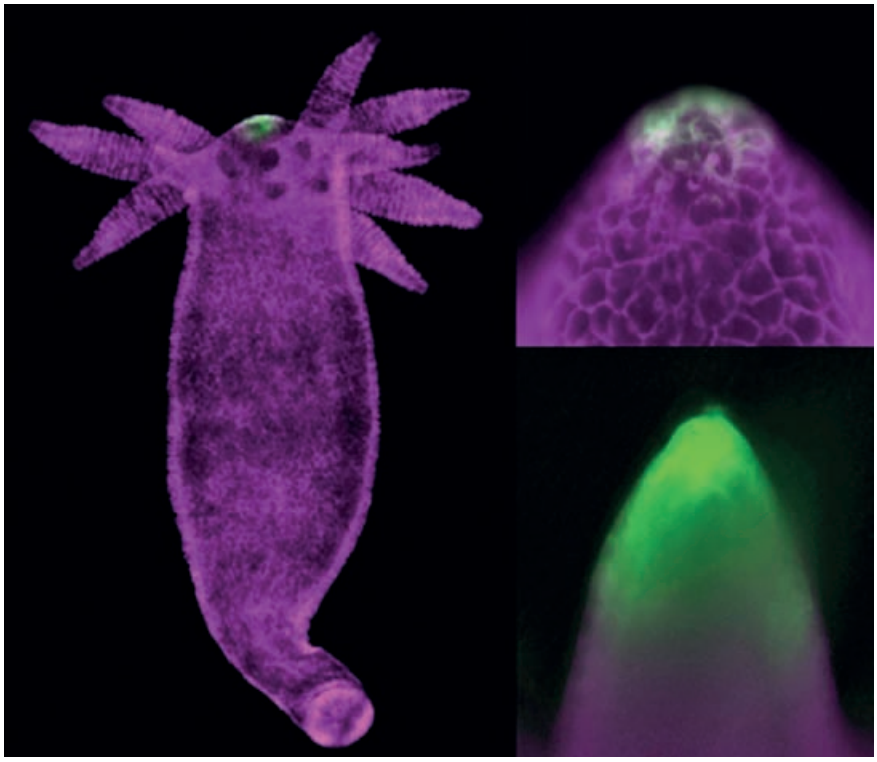

Figure 2
HyWnt3 promoter reproduces the endogenous expression. HyWnt3::EGFP (green) is activated exclusively in the HyWnt3-expressing cells in the apical tips of the adult hypostome. Reporter constructs lacking the HyWnt3 repressor sequence exhibit dramatic expansion of expression (From Nakamura et al. 2012, Proceedings of the National Academy of Sciences of the United States of America 108, 9137-9142).

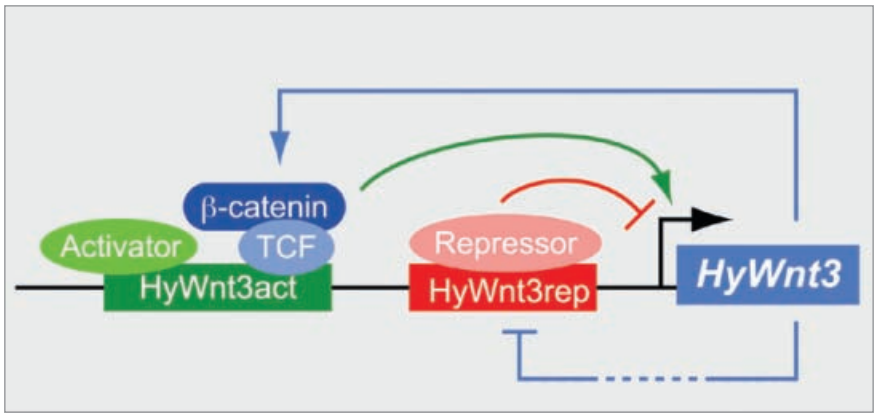

Figure 3
Model for the transcriptional control of organiser specific HyWnt3 expression. HyWnt3 is controlled by two cis-regulatory elements (HyWnt3act, green and HyWnt3rep, red), which are positively (light blue arrow) and negatively (light blue bar) regulated by Wnt/β-cantenin signaling. The β-Cantenin/Tcf complex (blue) and putative activators (light green) bind to HyWnt3act and their combinatorial inputs act in HyWnt3 transcription (green arrow). Potential repressors (pink) bind to HyWnt3rep and inhibit HyWnt3 expression. (From Nakamura et al. 2012, Proceedings of the National Academy of Sciences of the United States of America 108, 9137-9142).

Planned research and new directions
We will address three main questions:
(i) Neuronal network of *Hydra*
(ii) Stem cell recruitment in *Hydra* pattern formation
(iii) Evolutionary origin of Wnt ligand-receptor interactions

A main paradigm in neurobiology assumes that systemic behaviour can be understood as functional output of an integrating neuronal network needs. In a collaborative project (Ectop CellNetworks) we are testing and quantifying this hypothesis in *Hydra's* simple neuronal network (4000 neuronal cells). The nerve net can be manipulated or reconstituted by adding neuronal stem cells to a neuron-depleted »ghost«, and *Hydra's* behaviour will be analysed in quantifiable way.

Based on our transcriptome/proteome data demonstrating that the onset of *Hydra* regeneration is characterized by a large net up-regulation of proteins followed by an activation of patterning genes we aim to bring our analysis to the next level by elucidating how the new positional information of the regenerating tissue is generated. We will address the following questions: How is stemness regulated in a living regenerating tissue under the physically manipulated conditions of *de novo* pattern formation in a microarray format? How is positional information transmitted from an initial and local protein response to the onset of pattern formation on the transcriptional level? How is stem cell homeostasis and regeneration maintained by the interplay between β-catenin and Tor signaling pathways? Are there conserved stem cell specific signatures on the genomic level, which are addressed by the gene regulatory network at the onset of regeneration? As an *in vivo* assay we will analyze regenerates/re-aggregates from dissociated tissue under the microscope under large-scale conditions using the microfluidic technology (established in the framework of the HeiKA alliance with the Karlsruhe Institute of Technology, KIT) (CRC A1-873).

To understand the specific function of different Wnt ligands in animal evolution we study the regulatory network of Wnt transcriptional regulation. On the level of the receptor-ligand interactions we will focus our Wnt-related studies on the interactions of different Wnt ligands with receptor complex, a topic which is also under intensive investigation in vertebrates. Our project will help to understand how Wnt ligand specificity has evolved (DFG-FOR 1036).

Selected publications since 2009

Number of peer-reviewed articles 2009-2014: 21, number of citations 2009-2013: 643, h-index (2009-2013): 11, total h-index: 28 (according to Thomson Reuters).

Watanabe, H., Schmidt, H.A., Kuhn, A., Höger, S.K., Kocagöz, Y., Laumann-Lipp, N., Ozbek, S. and T. W. Holstein. (2014). Nodal signalling determines biradial asymmetry in *Hydra*. Nature in press: DOI 10.1038/nature13666

Nakamura Y, Tsiairis CD, Özbek S, Holstein TW. (2011). Autoregulatory and repressive inputs localize *Hydra* Wnt3 to the head organizer. Proc Natl Acad Sci U S A 108: 9137-42.

Chapman JA*, Kirkness EF*, Simakov O*, Hampson SE, Mitros T, Weinmaier T, Rattei T, Balasubramanian PG, Borman J, Busam D, el al. (2010) The dynamic genome of *Hydra*. Nature 464: 592-6. Note: *co-first authors

Lengfeld T, Watanabe H, Simakov O, Lindgens D, Gee L, Law L, Schmidt HA, Ozbek S, Bode H, Holstein TW. (2009). Multiple Wnts are involved in *Hydra* organizer formation and regeneration. Dev Biol. 330: 186-99 [this publication was among the top 5 cited Dev-Biol publications 2009-2013].

Philipp I, Aufschnaiter R, Ozbek S, Pontasch S, Jenewein M, Watanabe H, Rentzsch F, Holstein TW, Hobmayer B. (2009). Wnt/beta-catenin and noncanonical Wnt signaling interact in tissue evagination in the simple eumetazoan *Hydra*. 106: 4290-5.

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Fields of Interest
Microtubule dynamics, actin dynamics, adhesion, cytoskeleton, axon guidance, nervous system, live imaging

Brief summary of work since 2009
The Engel group utilizes light microscopy approaches to study cellular motility and polarized growth. We are interested in how effectors of guidance signaling regulate cytoskeletal effectors in axonal growth cones and more specifically how microtubule dynamics contribute to directed growth and turning. We found microtubule advance in the growth cone to correlate with axonal outgrowth rates in *Xenopus* spinal cord neurons. XCLASP1, a microtubule binding protein, promoted axon advance and microtubule growth (Marx et al., 2013).

Another focus of the group is actin dynamics. We use *Dictyostelium* as a model organism, which spontaneously polarizes in random directions. In a collaboration with G. Gerisch (MPI for Biochemistry, Munich) we have studied the role of actin and actin effectors in membrane organization, cell spreading, phagocytosis and intracellular transport by live confocal microscopy of *Dictyostelium* amebae (Gerisch et al., 2009, Clarke et al., 2010a, 2010b, Heinrich et al., 2014). Making use of the imaging resources of the Nikon Imaging Center, which is operated by the Engel group, we have worked together with groups of Heidelberg University to analyze mechanical properties of adhesion in plasmodium (Munter et al., 2009, Hegge et al., 2010) and the influence of substrate stiffness on cell morphology (Yoshikawa et al., 2010, 2011).

Major contributions
During my postdoc at Harvard Medical School in David Van Vactor's lab, we showed a role for orbit, the fly homologue of the CLASPs, in axon guidance. This finding was based on *Drosophila* genetics, which placed CLASP downstream of the Abelson kinase during guidance signaling (Lee H., Engel U. et al, Neuron 2004). In a recent follow-up study in collaboration with David Van Vactor, we demonstrated that indeed, vertebrate CLASP is a substrate of Abl in vitro and in vivo, and that CLASP binds not only to microtubules but also to actin (Engel et al., 2014). Here in Heidelberg, we set out to establish quantitative analysis of microtubule dynamics to study the role of CLASP in primary neurons. During her PhD, Astrid Marx used spinal cord neurons of *Xenopus laevis* expressing fluorescent EB3 to investigate microtubules dynamics. Movement of EB3 comets was 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 and microtubule advance. Interestingly, the effect of XCLASP1 depletion was very similar to treatment with very low doses of Taxol, which reduces microtubule dynamicity in

the growth cone. In line with the finding that CLASP can bind to actin, XCLASP1 knockdown also had an effect on the organization of the actin cytoskeleton (Fig. 1, Marx et al., 2013).

In a long standing collaboration with Günter Gerisch at the MPI for Biochemistry and Margaret Clarke at Oklahoma University, we analyzed actin dynamics at the cortex and during phagocytosis. As the phagocytic cup closes around the food particle, actin-dependent force transiently builds up to close the food vacuole. Using a trick, this state can be frozen and actin accumulation and its dependence on membrane curvature can then be studied in detail. When yeast with a bud is given as a food particle, the closing vacuole cannot open up again to enclose the bud. Instead actin accumulates in a frustrated attempt to close the vacuole. We used this system to study the role of I-BAR proteins and myosin 1, as well as the dynamics of actin assembly. (Fig. 2, Clarke et al., 2010).

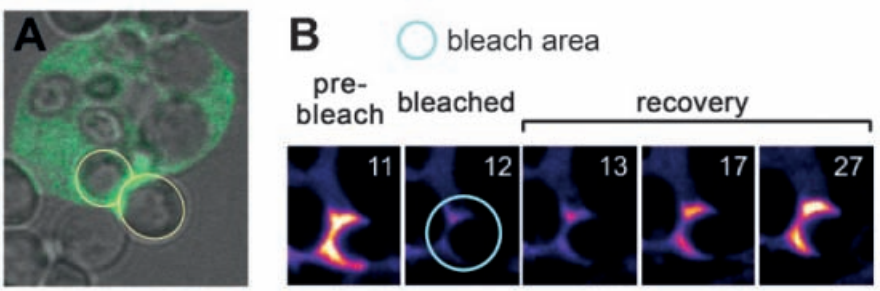

Figure 1
Curvature-dependent actin accumulation during phagocytic cup closure in *Dictyostelium*. (A) Cells expressing GFP-actin were fed with budded yeast (shape outlined in yellow). (B) In a cell with massive actin accumulation at the phagocytic cup, a FRAP experiment shows actin recovery within seconds, indicating the high turnover of actin at the closing phagocytic cup.

Planned research and new directions

In the future we will combine in-silico modelling with experiments to understand microtubule dynamics in neuronal growth cones. For the modeling, we are collaborating with F. Nedelec (EMBL) who has developed the software »Cytosim«, which provides a visual model for microtubules dynamics with all their biophysical properties, as well as motors acting on them. In this project a postdoctoral fellow, Carlo Beretta is funded within the collaborative project »Spatiotemporal signaling« (CellNetworks). An in-silico model for the growth cone is attractive to test the outcome of changing factors of dynamicity (e. g. catastrophe rate, retrograde transport of microtubules) for the overall outcome of neuronal morphology. In a second step we plan to extend model and experiments to growth cone turning during guidance, where signaling is thought to result in asymmetric regulation of microtubules. We are currently establishing experimental approaches to measure microtubule dynamics in turning growth cones that respond to the repellent Slit.

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 12, number of citations 2009-2013: 199, h-index (2009-2013): 8, total h-index: 14 (according to Thomson Reuters).

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. MBC *24*, 1544-1558.

Clarke, M., Engel, U., Giorgione, J., Muller-Taubenberger, A., Prassler, J., Veltman, D., and Gerisch, G. (2010). Curvature recognition and force generation in phagocytosis. BMC Biol. *8*, 154.

Munter, S., Sabass, B., Selhuber-Unkel, C., Kudryashev, M., Hegge, S., Engel, U., Spatz, J.P., Matuschewski, K., Schwarz, U.S., and Frischknecht, F. (2009). Plasmodium sporozoite motility is modulated by the turnover of discrete adhesion sites. Cell Host Microbe *6*, 551-562.

PROJECT LEADER: PD DR. SUAT ÖZBEK

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Fields of Interest
Molecular evolution in cnidarians, organelle morphogenesis, nematocyst biology, structure-function studies in signaling and ECM molecules, biochemistry and evolution of collagens

Brief summary of work since 2009
My research is focused on the evolution of extracellular matrix and signaling molecules. In particular, I'm interested in the evolution of complex features, as of organelles. In our lab we are applying biochemical, microscopic and structural techniques as well as systems biology approaches using functional genomics and proteomics. Our model organism is the cnidarian Hydra, which represents one of the most basal metazoan animals and a sister group to the bilateria, thus offering important implications for comparative evolutionary questions. 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. Nematocysts harbor a specialized form of extracellular matrix and their complex protein composition and morphogenesis offer an intriguing field of research. In addition, my lab is concerned with structure-function analyses and the evolution of Wnt signaling molecules. Wnt ligands comprise a large family of secretory cysteine-rich glycoproteins, which act as morphogens that control a variety of developmental and adult processes in all metazoans. Our research in this field is focused on the molecular interaction of Wnts with their cellular receptors and on characterizing novel regulators of Wnt signaling in cnidarians.

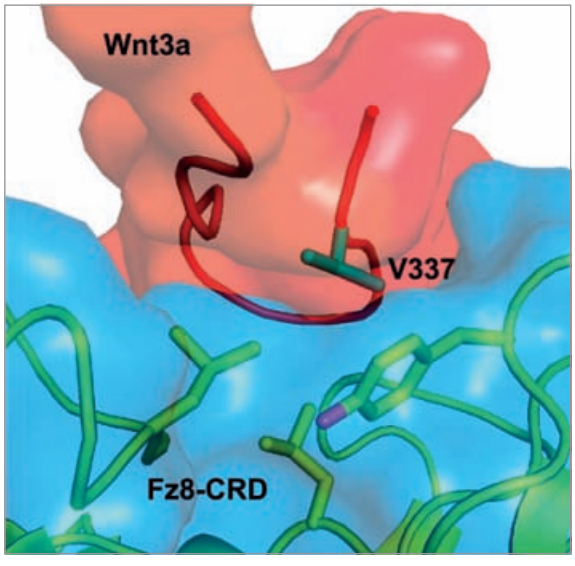

Figure 1
A sensitive site for canonical Wnt signaling defined by a molecular dissection of Wnt3a-Frizzled8 interaction.

Major contributions since 2009

In the time period since 2009 we have made major contributions to the research field of cnidarian genomics and proteomics. The analysis of horizontally transferred bacterial genes performed by my Ph.D. student P. Balasubramanian has been adopted for the Hydra genome paper (Chapman, 2012). The same student performed the first in-depth proteomic analysis of the Hydra nematocyst revealing the complex protein composition of this unique cnidarian organelle (Balasubramanian, 2012). Furthermore, work of two other Ph.D. students (P. Adamczyk, A. Beckmann) resulted in the identification and analysis of novel molecular components of the nematocyst that have important implications for its morphogenesis and biomechanical features (Adamczyk, 2010; Beckmann, 2012). The Ph.D. student S. Kumar has completed his detailed structure-function analysis of the mouse Wnt3a-Fz8-CRD interaction by testing a series of Wnt3a point mutants in diverse in vitro and in vivo settings. His findings allowed the molecular dissection of this interaction into sites relevant for physical binding and for signaling in canonical Wnt ligands (Kumar, 2014).

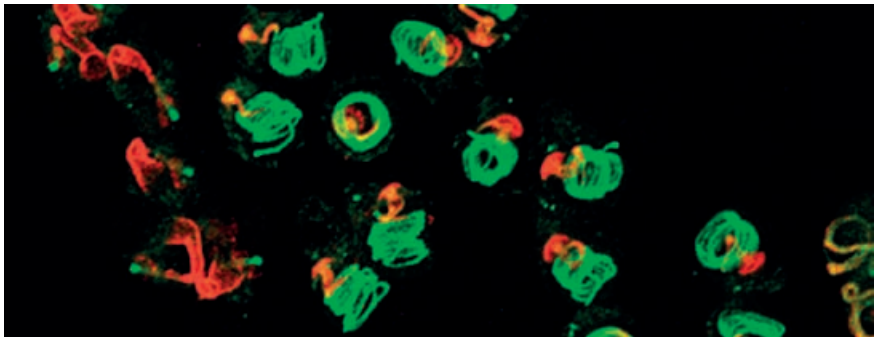

Figure 2
Immunostaining of developing nematocytes in Hydra. The staining highlights the tubules coiled inside the capsule matrix of the stinging organelle.

Planned research and new directions

Future research on nematocysts will focus on the subproteome of the operculum structure (PostDoc A. Reft) aimed at identifying the molecular basis of capsule discharge. Furthermore we will pursue the molecular and physiological characterization of mechanosensory TRP channels in Hydra started by A. Beckmann. As a translational project emanating from the nematocyst research, our project funded by the BW-Stiftung will be concerned with the application of polymerization domains from capsule matrix proteins for chimeric polymers from biological and synthetic components. This is a cooperative project with the KIT, Karlsruhe. We will also, on the basis of our findings of Wnt-receptor interaction, perform further studies on the stoichiometry of Wnt-Fz-LRP complexes in solution and the problem of receptor/co-receptor specificity as part of a SFB initiative.

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 20, number of citations 2009-2013: 452, h-index (2009-2013): 9, total h-index: 18 (according to Thomson Reuters).

Chapman, J.A., Kirkness, E.F., Simakov, O., Hampson, S.E., Mitros, T., Weinmaier, T., Rattei, T., Balasubramanian, P.G., Borman, J., Busam, D., et al. (2010). The dynamic genome of Hydra. Nature 464, 592-596.

Adamczyk, P., Zenkert, C., Balasubramanian, P.G., Yamada, S., Murakoshi, S., Sugahara, K., Hwang, J.S., Gojobori, T., Holstein, T.W., and Ozbek, S. (2010). A non-sulfated chondroitin stabilizes membrane tubulation in cnidarian organelles. J Biol Chem 285, 25613-25623.

Balasubramanian, P.G., Beckmann, A., Warnken, U., Schnoelzer, M., Schueler, A., Bornberg-Bauer, E., Holstein, T.W., and Ozbek, S. (2012). The Proteome of the Hydra Nematocyst. J Biol Chem.

Kumar, S., M. Zigman, T.R. Patel, B. Trageser, J.C. Gross, K. Rahm, M. Boutros, D. Gradl, H. Steinbeisser, T. Holstein, J. Stetefeld, and S. Ozbek. 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., Höger, S.K., Kocagöz, Y., Laumann-Lipp, N., Ozbek, S. and T. W. Holstein. (2014). Nodal signalling determines biradial asymmetry in Hydra. Nature. In press.

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

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Fields of Interest
Molecular (epi)genetics, developmental biology, evo-devo, plant biology, female and male germlines, reproduction, asexuality (apomixis), cell cycle, Retinoblastoma pathways, imprinting, transcriptional regulatory networks

Brief summary of work since 2009
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 with a specific focus on the Retinoblastoma (pRb) pathways. Of focus is the developmental biology of plant germlines – organs or cell populations that produce gametes such as the egg and the sperm cell. 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 (Figure 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. pRb-like proteins are evolutionarily ancient transcriptional regulators of the cell cycle throughout most, if not all eukaryotic systems. While complete depletion of pRb leads to cancer and lethality in most animal systems, we exploit plant model systems that efficiently tolerate mutation load, in order to dissect how the pRb regulatory network regulates cell fate determination and differentiation during germline and ensuing reproductive development. Higher plants such as *Arabidopsis* (sexual) and its apomictic relative *Boechera*, and a lower sexual plant *Physcomitrella* (moss) are our primary model systems (Figure 2). In addition to deciphering the basic molecular genetics of the pRb-associated pathways relevant to plant and animal systems, our applied goal is to deliver biotechnological know-hows of sexual and apomictic reproduction.

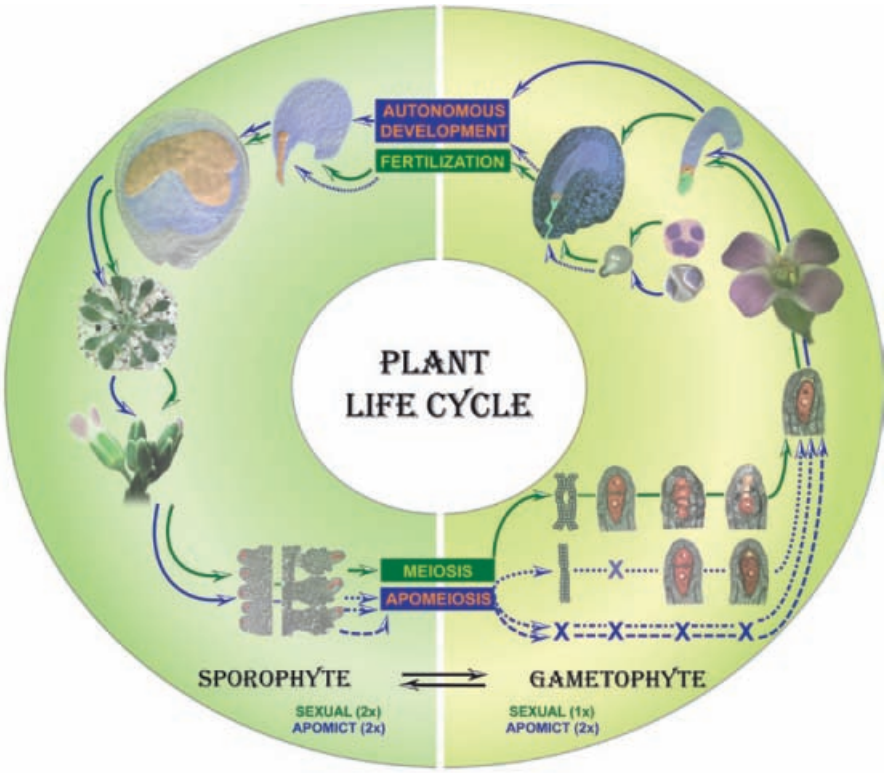

Figure 1
Sexual and asexual (apomictic) life cycle in higher plants. The plant life cycle is divided into two major phases: the sporophyte (left half) and the gametophyte (right half). Meiosis and fertilization (in case of sexual reproduction), OR apomeiosis and autonomous development (in case of apomicts) connect these two phases, facilitating continuous cycling between them. While the gametophyte is haploid in sexuals due to meiotic reduction, it is maintained with the sporophytic chromosomal number (diploid) in apomicts due to modifications in meiosis. Concurrent developmental stages during sexual and apomictic lifecycles are marked using green and blue arrows, respectively.

Major contributions since 2009

Following my doctoral work that established a genome-wide molecular frame-work towards inter-dependency between the female germlines and the surrounding sporophytic cells [a »highly accessed« publication Johnston et al., 2007], I characterised the function of RETINOBLASTOMA RELATED (RBR) – the *Arabidopsis* orthologue of pRb – in the control of germline-specific differentiation and development during my Post-doctoral work at ETH Zurich, Switzerland. It must be noted that »female germline biology« is one of the difficult research area in plant developmental biology due to a) the miniature size of the cells and organs (egg cell ~10µm in diameter; encased by a female gametophyte structure of ~25×10^3 μm^3); and b) difficulties in reaching the cell types that are buried inside hundreds of surrounding ovular cells. Despite these challenges, we could demonstrate that i) RBR controls cell fate determination and differentiation during terminal female germline development; ii) RBR directly targets a *de novo* maintenance methylase *METHYLTRANSFERASE 1* – an orthologue of the mammalian Dnmt1 – in order to control hetero-chromatinisation; and iii) an evolutionarily conserved feed-back loop between RBR and members of the POLYCOMB REPRESSIVE COMPLEX 2 (PRC2) operates during germline and ensuing seed development. This work was published in November 2008 [Johnston et al., 2008; Johnston and Gruissem, 2009]. RBR seems to differentially regulate PRC2 loci in the germlines depending on their imprinting status. Whereas most of the genes required for embryogenesis and endosperm (embryo nourishing tissue, analogous to mammalian placenta) are expressed bi-parentally (i.e. both from male and female), imprinting refers to an epigenetic phenomenon by which certain developmental genes are only mono-allelically expressed due to silencing of one of the two alleles by epigenetic modifications. Developmental-context dependent regulation of and by RBR, and the inter-connection between RBR and imprinting; were very stimulating findings. Subsequently, a collaborative work described positional cloning of an inner centromeric protein (INCENP) orthologue that controls terminal female germline development (Kirioukhova, Johnston et al., 2011).

I relocated to the Leibniz Institute of Plant genetics in order to explore the germline-specific RBR regulatory network operating during germline development and embryogenesis. It must be noted that the sporophytic (vegetative development) function of *RBR* could not be genetically demonstrated at that time since amorphic mutations in *RBR* are homozygous lethal. We could overcome this problem by tetraploid genetics in which

75 % of RBR function was abolished, leading to dramatic vegetative phenotypes including homeotic changes in floral organs (Johnston et al., 2010). We found that the overall RBR function was dosage-dependent and that the loss of germline-specific function of RBR could lead to problems in genome integrity. In an additional collaborative work we identified a conserved *trithorax*-Group (*trx*-G) protein known as TRAUCO (TRO) for its essential role during embryogenesis (Aquea, Johnston et al., 2010). TRO is a plant orthologue of the yeast BRE2P and it shares sequence similarities with ASH2 type metazoan *trx*-G proteins. In addition to this work, we identified a plant-specific transcription factor family down-stream of RBR, which seems to activate a subset of egg cell-specific transcription program (Koszegi, Johnston et al., 2011).

Prior to our lab establishment in Heidelberg, our small research team at the Leibniz Institute worked towards the following technology development and projects: a) automated mRNA *in situ* hybridisation platform and laser-capture micro-dissection of germline tissues; b) acquired training in *Physcomitrella* work in Japan (Hasebe lab, NIBB, Okazaki) and the U.K. (Cumming lab, University of Leeds) in order to dissect the adaptive function of RBR pathway during plant evolution (Figure 1); d) completion of a Bachelor project at the Halle University in 2011, which established a hypomorphic approach to characterise a germline-specific Retinoblastoma pathway; and e) co-supervision of a project aiming at characterising molecular events during asexual germline development; here a collaborative work described cloning of a genomic loci associated with apomixis (Schallau et al., 2010). We relocated our lab to Heidelberg since spring 2013, thanks to the funding by the DFG's Emmy-Noether program. Most of the technology aspects, projects and trained personnel described above have been fully transferred here. Amidst the hiatus and delays in lab establishment, two Masters students successfully completed their practical work in our lab and defended their thesis; the first one reported a non-cell Retinoblastoma pathway operating during germline development, and the second one uncovered a novel RBR-associated female-specific transcription factor controlling female germline development. Both these work have been intensively scrutinized in the lab and will soon be published in competitive journals.

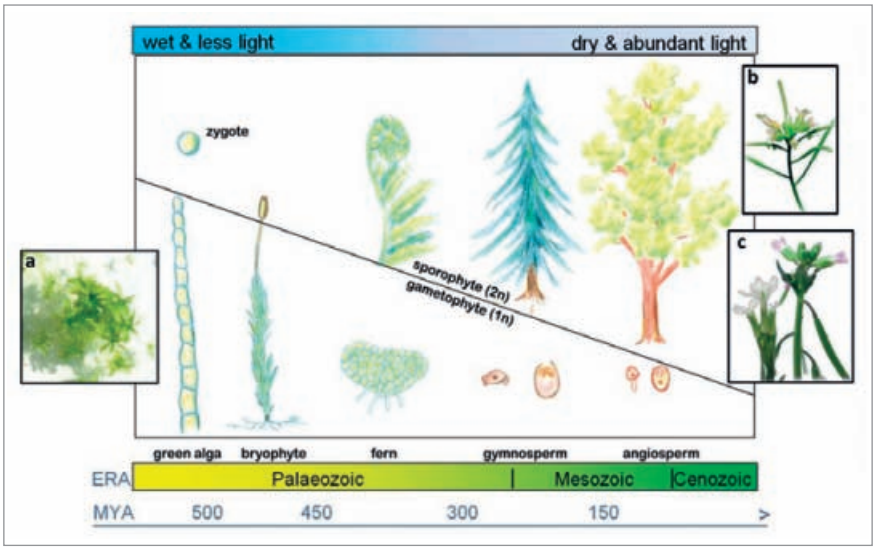

Figure 2
Evolution of land plants. Reduction of gametophytes and concomitant expansion of the sporophyte in plant evolution. Shown in insets are the model systems being used: (a) the moss *Physcomitrella*, the flowering plants *Arabidopsis* (b) and *Boechera* (c). MYA – million years ago; Paleozoic: 542-251 MY; Mesozoic: 251 – 65.5 MY; Cenozoic: 65.5 MY to date.

Planned research and new directions

It is still an enigma how the highly specialised higher plants of today evolved from lower plants or other early life forms. Plants with large gametophytes such as the bryophytes (e. g. mosses) dominated plant evolution starting early paleozoic era. When the land plants were confronted with increasing light and dry environments, there was a clear size reduction in the gametophytic forms and a concomitant expansion of the sporophyte (Figure 2). These observations supported the views of Prof. Wilhelm Hofmeister (1851), a proud alumnus of the Heidelberg University, on alternation-of-generations and gametophyte reduction. The present day sporophyte represents, therefore, a functional replacement of the earliest »leafy« gametophytes, leaving today's gametophytes extremely reduced. Consequently, a shift in gene regulation must have occurred in evolution of the land plants; our research on *Arabidopsis*-to-moss germline-specific pRb network aims to provide strong evo-devo clues along these lines.

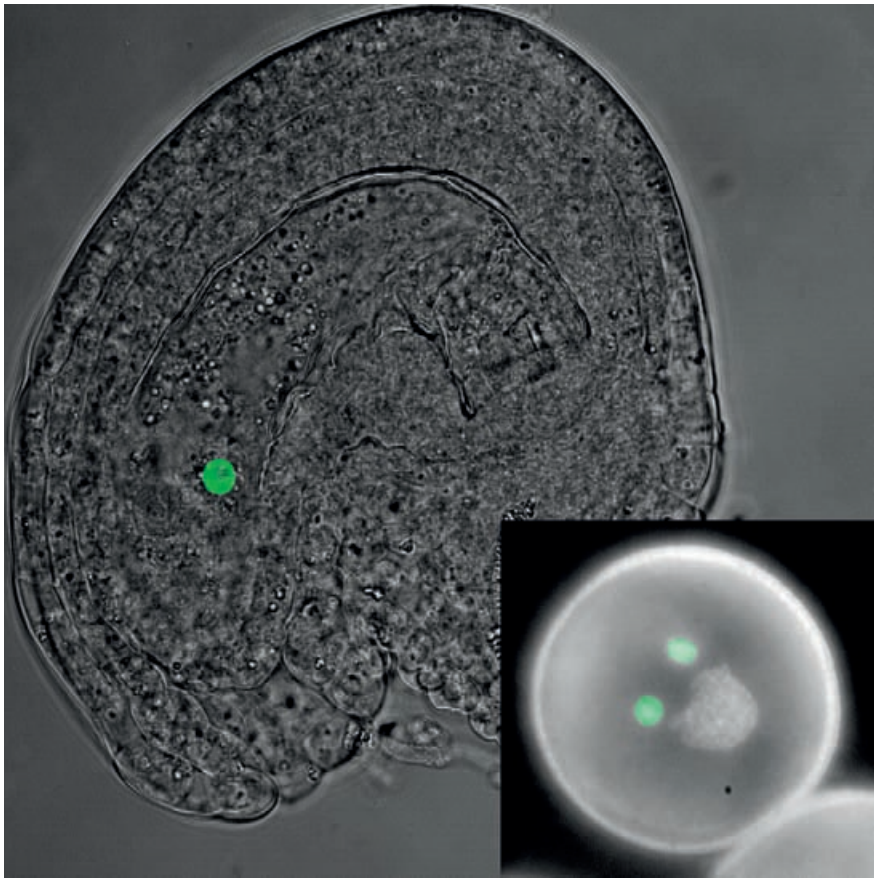

Figure 3
Arabidopsis female and male germlines. Shown are a mature ovule (female) with a green fluorescent protein (GFP) tagged egg cell and a pollen grain (male) with GFP-expressing sperm cells (inset).

In higher plants, fertilisation of an egg cell by a sperm cell gives rise to a diploid embryo, which further develops distinct plant morphologies such as leaf, stem and root; commonly known as the sporophyte (Figure 1,3). Thus, the plant life cycle is characterized by alternation of both gametophyte and sporophyte generations. Genetic and molecular dissection of gametophyte development is critical not only to improving our knowledge of plant evolution; it also provides the first step to understand and to manipulate the reproductive systems such as asexual (apomixis) embryo development (parthenogenesis) in agriculture that are crucial for securing food and feed security. Given that introduction of apomixis in cultivated plants is anticipated to provide social and economic benefits exceeding those of green revolution, (female) germline biology has become an important research area.

Current priorities:

1. Establishment of moss work (eg. gene targeting via homologous recombination), exploring adaptive evolution of germline-specific developmental events
2. Micro-manipulation and single cell isolation procedures for transcriptional and epigenomic work
3. Advanced microscopy of germlines, and modelling using bioinformatics and biophysical data
4. Function of cell-autonomous and non-cell autonomous RBR pathways (eg. for those identified via RBR protein interaction assays) operating prior to, at the onset of, and at the terminal stage of female germline development. Genome-wide analyses of the underlying transcriptional and epigenetic network
5. Positional cloning of *REPRESSORS OF RETINOBLASTOMA IN REPRODUCTION* (*ROR*s) that were identified in a suppressor mutant screen deregulating a *RBR* reporter and general imprinting status
6. Transcriptional and epigenetic regulatory network in case of sexual versus asexual female germline development in *Boechera*

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 5, number of citations 2009-2013: 146, h-index (2009-2013): 5, total h-index: 5 (according to Thomson Reuters).

Kirioukhova, O., Johnston, A.J., Kleen, D., Kägi, C., Baskar, R., Moore, J.M., Bäumlein, H., Gross-Hardt, R., Grossniklaus, U. (2011). Female gametophytic cell specification and seed development require the function of the putative *Arabidopsis* INCENP ortholog WYRD. Development *138*, 3409–3420.

Koszegi, D., Johnston, A.J., Wuest, S.E.J., Rutten, T., Kirioukhova, O., Altschmied, L., Kumlehn, J., Grossniklaus, U., Baumlein, H. (2011). Members of the RKD transcription factor family induce an egg cell-like gene expression program. Plant J *67*, 280–291.

Johnston, A.J., Kirioukhova, O., Baskar, R., Barrell, P.J., Moore, J.M., Grossniklaus, U., Gruissem, W. (2010). Dosage-sensitive function of RETINOBLASTOMA RELATED and convergent epigenetic control are required during the *Arabidopsis* life cycle. PLoS Genet *6*, e1000988.

Schallau, A., Arzenton, F., Johnston, A.J., Hähnel, U., Koszegi, D., Blattner, F., Altschmied, L., Haberer, G., Barcaccia, G., Bäumlein, H. (2010). Identification and genetic analysis of the APOSPORY locus in *Hypericum perforatum* L. Plant J *62*, 773–784.

Aquea, F., Johnston, A.J., Cañon, P., Grossniklaus, U., Arce-Johnson, P. (2010). TRAUCO, a trithorax group protein homologue, is essential for early embryogenesis in *Arabidopsis thaliana*. J Exp Bot. 61, 1215–1224.

2.11 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, Brassicaceae

Brief summary of work since 2009
The most important achievement since 2009 was preparing and launching the DFG priority programme »Adaptomics« (DFG-SPP 1529) together with Ute Krämer (Heidelberg, now Bochum). The 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 not only 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. Additional international research frameworks concerning non-Brassicaceae systems were established, for example, focusing on Cheddar Pink (*Dianthus gratianopolitanus*) as an important conservation target species of highest European priority, St. John´s wort (*Hypericum perforatum*) as an example of the evolution of cryptic gene pools and associated non-sexual reproduction, the Ginkgo-tree as a »living fossil« or the evolution of laurel forest in Tenerife. For all of these taxa and projects we have fully established population genomic approaches and are moving towards analyzing (bioinformatically) all three plant genomes. Towards the middle of this year (2014), we will have well established bioinformatic pipelines to operate at these levels. This allows us to characterize evolutionary processes in space and time with the highest resolution and will lead to the development of various important new model systems available to the wider scientific community.

Figure 1
Arabis alpina (Brassicaceae) in its alpine environment.

Major contributions and research highlights since 2009

Our research focus on cruciferous plants will substantially contribute 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. Particular focus has been given to the tribe Arabideae, and we have presented the first global comprehensive phylogenetic-perspective on the evolution of the largest monophyletic lineage within the Brassicaceae (comprising more than 550 species; Karl & Koch 2013).

We continue to focus on the evolution of wild relatives of *Arabidopsis*. The most important achievements were the development of a working 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 (bedrock) adaptation (Schmickl and Koch 2011). Using whole genome sampling of individuals from the hybrid 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 these signatures of bedrock adaptation.

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 first release of *BrassiBase* (version 1.1, june 2012) which was dedicated to Brassicaceae and Brassicales systematics and taxonomy. Earlier versions of the database introduced the underlying taxonomic framework while the second release

(version 1.1.9, August 2013) builds on the taxonomic content and synergizes taxonomic information into a »taxonomy tool« with two major new features: A »Phylogenetics Tool« and a »Cytogenetics Tool« (Kiefer et al. 2013).

Evolutionary breeding systems have been studied on large spatial continental scales in detail. Our *HypEvol* research network focusing on *Hypericum perforatum* 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 (Koch et al. 2013). Our studies on the crucifer *Boechera* highlighted Pleistocene differentiation patterns at a North American and continental scale (Kiefer et al. 2009), and this work elucidated the dynamics of the evolution of apomixis in the widest sense, indicating multiple independent realizations of this trait.

An additional knowledge database system is focusing on the collections and associated information which are based on the life-time work of Prof. Rauh, director of the Botanical Garden. During his time as director (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 Gardens in Heidelberg, especially succulents, bromeliads and orchids, 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 of the regions he visited. As part of this record, 8,776 hand written pages providing more than 32,000 entries, (previously inaccessible for research), were made available and are now being processed within »The Werner Rauh Heritage Project«.

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 two genera to develop concepts and research frameworks: 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. The second important study system is the genus *Arabidopsis*. We are aiming to present the first genus-wide »three genomes« phylogeny and are actively analyzing 100+ accessions. A third and major contribution will be to develop a timeline for Brassicaceae evolution and further elaborate the principles of crucifer evolution at the familial level, which continues to be controversial (and requires a timely resolution). Finally, the *Arabidopsis* hybrid zone in Austria continues to be source of multiple and varied evolutionary processes ripe for cutting edge research. We plan to study the meiotic evolution in response to genome doubling that the tetraploids have undergone in the process of stabilizing their whole genome duplication. In parallel with these genomic investigations, we aim to understand the local bedrock adaptation by conducting ecological crossing experiments to look for evidence of environmental factors maintaining adaptive divergence between the bedrock types.

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 of 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.

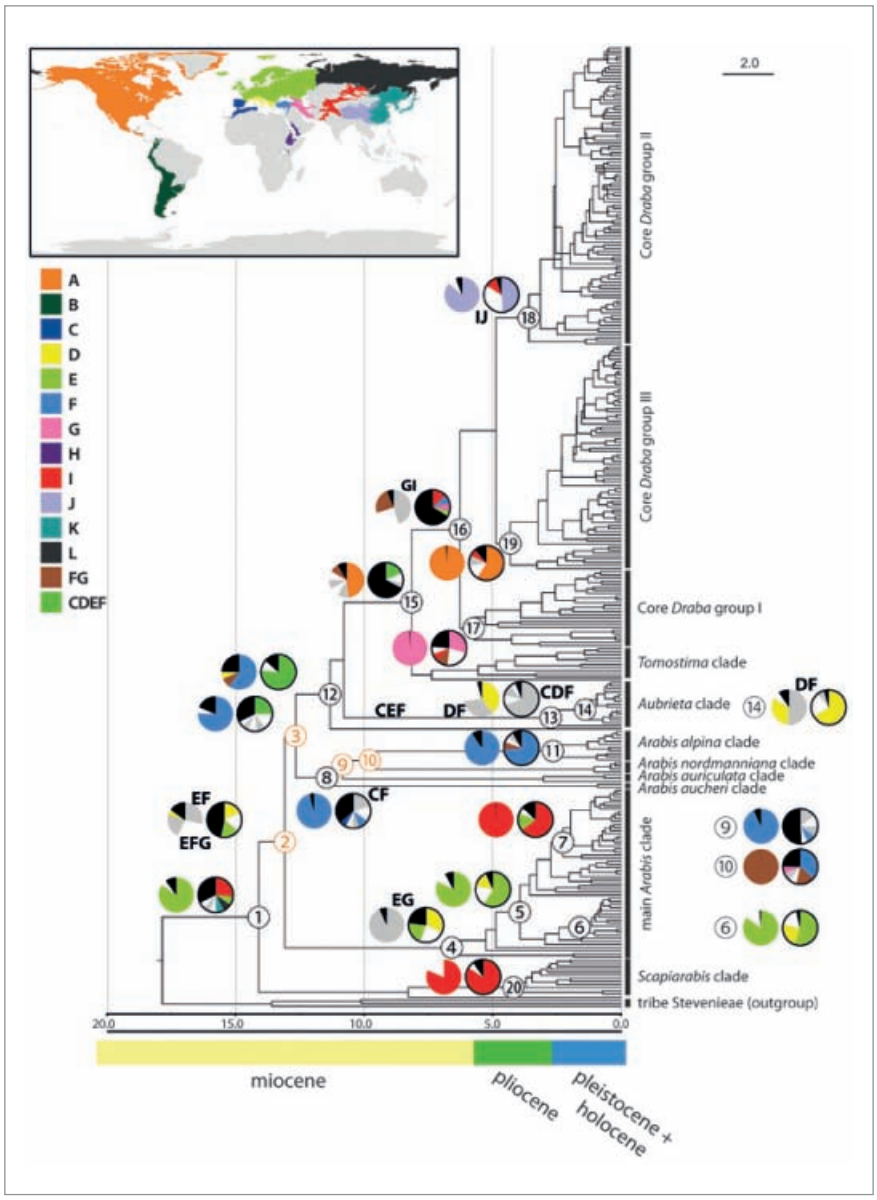

Figure 2
Maximum clade creditability (MCC) tree from the divergence time estimation in BEAST based multiple DNA sequence information of the tribe Arabideae (appr. 550 taxa), and ancestral area reconstructions.

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 41; number of citiations 2009-2013: 550, h-index (2009-2013): 12, total h-index: 35 (according to Thomas Reuters).

Kiefer M., Schmickl R., German D., Lysak M., Al-Shehbaz I.A., Franzke A., Mummenhoff K., Stamatakis A., Koch M.A. (2013) *BrassiBase:* Introduction to a Novel Knowledge Database on Brassicaceae Evolution. Plant Cell and Physiology 55 (1):e3.

KARL R, KOCH M.A. (2013) A world-wide perspective on crucifer speciation and evolution: phylogeny, biogeography and trait evolution in tribe Arabideae. Annals of Botany 112: 983-1001.

Koch M.A., Scheriau C., Betzin A., Hohmann N., Sharbel T.F. (2013) Evolution of cryptic gene pools in *Hypericum perforatum*: the influence of reproductive system and gene flow. Annals of Botany 111 (6): 1083-1094.

Schmickl R., Koch M.A. (2011) *Arabidopsis* hybrid speciation processes. Proceedings National Academy Science, USA 108 (34): 14192-14197.

Kiefer, C., Dobes, C., Sharbel, T., Koch, M.A. (2009) Phylogeographic structure of the chloroplast DNA gene pool in North American *Boechera* – a genus and continental wide perspective. Mol. Phyl. Evol. 52 (2): 303-311.

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

Brief summary of work since 2009
A first focus was directed on floral developmental features for understanding relationships between structure, function, systematics, and phylogeny. A second focus was on floral ecology. Floral developmental patterns have been proven as important characters when testing the predictions of relationships of flowering plants by molecular data. Current projects concerning ontogeny are the study of corolla tube formation in euasterids and the search for non-molecular data characterizing families resulting from the disintegration of Scrophulariaceae. Other projects are the style diversity in Asteraceae (with regard to function and phylogeny) and the diversity of nectaries. The in depth research results on style morphology and mode of secondary pollen presentation in Asteraceae will be presented in an atlas-like publication and work is in progress.

Major contributions since 2009
Disintegration of the Scrophulariaceae due to molecular data resulted in recircumscriptions, resurrection and new descriptions of families to encompass the monophyletic lineages. In some of these families floral morphological/ontogenetic data, however, are largely missing, as for example in Paulowniaceae. In our study we could reveal a set of distinctive ontogenetic features that were up to now unknown in the family and, in addition, we found the tubular stigma with papillae inside a dilated chamber as a distinctive character (Erbar & Gülden 2011).

The observation that bee-flies (*Bombylius major*) visit flowers of the wood *Anemone* (*Anemone nemorosa*, Ranunculaceae) for nectar as reward, was unexpected, because *Anemone* is thought to be a true pollen flower and, at first sight, the mouthparts of *Bombylius* do not seem well suited to true pollen flowers. Our investigations in *Anemone nemorosa* proved for the first time nectar secretion in the genus *Anemone* s.s. (i. e. other than the *Pulsatilla* group), and in addition, within the family, a new type of a carpellary nectary. Nectar production is mainly limited to the female phase of the proterogynous flower. 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 gynoecium (Erbar & Leins 2013). The conclusions inspired a comparative study on nectar secretion and nectaries in basal angiosperms (Erbar 2014; DOI: 10.1127/1869-6155/2014/0131-0075).

The decades of experience and own research on flower ontogeny, pollination and dispersal of Peter Leins and myself substantially affected the successful German textbook »Blüte und Frucht. Morphologie, Entwicklungsgeschichte, Phylogenie, Funktion und Ökologie« (2nd ed. 2008; Stuttgart: Schweizerbart). In 2010, we published an updated and enhanced English version »Flower and Fruit. Ontogeny, Phylogeny, Function and Ecology«, that received widespread recognition and commendation. I only quote from the review of Rudolf Schmid (Taxon 60: 935-396): »In summary, this is a significant work placing the reproductive morphology of angiosperms into a modern phylogenetic, functional, and ecological context. This book is an essential reference for research libraries and for all persons interested in flowers and fruits.«

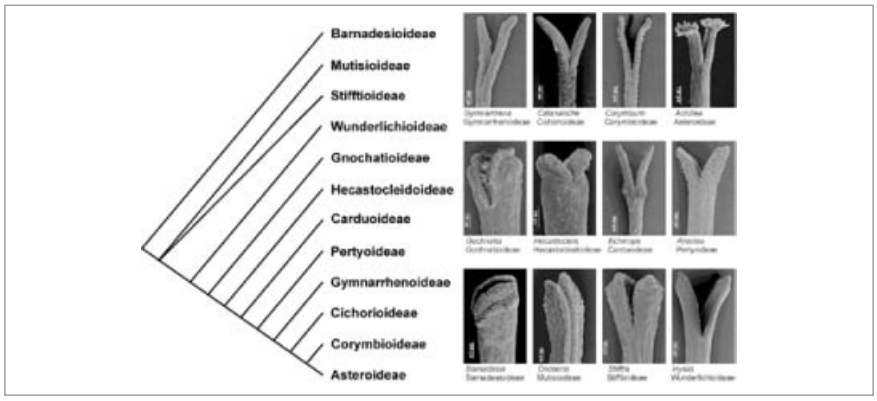

Figure 1
Style diversity in the Asteraceae.

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. The scope will be extended to basal asterids in which in many sympetalous families information on the ontogeny is missing. Another focus will be the search for distinguishing non-DNA characters in several new or more broadly circumscribed families resulting from the disintegration of the Scrophulariaceae.

To some degree the histology and position of nectaries may contribute to systematics. 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. First results have shown that we can recognize 8 different main diagrams (considering only number and position of the nectaries and disregarding shapes). However these diagrams are distributed across the 49 tribes currently recognized. Nevertheless, 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.

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 8, number of citations 2009-2013: 8, h-index (2009-2013): 2, total h-index: 12 (according to Thomson Reuters).

Erbar, C. (2010). Floral organ determination and ontogenetical patterns during Angiosperm evolution. Int. J. Plant Dev. Biol. (IJPDB) *4* (Special Issue), 1–16.
Erbar, C. & Leins, P. 2010: Nectaries in Apiales and related groups. Plant Div. Evol. *128*, 269–295.
Erbar, C. & Gülden, C. (2011). Ontogeny of the flowers in *Paulownia tomentosa* – a contribution to the recognition of the resurrected monogeneric family Paulowniaceae. Flora *206*, 205–218.
Erbar, C. & Leins, P. (2011). Synopsis of some important, non-DNA character states in the asterids with special reference to sympetaly. Plant Div. Evol. *129*, 93–123.
Erbar, C. & Leins, P. (2013). Nectar production in the pollen flower of *Anemone nemorosa* in comparison with other Ranunculaceae and *Magnolia* (Magnoliaceae). Org. Divers. Evol. *13*, 287–300.

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Fields of Interest
Plant systematics and evolution, phylogenetics, divergence time estimation, biogeography, species diversification dynamics, character evolution, Hypericum

Brief summary of work since 2009
We investigate macroevolutionary phenomena using the hyper-diverse and geographically widespread plant genus *Hypericum* (St. John's worts, Hypericaceae). With 500 species and several radiations in very different biomes, e. g., high Andean cloud grasslands, or the western Mediterranean, the genus is one of the 100 largest genera of flowering plants. Comprehensive phylogenetic analyses identified main evolutionary lineages and morphological/ecological characteristics of clades. Fossil calibrated divergence time estimations and biogeographic analyses placed major radiations in time and space. Ongoing investigations of South American *Hypericum* aim at detailed understanding of species diversification dynamics in one of the world's major hotspots of biodiversity.

Major contributions
During the past two decades, the use of molecular phylogenetic methods has increasingly contributed to plant systematics, for example, by resolving phylogenetic relationships within difficult taxonomic groups. Our investigation on the evolution of *Hypericum* clarifies systematic issues (Nürk & Blattner 2010; Nürk et al. 2013a; 2013b) and highlights the effect of a biome shift, which has taken place during global climate cooling after the Eocene Thermal Maximum (Nürk et al. submitted). We were able to locate ancestral population distributions in western Laurasia, which invert the classical out-of-Africa hypothesis. Thus, our evolutionary scenario contributes to a more detailed understanding of plant diversification dynamics during global climate change. Diversification rate shifts in *Hypericum* have been shown to be associated with a niche shift from tropical to temperate climates. This highlights *Hypericum* as a remarkable example, in which global climate change acts as a driver creating biodiversity.

Comparative investigation of apomixis (seed production without fertilization), which was placed in an explicit phylogenetic framework, revealed repeated evolution of this asexual mode of reproduction in the genus (Nürk et al. 2013a). This indicates a kind of preadaptation for apomixis in the seed development pathway in *Hypericum*.

A rapid radiation has been identified in high Andean *Hypericum* (Nürk et al. 2013b). Since the páramos (high elevation cloud grasslands in Andean South America) are one of the fasted evolving biodiversity hotspots in the world (Madriñán et al. 2013), time-calibrated phylogenies of páramo flora elements are essential in understanding mechanisms causal for the evolution of the present diversity. During several month of fieldwork in the Andes, we accumulated comprehensive geo-referenced collections, which we used to generate molecular phylogenetic hypotheses. Detailed analyses of biogeography, niche shifts, and

associated diversification rates are in progress. The results will contribute to our understanding of evolutionary drivers and patterns creating biodiversity.

Planned research and new directions

Within the next two years, we focus on phylogeographic patterns of *Hypericum* in the New World. Since evolution in the Andean hotspot was recent and rapid, next generation sequencing (NGS) approaches, including »genome skimming«, targeted sequencing, and genotyping by sequencing (GBS), are promising to obtain resolved phylogenies and associated population parameters. Multi-species coalescent models will be employed to generate species trees, and Bayesian and maximum likelihood methods will be used to estimate population histories. This allows us to reveal detailed insights into the evolutionary history of our model group, and thus, to identify causal cohesive motives underlying the observed species richness in the tropical montane biodiversity hotspot.

Selected publications since 2009

Number of peer-reviewed articles 2010-2013: 4, number of citations 2010-2013: 26, h-index (2010-2013): 3, total h-index: 3 (according to Thomson Reuters).

Nürk, N.M., Scheriau, C., and Madriñán, S. (2013b). Explosive radiation in high Andean *Hypericum* – rates of diversification among New World lineages. Front. Genet. 4(175), 1–14.

Nürk, N.M., Madriñán, S., Carine, M.A., Chase, M.W., and Blattner, F.R. (2013). Molecular phylogenetics and morphological evolution of St. John's wort (*Hypericum*; Hypericaceae). Mol. Phylogen Evo. *66*, 1–16.

Nürk, N.M., and Blattner, F.R. (2010). Cladistic analysis of morphological characters in *Hypericum* (Hypericaceae). Taxon *59*, 1495–1507.

Weigend, M., Gottschling, M., Hilger, H.H., and Nürk, N.M. (2010). Five new species of Lithospermum L. (Boraginaceae tribe Lithospermeae) in Andean South America: another radiation in the Amotape-Huancabamba zone. Taxon *59*, 1161–1179.

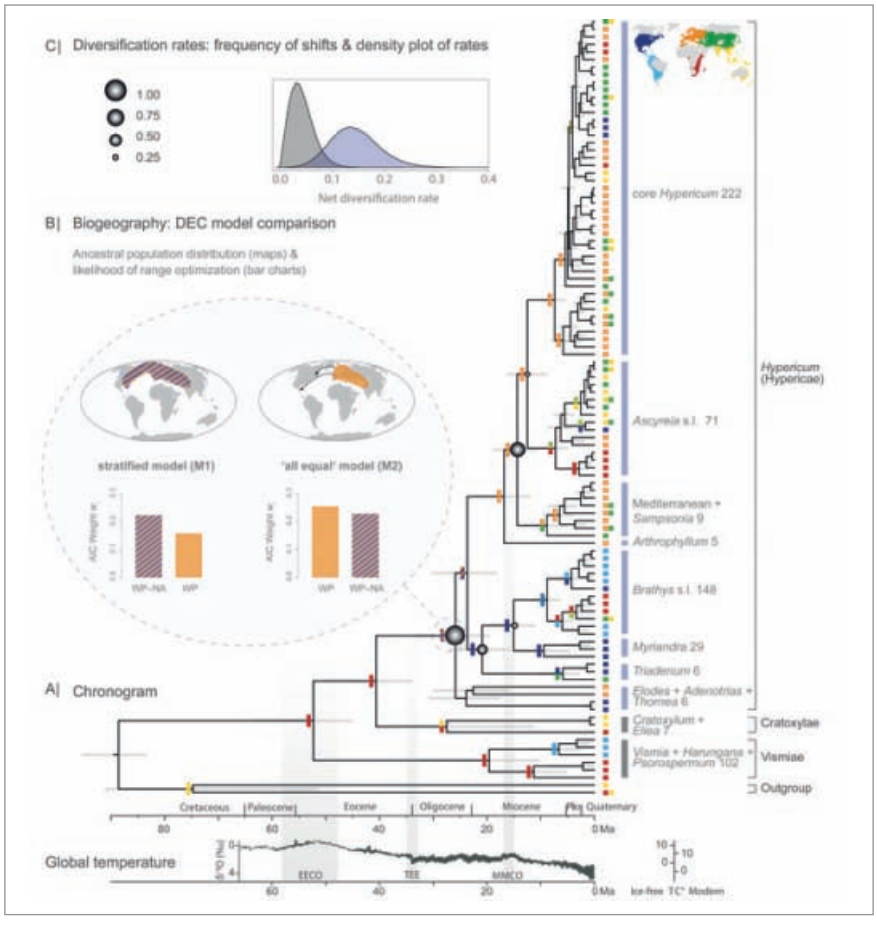

Figure 1
Ultrametric time-calibrated phylogeny of Hypericaceae, detailing (A) the 95%highest posterior density maximum clade credibility chronogram, (B) historical biogeography (parametric model-based, and (C) diversification rate shifts with associated net diversification rates. Note that Hypericum is the sole lineage within the family that does occur in cold-temperate climes (indicated by light-blue colors) and that the niche shift from tropical to temperate climates correlates with accelerated diversification rates in cold-adapted Hypericum (Nürk et al. submitted, New Phytol., Fig. 2).

2.12 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 2009
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. Thus, on the methodological side, during the last five years, algorithms for the complexity reduction of large systems, as well as improved methods for the stochastic simulation of these have been developed and integrated in our software package COPASI. In addition, global sensitivity analysis approaches have been analysed. The software COPASI itself is increasingly used in computational systems biology with current download numbers per release of about 8000. This heavy use also led to the establishment of a user forum which is currently used by ca. 600 registered users. The group is also heavily involved in the development of standardized data exchange, e. g. in the development of SBML. Apart from method development, we have been applying the methods in projects modeling both signalling as well as metabolic networks in different organisms. Thus, understanding information processing in calcium signalling has been one of the major topics. However, also information processing in other signalling pathways like NFkB and IFNa was under investigation. Finally, understanding the determining factors in metabolic networks like the central metabolism in microbial species and the sulfur assimilation pathways in plants was another goal.

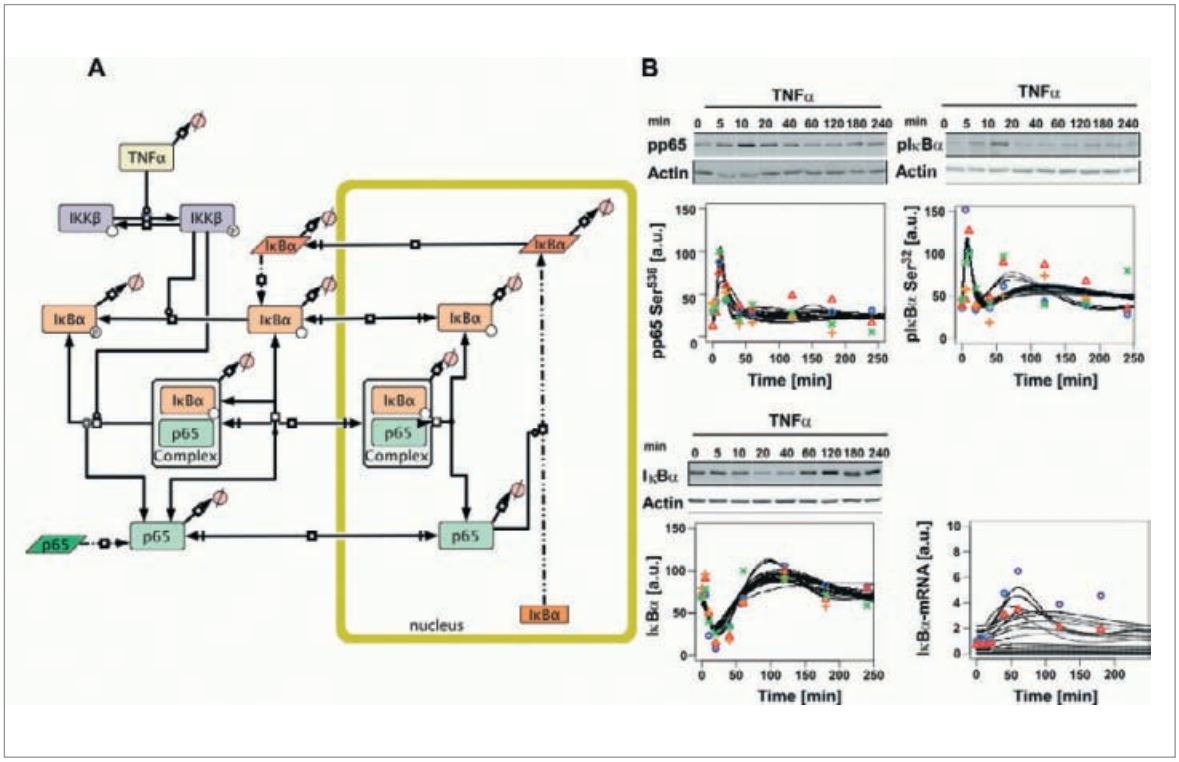

Figure 1
Schema of a computational model of NFkB signalling in hepatocytes (left). Experimentally measured time-course data plus model fit (right). As published in Pinna et al., 2012.

Major contributions since 2009

Method developement:
The growing complexity of biochemical models asks for means to rationally dissect the networks into meaningful and rather independent subnetworks. Such foregoing should ensure an understanding of the system without any heuristics employed. Important for the success of such an approach is its accessibility and the clarity of the presentation of the results. We developed methods (based on time-scale separation and on CSP. These methods have been implemented for time-dependent application within COPASI. The implementation includes different possibilities for the representation of the results including 3D-visualization (Surovtsova et al., 2009 and 2012). COPASI is developed in conjunction with the groups of Pedro Mendes (University of Manchester and Connecticut).

Information processing in signalling networks:
We investigated the mechanisms how information is encoded and decoded in calcium signal transduction in different cell types and species. In collaboration with the group of Thomas Braunbeck (COS), we discovered that – in contrast to the respective mammalian cells – calcium signalling works via an amplitude encoded mechanisms in fish liver cells (Seitz et al., 2011). This means that increasing agonist concentrations result in increasing calcium oscillation amplitudes in fish cells, whereas increasing agonist concentrations are translated in mammalian cells into increasing calcium oscillation frequencies.

In human neutrophilc leukocytes, we found a tight coupling between calcium dynamics and function (Hübner et al., 2013). Thus, neutrophils exhibiting specific functional behaviour, e. g. during cell migration, cell adherence or blebbing simultaneously show distinct calcium dynamics. We studied this system by means of multi-variate live cell imaging (performed by our group with support of the Nikon Imaging Center at BIOQUANT) on isolated primary human neutrophils (provided by the group of Maria Hänsch (Medical Faculty). During imaging we followed both the calcium concentration, as well as different morphological parameters that later allowed us to semi-automatically classify different functional states of the cells. We started to establish a model for integrin mediated calcium signalling in this cell type.

Apart from calcium signalling, we performed studies on several other signalling pathways:
Interferon signalling plays a central role in the antiviral response of cells, e. g. during hepatitis C infection of hepatocytes. A fast and strong (amplitude) reply seems to be a prerequisite for a successful virus defense. In collaboration with experimental groups (lab of Ursula Klingmüller), we studied factors that are mainly responsible for the speed and strength of the induction of antiviral genes. Here, we were able to identify IRF9 as a crucial factor in IFNa which was confirmed by validation experiments (Maiwald et al., 2011).

TNFa induced NFkB signalling plays a central role in liver regeneration. In collaboration with experimental partners (group of Kai Breuhahn, Medical Faculty), we investigated the role of protein turnover in this pathway. We also predicted that an additional phophorylating enzyme for NFkB in the nucleus – apart from the canonical pathway – is needed in order to be able to explain the measured data (Pinna et al., 2012). This has been experimentally confirmed meanwhile.

Understanding metabolic networks:
The central metabolism of different lactic acid bacteria was studied to shed light into the adaptation of these different species to their respective different environments. We studied the interactions of the central metabolism in Lactococcus lactis, being of great biotechnological importance and of Streptococcus pyogenes, a human pathogen. Computational models revealed an important role for extracellular phosphate in the regulation of central metabolism and the efficient use of glucose in both species, but with different impact (Levering et al., 2012). The study was done in a large collaborative effort with the groups of Bas Teusink (Vrije Universitet Amsterdam) and Bernd Kreikemeyer (University of Rostock), among others.

Planned research and new directions

Method development
On the methodological side we certainly will continue developing and maintaining our software package COPASI. This will include the integration of new algorithms currently under development in the group, e. g. for the analysis of nonlinear dynamics phenomenon. In addition, we are currently working on the possibility to to 2D-simulations, mimicking live-cell imaging data (in collaboration with Peter Bastians, IWR).

Information processing in signalling networks
W.r.t. calcium signalling and information processing within, we are conducting new experiments on human neutrophils which should elucidate the exact tie between cell adhesion, calcium dynamics and metabolism. This will be accompagnied by computational models. Moreover, (in collaboration with Karin Schumacher, COS, amongst others) we are planning to also study information processing via calcium in plant cells.

Thanks to reporter mice, we will be able to follow NFkB signalling in single hepatocytes and investigate, whether oscillations play a role for information transfer or not.

Understanding metabolic networks
We have established a collaboration with Ruedi Aebersold's lab at the ETH Zurich and are currently establishing the means to map proteome data on whole-genome scale metabolic models.

We will also further investigate, how to deal with unidentifiable parameters in kinetic models, e. g. in the context of modeling sulfur assimilation in plants (together with the group of Rüdiger Hell, COS).

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 14, number of citations 2009-2013: 213, h-index (2009-2013): 8, total h-index: 20 (according to Google Scholar).

P. Mendes, S. Hoops, S. Sahle, R. Gauges, J. Dada, and U. Kummer Computational Modeling of Biochemical Networks Using COPASI., Methods in Mol. Biol. 500, 17-59, 2009

I. Surovtsova, N. Simus, T. Lorenz, A. König, S. Sahle, and U. Kummer. Accessible methods for the dynamic time-scale decomposition of biochemical systems. Bioinformatics. 2009; 25(21):2816-23.

T. Maiwald, A. Schneider, H. Busch, S. Sahle, N. Gretz, TS Weiss TS, U. Kummer, and U. Klingmüller. Combining theoretical analysis and experimental data generation reveals IRF9 as a crucial factor for accelerating interferon α-induced early antiviral signalling. FEBS J. 277, 4741-54, 2010

J. Levering, M. Musters, M. Bekker, D. Bellomo, T. Fiedler, W.M. de Vos, J. Hugenholtz, B. Kreikemeyer, U. Kummer and B. Teusink Role of phosphate in the central metabolism of two lactic acid bacteria – a comparative systems biology approach., FEBS J., 279, 1274-1290, 2012

K. Hübner, I. Surovtsova, K. Yserantant, M. Hänsch, U. Kummer Ca^{2+} dynamics correlates with phenotypes and function in primary human neutrophils., Biophys. Chem., 184, 116-125, 2013


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2.13 MORPHOGENESIS AND THE EVOLUTION OF FORM
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Fields of Interest
Evo-Devo, Gastrulation, Morphogenesis,
Gene regulatory networks

Brief summary of work since 2011
Morphological differences between species are shaped by diverging developmental trajectories, and ultimately the adult form of an organism rests on morphogenetic changes in shape and behavior of cells and tissues. To explore how changes in developmental regulatory networks have led to the evolution of novel form and function, we have established gastrulation in the insect order Diptera (flies) as a framework for comparative morphogenetic studies: in flies, gastrulation comprises the initial morphologically dynamic events during embryonic development, it is directly influenced by preceding genetic patterning, it is morphologically sufficiently complex and differs between species, and yet it can be readily compared between Drosophila *melanogaster* and satellite species on an evolutionary scale ranging from population level to up to 250 million years, because all flies are essentially long germ insects and their main morphogenetic transformations like germ band extension are conserved during gastrulation. Focusing on two selected fly species at informative positions in the phylogenetic tree (*Chironomus riparius, Megaselia abdita*), we and collaborating labs generated genome and transcriptome sequence assemblies, we established protocols, molecular tools, and markers for live imaging, and for both species we have generated protocols and vector sets for transgenesis. Using these tools, we have focused on two distinct genetic networks that have changed significantly during the course of dipteran evolution, i.e. mesoderm invagination under control of the basic helix-loop-helix transcription factor Twist and extraembryonic tissue formation under control of the homeodomain transcription factor Zen.

Research highlights since 2011

From stochastic cell ingression to coordinated tissue involution
What is the minimal regulatory network needed to coordinate 1200+ cells folding into a tube, and how did such coordination evolve? We study this question by focusing on mesoderm formation in the midge *Chironomus riparius*, which shared a last common ancestor with *D.melanogaster* 250 million years ago: like in *D.melanogaster*, the presumptive mesoderm in *C.riparius* is patterned on the ventral side of the embryo through the expression of the basic helix-loop-helix transcription factor Twist. From this Twist domain, we could show that cells leave the blastoderm epithelium individually as they undergo, apparently randomly, an epithelial-to-mesenchymal transition. This stochastic ingression of mesodermal precursors is reminiscent of how mesoderm is forming in chicks and fish embryos, but it is markedly different from Drosophila, where tightly coordinated apical

actomyosin constriction leads to the concerted involution of all mesodermal precursors, the formation of a mesodermal tube, and only once all cells are internalized, an epithelial-to-mesenchymal transition. By comparing genetic components of the developmental regulatory network for mesoderm formation in *C.riparius* and *D.melanogaster*, we have identified folded gastrulation (fog) and T48 as two candidate genes that are very likely involved in the evolutionary origin of the coordinated mesoderm involution described for *D.melanogaster*. In Drosophila, fog and T48 are expressed ventrally within the domain of Twist activity and necessary for the apical activation of actomyosin constriction. For both genes we could identify homologs in the genome of *C.riparius* and related mosquitoes like *Anopheles gambiae*, and we could show that both genes after mesoderm internalization are expressed similar to *D.melanogaster*. However, neither fog nor T48 homologues were expressed ventrally in *C.riparius* blastoderm embryos. To test whether ventral expression of these genes is sufficient to recapitulate *D.melanogaster* mesoderm behavior in *C.riparius* embryos, we have cloned full length cDNAs for both genes and currently test their functional effect on mesoderm behavior after ventral expression in the mesoderm. We have developed image analysis tools to quantify the stochasticity vs. coordination of mesoderm cell behavior, which will allow us to measure how ventral expression of fog and T48 contributed to the origin of coordinated mesoderm involution.

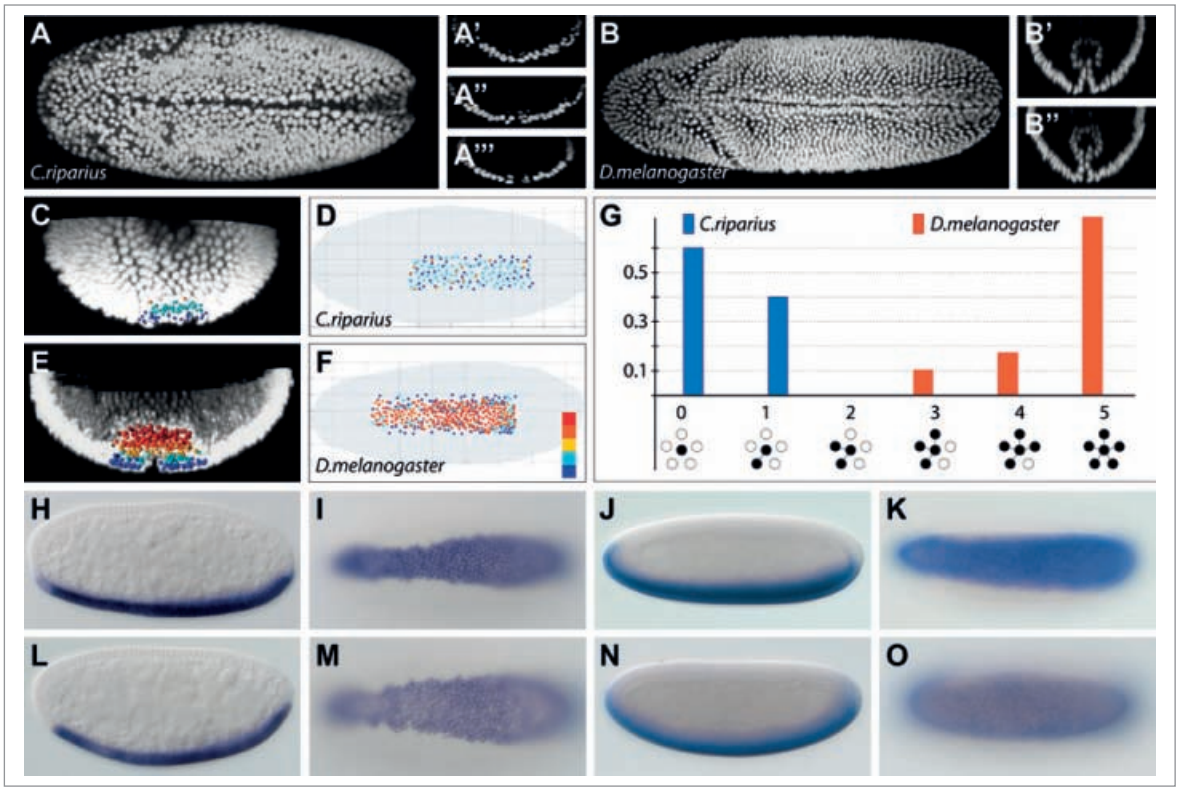

Figure 1
Comparison of mesoderm internalization in C.riparius and D.melanogaster. (A-B''') Mesodermal precursors (nuclei visualized by DAPI stain + confocal microscopy) enter the embryo along the ventral midline: in C.riparius by ingression of single cells (A-A''), in D.melanogaster by tissue involution (B-B'''). (C-F) Following segmentation, nuclear positions of mesodermal cells in a given region of interest were plotted as spheres into maximum intensity projections of confocal image stacks with color-coded ingression depth (blue, ingression less than 0.5 nuclear diameter; cyan, between 0.5 and one nuclear diameter; yellow, 1-2 nuclear diameter; orange, 2-3 nuclear diameter; red, more than 3 nuclear diameter). (G) Similarity in cell behavior was scored as proportion of the top 5% of internalized cells in *C.riparius* and *D.melanogaster* and counting how many of the five closest cell neighbors fall into the same ingression depth category. (H-O) Whole mount in situ hybridization shows blastoderm expression of twist (H-K) and snail (L-O) in C.riparius (H,I,L,M) and D.melanogaster (J,K,N,O). Anterior is to the left, views are lateral (H,J,L,N), ventral (A,B,I,K,M,O), or cross sections at selected points along anterior posterior axis (A'-A''',B'-B'').

From a stretching fold to a separated epithelium

By which mechanism can an epithelium separate as a whole from the rest of the embryo in order to become squamous and envelope the entire organism as a protective seal? We address this question in the scuttle fly *Megaselia abdita*, which shared a last common ancestor with *D.melanogaster* 150 million years ago. *Megaselia abdita*, like most other insects, develops two distinct extraembryonic tissues, the amnion and the serosa. At the blastoderm stage, the serosa anlage is defined by expression of the homeodomain transcription factor Zen (Zerknüllt) and consists of a narrow dorsal band of about 5 cells wide and 40 cells long. Midway through germband extension, this set of cells changes the morphology as the cells shrink in height and increase their apical surface area, and the initially columnar epithelium turns into a thin sheet of non-dividing squamous cells. With this increase in apical surface area, the extraembryonic tissue folds and extends over the neighboring embryo proper. This so-called amnioserosal fold is eventually torn by the continuously expanding serosa, which then detaches from the embryo and fuses on the ventral side to eventually enclose the entire embryo. Concurrently, the amnion fuses underneath the serosa and closes the embryo dorsally. In *D.melanogaster*, the extraembryonic anlage is similarly defined by expression of the homeodomain transcription factor Zen in the dorsal domain of the blastoderm embryo. Extraembryonic development proceeds through germband extension as in *M.abdita* and the tissue increases in apical surface area. However, the extension of the amnioserosal fold is extremely limited, it does not tear apart, and thus only a single epithelium is formed to close the embryo dorsally, the amnioserosa. By comparing genetic components of the developmental regulatory network downstream of Zen in *M.abdita* and *D.melanogaster*, we have identified the Dorsocross genes as candidates that are very likely involved in the evolutionary transition from a two-epithelia to a single-epithelium extraembryonic development: Dorsocross acts downstream of Zen and is known to be fundamental for fold formation in wing imaginal discs in D. melanogaster. We have studied expression and function of Dorsocross in embryos of the scuttle fly *M.abdita*, and our data suggests that Dorsocross function and changes in the regulation played a critical role in the divergence of extraembryonic morphogenesis.

Planned research and new directions

In the coming years, we will continue to focus on comparative analyses of known genetic cascades underlying morphogenesis during fly gastrulation. On the one hand, we will extend our analyses to newly described morphological differences that we recently discovered along the way, on the other hand, we are interested to explore gastrulation differences more systematically and also at smaller evolutionary time scales.

Morphogenetic variation and quantitative differences in closely related species

We see several questions as key to a comparison of gastrulation *in toto* and between more closely related species: what is the amount of variability within a stable, homogeneous genetic background (single line), how much does it differ within a species (population), and which of these differences are eventually fixed (e. g. within the genus Drosophila). To address these questions, we will use light-sheet microscopy to obtain time lapse recordings of gastrulation in the entire embryo and at a spatial and temporal resolution of individual cells. These large scale quantitative data sets are processed by automated image analysis and tracking pipelines in collaborating with the groups of Fred Hamprecht at HCI and Heike Leitte at IWR. The first quantitative description of gastrulation will allow us to define areas of low inner-strain variability but high inter-specific differences that are likely genetically encoded. This approach complements the macroevolutionary questions addressed by the comparison of distantly related species with gross morphogentic differences; our long-term goal is to bridge the gap between macroevolutionary and microevolutionary analyses to recapitulate at various time scales how and at which level evolution acted on genetic regulatory networks to generate morphogenetic novelty.

Selected publications since 2009

Number of peer-reviews articles 2009-2013: 8, Number of citations 2009-2013: 69
h-index (2009 – 2013): 6, total h-index: 9 (according to Thomas Reuters).

Rafiqi AM, Park CH, Kwan CW, Lemke S, Schmidt-Ott U (2012). BMP-dependent serosa and amnion specification in the scuttle fly *Megaselia abdita*. Development *139*, 3373.
Lemke S, Antonopoulos DA, Meyer F, Domanus MH, Schmidt-Ott U (2011). BMP signaling components in embryonic transcriptomes of the hover fly Episyrphus balteatus (Syrphidae). BMC Genomics *12*, 278.
Schmidt-Ott U, Rafiqi AM, Lemke S (2010). Hox3/zen and the evolution of extraembryonic epithelia in insects. Adv Exp Med Biol *689*, 133.
Lemke S, Busch SE, Antonopoulos DA, Meyer F, Domanus MH, Schmidt-Ott U (2010). Maternal activation of gap genes in the hover fly Episyrphus. Development, 137:1709-19.
Lemke S, Schmidt-Ott U (2009). Evidence for a composite anterior determinant in the hover fly Episyrphus balteatus (Syrphidae), a cyclorrhaphan fly with an anterodorsal serosa anlage. Development. 136:117-27.

2.14 DEVELOPMENTAL BIOLOGY
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Fields of Interest
Hox genes, cell- and tissue-specific function of developmental TFs, stem cell maintenance and differentiation, motor control and behavior, regulatory networks, regulatory precision of Hox transcription factors

Brief summary of work since 2009
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 have applied genomic approaches and developed bioinformatics tools to elucidate the regulatory code imprinted in Hox *cis*-regulatory modules (CRMs). Our work changed the picture of Hox target gene regulation, which previously relied solely on the cooperative binding of Hox proteins and a few dedicated Hox cofactors to DNA, and which is now expanded to a large pool of co-regulators that modulate target gene expression in a combinatorial manner. On the other hand, my lab also studies how Hox proteins translate their regulatory capacity 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 behaviours, to elucidate how Hox proteins regulate downstream processes in a stage- and context-dependent manner thereby achieving a highly specific architectural/morphological output.

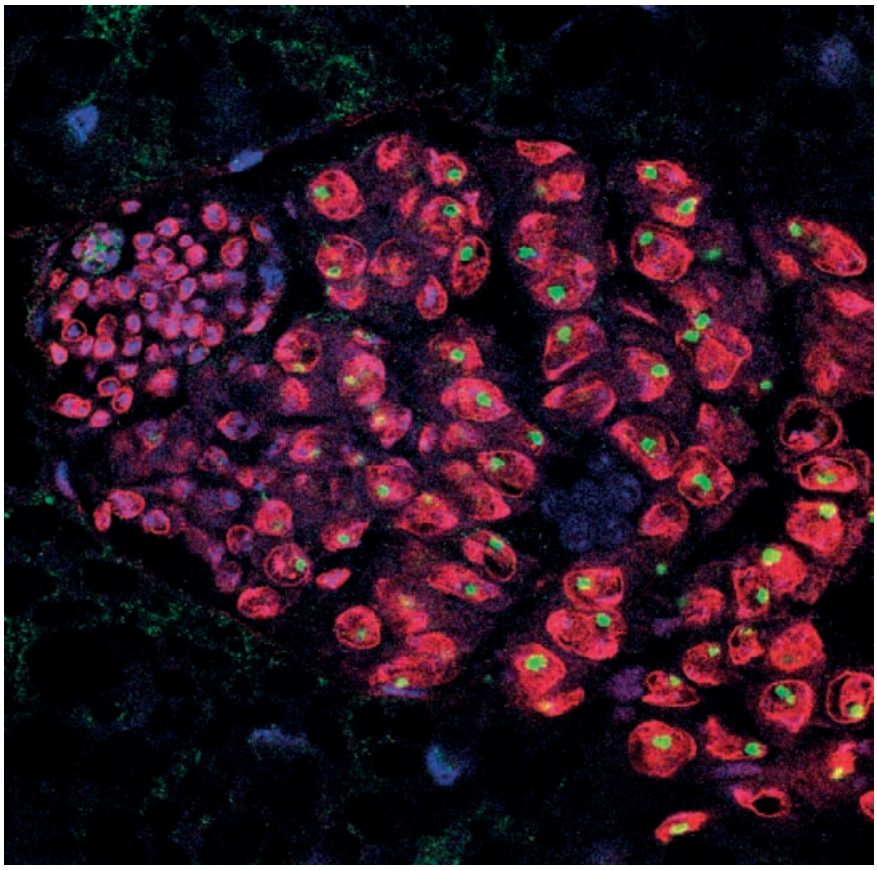

Figure 1
Staining of wild-type 3rd larval testis with Kugelkern (red) for labeling the nuclear membrane, DAPI (blue) for the DNA, and the Hox protein Abdominal-B (Abd-B) (green). Abd-B is expressed in the nucleolus of premeiotic spermatocytes and required for the positioning of the niche to the testis anterior (Papagainnouli et al., Dev. Cell, 2014).

Major contributions since 2009

Despite their broad expression, transcriptional regulators of the Hox class activate or repress transcriptional programs with extreme spatial and temporal resolution, making them ideal models to study the mechanisms underlying TF cell- and tissue-specificity. However, due to the paucity of known Hox CRMs the regulatory code underlying the strict spatio-temporal control of Hox target genes was only poorly understood. To address this question, we have quantitatively characterized hundreds of binding regions of the *Drosophila* Hox protein Deformed, which we independently identified by ChIP-seq and by computational methods. Our analysis revealed specific architectural features like motif-pair associations and motif distance preferences to be essential for cell-type specific expression of associated target genes. To this end, my lab has developed a specific bioinformatics tool, COPS (Co-Occurrence Pattern Search), which detects co-occurring TF binding sites (BSs) on defined genomic regions CRM. By comparing CRMs regulated by Dfd and Ubx, two different Hox proteins with different embryonic regulatory specificities, we showed that while similar design principles apply, specificity is encoded by distinct sets of co-occurring DNA motifs. Our work also revealed that due to the highly dynamic regulatory output of Hox transcription factors in space and time cell-type specific approaches are required to elucidate all relevant aspects of Hox-chromatin and Hox-cofactor interactions (Sorge et al., 2012; Ha et al., 2012).

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 now 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).

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 are largely unknown. We have 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 have 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 uncovers 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 neuromuscular units required for feedteaching (Friedrich et al., in preparation).

Besides elucidating the mechanistic basis of Hox proteins, my lab is also interested in understanding tumour formation and progression. To this end, we have used the *Drosophila* posterior spiracle (PS) as a model and have identified a hard-wired program through which the cell-type specifying transcription factor Cut (Ct) controls in a subset of PS cells initiation of differentiation and simultaneous repression of apoptosis via the direct transcriptional regulation of the pro-apoptotic gene rpr. Since loss of differentiation is one hallmark of cancer, we asked whether the combined transcriptional regulation of differentiation and apoptosis repression by Ct could represent a cancer prevention mechanism. Using two well-established *Drosophila in vivo* eye cancer models, we could demonstrate that eye-specific inhibition of Ct activity induced in a few cases primary and secondary tumour formation in both sensitized backgrounds, however, these numbers were dramatically increased when Ct function and the ability to activate apoptosis were simultaneously inhibited. The coupled regulation of differentiation and apoptosis by a single transcription factor for cancer prevention has been meanwhile confirmed in vertebrates (Zhai et al., 2009).

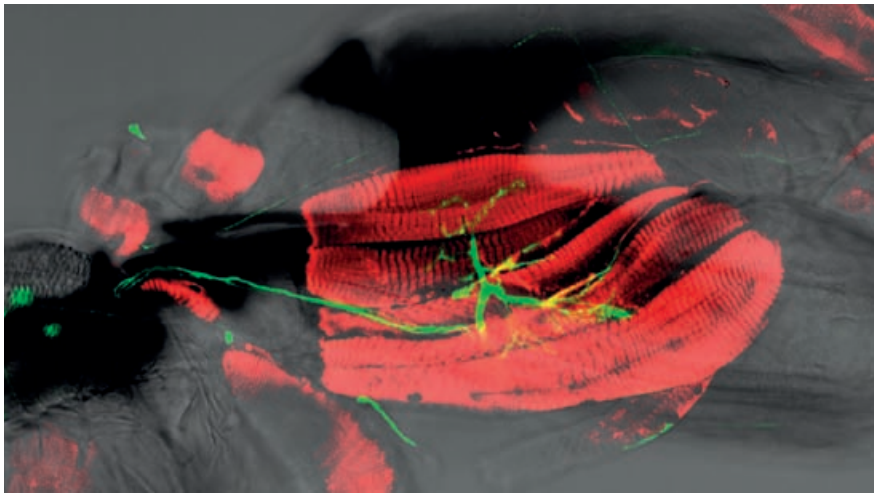

Figure 2
Mouth hook elevator (MHE) and mouth hook depressor (MHD) muscles marked by Myosin (red) in 3rd instar larvae expressing mCD8-GFP (green) by means of the OK371-GAL4 motoneuronal driver. MHD and MHE muscles (red) associated to the mouth hooks are innervated by maxillary nerve motor projections (green), formation of this motor unit is under the control of the Hox transcription factor Deformed and essential for feeding.

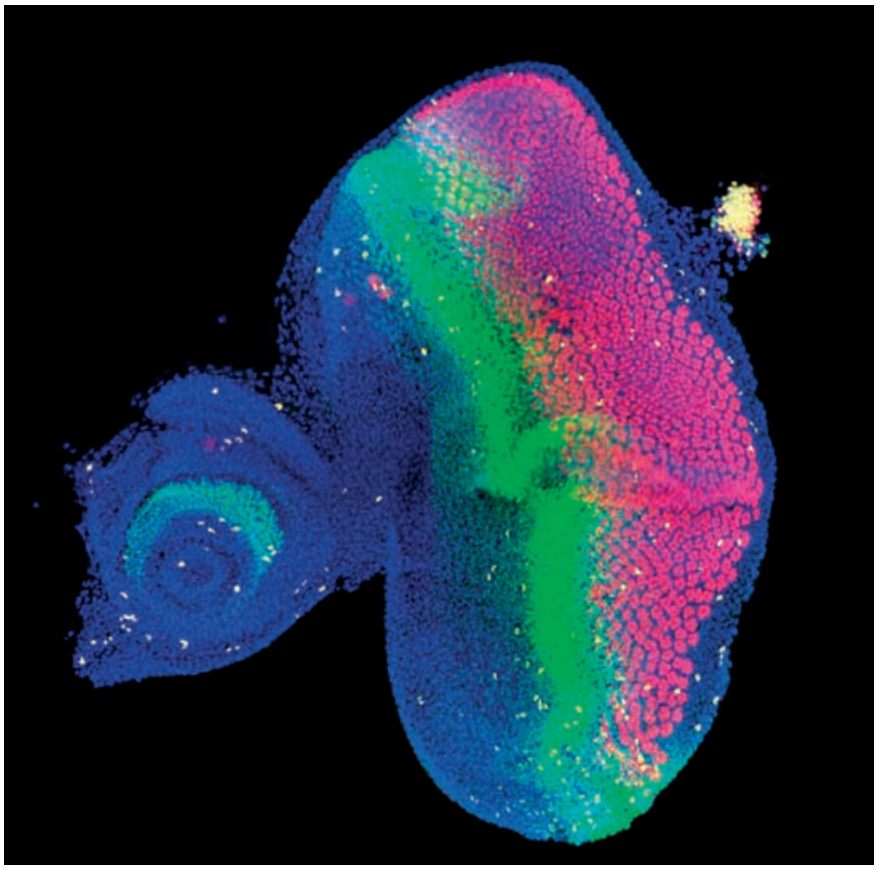

Figure 3
Staining of 3rd instar eye imaginal disc displaying over-proliferation phenotype (ey-GAL4, UAS-Dl/ UAS-CG9603RNAi) with DAPI (blue) for the DNA, Dachshund (green) for labeling differentiating cells, ELAV (red) for labeling neurons and phospho Histone 3 (yellow) to mark cell proliferation.

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 identify Hox-DNA and Hox-cofactor interactions, chromatin modifications and CRM-associated Hox complexes (Hoxosomes), which will allow us to correlate Hox input requirements with cell-type specific activity outputs in a so far unprecedented manner. Specifically, we will elucidate tissue-specific *cis*-acting control elements and transcriptional changes to understand the regulatory logic allowing widely expressed developmental master-regulators to control segment- and cell type-specific functional outputs. Furthermore, we will decipher tissue-specific Hox interactomes by analyzing *in vivo* interaction properties of the Hox proteins Dfd, Ubx, and Abd-A with around 300 pre-selected TFs expressed in the mesoderm and/or the nervous system, using the established Bimolecular Fluorescence Complementation (BiFC) approach. Here, we expect to identify putative Hox cofactors that will directly impinge on Hox DNA-binding properties in a tissue-specific manner. And finally, we plan to study specific Hox-cofactor interactions in more detail, to elucidate the *cis*-dependent *trans*-requirements necessary for tissue-specific Hox transcriptional output. To this end, we aim at characterizing chromatin as well as additional protein interactions of selected tissue-specific Hox/cofactor combinations by bimolecular ChIP (biChIP) and mass-spectrometry, respectively.

On the other hand, we will continue to elucidate the mechanistic basis of Hox function in selected biological processes. Here, we will focus on Hox-dependent stem cell control in the *Drosophila* testis and the Hox-dependent regulation of regional motor patterns, with a focus on feeding- and crawling-related motor activities. In the *Drosophila* male stem cell system, we will focus on the Hox protein Abd-A, which is critical for the differentiation of somatic stem cells. We will use the quantitatively identified testis specific chromatin binding sites, which gives us direct access to Hox responsive *cis*-regulatory motifs, as well as target genes. We will use this resource to elucidate biological function of a group of selected Hox targets involved in testis stem cell control at the mechanistic level. Furthermore, we will study selected testis-specific Hox CRMs to elucidate how the activity of the

widely expressed developmental master-regulator Abd-A is translated into cell-type specific outputs in the male stem cell niche. Concerning the Hox-dependent control of motor patterns, we will analyze in detail Dfd and Ubx target genes specifically controlled in the neuronal and mesodermal tissues, which we will have identified by cell-type specific approaches described above. Our goal is to understand how Hox proteins region-specifically guide the interaction of appropriate synaptic partners, motoneurons and muscles, to achieve highly specific behaviours, like feeding and crawling.

The specific aims we have for the next years are:
1. Decipher *cis*- and *trans*-requirements for tissue-specific Hox activity
2. Determine tissue-specific interaction networks of Hox proteins in live embryos
3. Comprehensively study stem cell maintenance and behaviour under the control of Hox proteins

In sum, these studies will allow us to elucidate how the developmentally critical class of TFs, the Hox proteins, acquires context dependent activities and functions at the mechanistic level.

Selected publications since 2009
Number of peer-reviewed articles 2009-2013: 13, number of citations 2009-2013: 81, h-index (2009-2013): 6, total citations: 529, total h-index: 10 (according to Thomson Reuters).

Papagiannouli, F., Schardt, L., Grajcarek, J., Ha, N., Lohmann, I. (2014). The *Hox* gene *Abd-B* controls stem cell niche function in the *Drosophila* testis. Dev. Cell *28(2)*, 189-202.
Sorge, S., Ha, N., Polychronidou, M., Friedrich, J., Bezdan, D., Kaspar, P., Schaefer, M.H., Ossowski, S., Henz, S.R., Mundorf, J., Rätzer, J., Papagiannouli, F., Lohmann, I. (2012). The *cis*-regulatory code of Hox function in *Drosophila*. EMBO J. *31(15)*, 3323-3333.
Ha, N., Polychronidou, M., Lohmann, I. (2012). COPS: detecting co-occurrence and spatial arrangement of transcription factor binding motifs in genome-wide datasets. PLoS One 7(12), e52055.
Zhai, Z., Ha, N., Papagiannouli, F., Hamacher-Brady, A., Brady, N., Sorge, S., Bezdan, D., Lohmann, I. (2012). Antagonistic regulation of apoptosis and differentiation by the Cut transcription factor represents a tumor-suppressing mechanism in *Drosophila*. PLoS Genet. *3(3)*, e1002582.
Stöbe, P., Stein, M.A., Habring-Müller, A., Bezdan, D., Fuchs, A.L., Hueber, S.D., Wu, H., Lohmann, I. (2009). Multifactorial regulation of a Hox target gene. PLoS Genet. *5(3)*, e1000412.

2.15 STEM CELL BIOLOGY
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Fields of Interest
Molecular and developmental genetics, stem cell regulation, plant development and environmental adaptation, transcriptional networks

Brief summary of work since 2009
As sessile organisms, plants are exposed to extreme variations in environmental conditions over the course of their lives. Since plants grow and initiate new organs continuously, they have to modulate the underlying developmental program accordingly to cope with this challenge. At the heart of this extraordinary developmental plasticity are pluripotent stem cells, which are maintained during the entire life-cycle of the plant and that are embedded within dynamic stem cell niches. Work in my lab during the past five years has focused on four major lines of investigation using the shoot apical meristem of the reference plant *Arabidopsis thaliana* as a model: 1. Define the molecular mark-up of stem cells and niche cells. 2. Elucidate the molecular mechanisms of the stem cell inducing WUSCHEL transcription factor. 3. Delineate the regulatory networks of cell-cell communication in the apical stem cell system. 4. Trace stem cell behavior in response to environmental variation. Our work revealed that WUSCHEL protein function is dependent on plasmodesmata mediated movement from organizing cells into stem cells and that WUSCHEL acts via histone de-acetylation. We identified novel WUS executor genes, which play important roles in maintaining meristem activity and uncovered unsuspected regulatory interplay between the auxin and cytokinin phytohormone pathways serving as essential integration hub for interregional signaling. Finally, we have made strides in developing methods for cell type specific chromatin profiling, live imaging and quantitative analysis of the apical meristem, as well as in establishing a novel cloning system for high throughput generation of complex plant transformation constructs for functional analysis *in vivo*.

Major contributions since 2009
Induction and maintenance of stem cell fate by non-cell autonomous signals is a shared feature of most biological systems. 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. We were able to show that plasmodesmata dependent symplastic connectivity within the apical stem cell niche of *Arabidopsis* is required for stem cell activity. We found that the stem cell inducing transcription factor WUSCHEL (WUS), expressed in the niche, moves to the stem cells via plasmodesmata in a highly regulated fashion and that this movement is required for stem cell activity. WUS mobility is encoded in its protein sequence and mediated by multiple domains. Interestingly, parts of the protein that restrict movement are required for WUS homo-dimerization, suggesting that formation of WUS dimers might contribute to the regulation apical stem cell activity (Daum et al. under review).

Another important focus of our work was the elucidation of the regulatory networks downstream of WUS. Consequently, we recorded the genome-wide regulatory potential of WUS by transcriptional and genomic profiling including WUS chromatin binding, histone H3 acetylation and H3K27 tri-methylation. By meta-analysis, we were able to show that WUS acts by directly binding to at least two distinct DNA motifs in more than a thousand target promoters and preferentially affects the expression of genes with roles in hormone signaling, metabolism and development. Striking examples are the direct transcriptional repression of *CLAVATA1*, which is part of a negative feedback regulation of *WUSCHEL* and the immediate regulation of transcriptional repressors of the TOPLESS family, which are involved in auxin signaling. It also became clear that WUS acts via de-acetlylation of histone H3 providing a mechanistic explanation for its repressory activity (Busch et al. 2010; Miotk et al. in preparation).

Among the direct WUS targets, we identified the bHLH transcription factor HECATE1 (HEC1), which we found to contribute to meristem function by promoting stem cell proliferation while antagonizing niche cell activity. HEC1 represses the stem cell regulators *WUS* and *CLAVATA3* and, like WUS, controls genes with functions in metabolism and hormone signalling. Among the targets shared by HEC1 and WUS are phytohormone response regulators, which we showed to act as mobile signals in a universal feedback system (Schuster el al. 2014).

The classical phytohormones cytokinin and auxin play essential roles for the maintenance of stem cell systems embedded in shoot and root meristems and exhibit complex functional interactions. We were able to show that the activity of both hormones directly converges on the promoters of two A-type *ARABIDOPSIS RESPONSE REGULATOR (ARR)* genes, *ARR7* and *ARR15*, which are negative regulators of cytokinin signaling and have important meristematic functions that we had described earlier (Leibfried et al. 2005). While *ARR7* and *ARR15* expression in the shoot apical meristem is induced by cytokinin, auxin has a negative effect, which is, at least in part, mediated by the *AUXIN RESPONSE FACTOR5/ MONOPTEROS* transcription factor. Our results were the first to provide a mechanistic framework for hormonal control of the apical stem cell niche and demonstrated how root and shoot stem cell systems differ in their response to phytohormones (Zhao et al. 2010).

Flowers develop from floral meristems, which harbour short-term stem cells that support the growth of floral organs making them an attractive model to study stem cell termination. Using a highly conserved cis-regulatory motif as bait, we identified the bZIP transcription factor PERIANTHIA (PAN) as a direct regulator of AGAMOUS (AG), which in turn is required for termination of stem cell fate. Mutations in *PAN* have environmentally dependent stem cell phenotypes and *PAN* expression persists in ag mutant flowers, suggesting that *PAN* and *AG* are engaged in a negative feedback loop, which might be mediated by WUS (Maier et al. 2009).

While we extensively use genomic profiling or live cell imaging, functional analysis *in vivo* by genetics and transgenics remains at the heart of our work. Therefore, we established the GreenGate system, which is based on Golden Gate cloning to facilitate the assembly of complex plant transformation constructs. GreenGate cloning is simple and efficient since it uses only a single endonuclease, depends on only six types of insert modules (plant promoter, N-terminal tag, coding sequence, C-terminal tag, plant terminator and plant resistance cassette), but at the same time allows assembling several expression cassettes in one binary destination vector from a collection of pre-cloned building blocks. Thus, GreenGate considerably speeds up cloning and transgene stacking for analysis of gene function *in planta* (Lampropoulos et al. 2013).

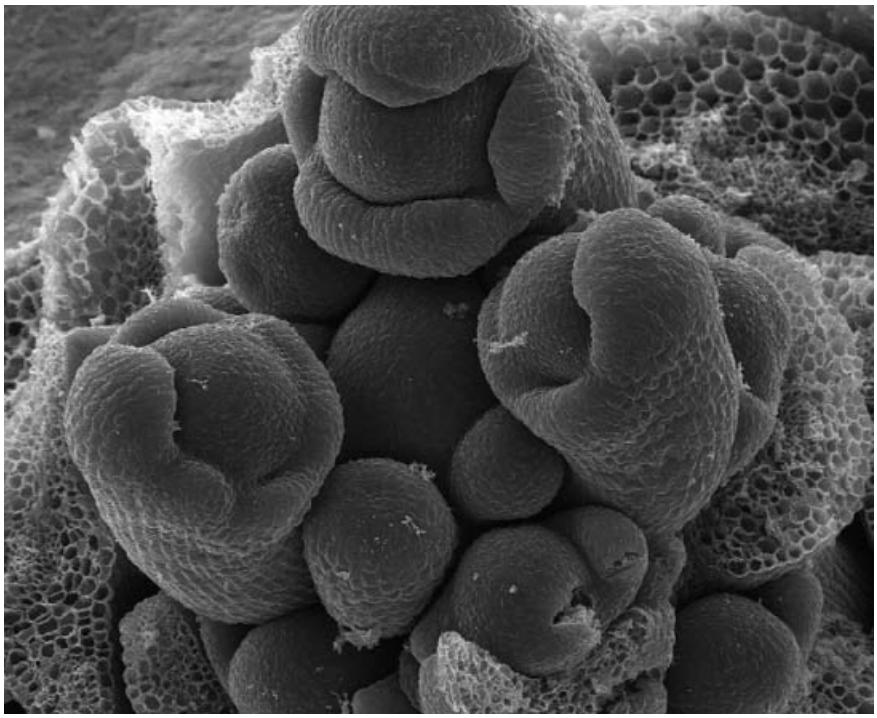


Figure 1
Scanning electron micrograph of an Arabidopsis shoot apex. The dome-shaped inflorescence meristem in the center encompasses stem cells, while at the periphery flowers develop.

Planned research and new directions

The aim of our work in the coming years is to define a regulatory framework for plant stem cell control, which underlies the balance between proliferation and differentiation under changing environments in the continuously active shoot apical meristem. To this end we will follow an interdisciplinary approach build on live imaging, molecular analyses, genetics and genomics, biochemistry and structural biology, as well as mathematical modeling. We will continue to move towards cell-type specific analyses to increase the resolution of our experiments and to circumvent problems of pleiotropic or essential gene functions. We have initiated collaborations with groups focusing on mathematical modeling of cell populations and biochemical processes, respectively, and are working towards quantitative experimental data at both levels to provide a solid baseline for their efforts. Due to the increasing complexity of meristem regulation, which precludes intuitive interpretation, I see great potential in computational analysis and simulation, on the one hand to understand the regulatory wiring and on the other hand to help us to derive new and testable hypotheses for future research. Particular focus for the coming years will be on genome profiling of specific meristematic cell types including stem cells and organizing cells; a thorough structure-function analysis of WUS including crystal structure and *in vivo* protein behavior; detailed functional studies of WUS downstream processes, such as the one controlled by HEC1; mechanistic analyses of the integration of interregional hormone signaling and local transcriptional programs and finally a top level understanding of the influence of light and temperature on the regulatory system of stem cell control.

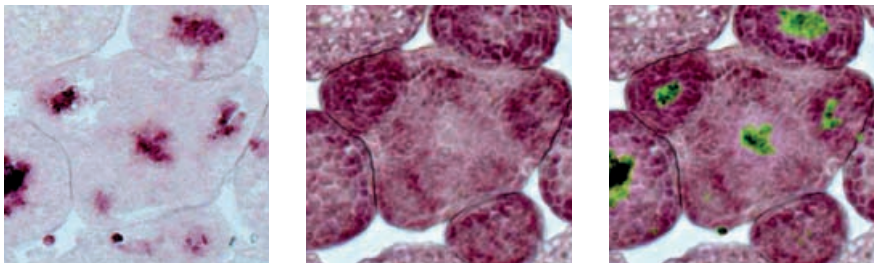


Figure 2
Activity patterns of the stem cell regulators WUSCHEL (top panel) and HECATE1 (middle panel). RNA in situ hybridization on consecutive cross-sections of an Arabidopsis shoot apex reveals non-overlapping expression domains (bottom panel), which correlates well with the functional antagonism observed between WUSCHEL and HECATE1.

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 15, number of citations to papers published 2009-2013: 227, h-index (2009-2013): 5; total h-index: 23 (according to Thomson Reuters).

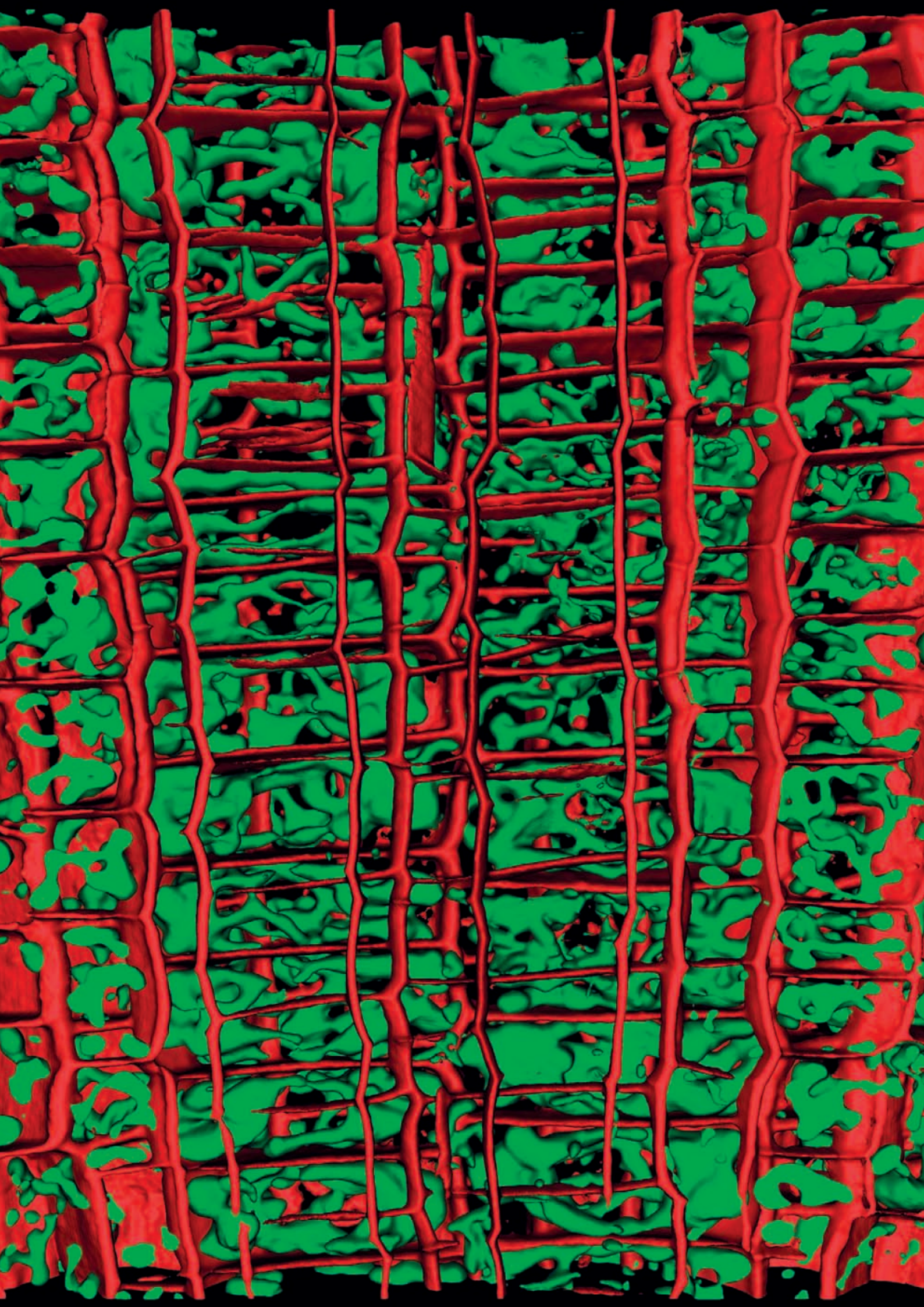
Schuster, C., Gaillochet, C., Medzihradszky, A., Busch, W., Daum, G., Krebs, M., Kehle, A., and Lohmann, J.U. (2014) A regulatory framework for shoot stem cell control integrating metabolic, transcriptional, and phytohormone signals. Developmental Cell, 28, 438-49

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

Zhao, Z., Andersen, S. U., Ljung, K., Dolezal, K., Miotk, A., Schultheiss, S. J., and Lohmann, J. U. (2010) Hormonal control of the shoot stem cell niche. Nature, 465, 1089-92.

Busch, W., Miotk, A., Ariel, F. D., Zhao, Z., Forner, J., Daum, G., Suzaki, T., Schuster, C., Schultheiß, S. J., Leibfried, A., Haubeiß, S., Ha, N., Chan, R. L., and Lohmann, J. U. (2010) Transcriptional regulation of a plant stem cell niche. Developmental Cell, 18, 849-61.

Maier, A. T., Stehling-Sun, S., Wollmann, H., Demar, M., Hong, R. L., Haubeiß, S., Weigel, D., and Lohmann, J. U. (2009) Dual roles of the bZIP transcription factor PERIANTHIA in the control of floral architecture and homeotic gene expression. Development, 136, 1613-20.



2.16 DEVELOPMENTAL PLASTICITY IN PLANTS

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INDEPENDENT RESEARCH GROUP

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Fields of Interest
Molecular and developmental genetics of plants, morphogenesis, plasticity, lateral root, small RNAs

Brief summary of work since 2009
My laboratory is interested in the cellular and molecular bases of plant developmental plasticity. Our goal is to understand how, at the cell scale, plants are able to adapt their post-embryonic development to their environment. Developmental plasticity is best illustrated in the root system, as genetically identical plants display highly distinct root morphologies under different growth conditions. My group therefore uses as a model the root system of *Arabidopsis thaliana*, which continuously forms new lateral roots.

Work in my group develops into two major directions. On the one hand, we study at the organ and cell level how small RNA molecules (miRNAs and ta-siRNAs) are produced and consequently fine tune lateral root growth. On the other hand, we study the morphodynamics of lateral root formation, in particular the role played by physical constraints on shaping this organ. Current research in my lab is based on an integrated approach of cell biology, molecular genetics, advanced microscopy, and biochemical methods together with computational and systems analysis.

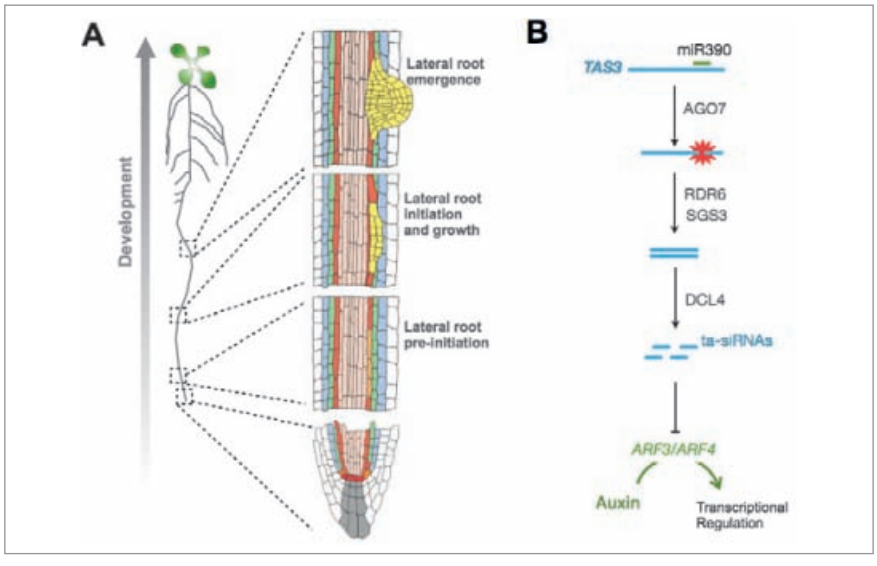


Figure 1
(A) General organization of the root system in Arabidopsis and development of lateral roots. Lateral roots are formed by priming of cells that are deep in the main root (pre-initiation). Primed cells then divide and form the lateral root primordium (intiation and growth) which emerges. (B) Biogenesis of TAS3-derived ta-siRNAs. TAS3 is cleaved by the AGO7/miR390 complex and the cleavage products are converted into dsRNA by RDR6 and SGS3. DCL4 then catalyses the formation of ta-siRNAs from the dsRNA. The ta-siRNAs inhibit the expression of ARFs transcription factors that mediate the effects of the hormone auxin.

Major contributions

1. Control of lateral root growth by trans-acting small interfering RNAs
Trans acting siRNAs (ta-siRNA) are plant specific endogenous small regulatory RNAs that are produced from non-coding *TAS* genes and that guide the cleavage of specific mRNA targets. Their biogenesis requires an initial cut by a miRNA and the conversion of one of the resulting cleavage products into a double-stranded RNA. This double-stranded RNA is subsequently processed into 21nt long ta-siRNAs (see Figure). The ta-siRNAs produced by miR390-mediated cleavage of the phylogenetically conserved *TAS3* locus regulate the abundance of several *ARF* genes. *ARF* genes control the transition between juvenile and adult leaves as well as leaf polarity. Work in my group has established that (i) ta-siRNAs can act in a non-cell autonomous manner and that (ii) the *TAS3* pathway controls lateral root growth. In roots, modification of *TAS3* transcript levels leads to a modification of the lateral root length. *MIR390,* needed for ta-siRNA production, is expressed at the sites of lateral root formation and the lateral root primordium itself. We have demonstrated the existence of a complex set of feed-back mechanisms between ARFs and miR390. These findings establish that activation of a small RNA cascade during organogenesis results in the quantitative modulation of lateral root growth and provide a mechanistic basis for the developmental plasticity and robustness of lateral root growth.
Major associated publication: (Marin et al., 2010)

Cellular architecture of ta-siRNA production and RNA quality control
The formation of ta-siRNAs from the *TAS3* precursor is triggered by the AGO7/miR390 complex which primes *TAS3* for conversion into double stranded RNA by the RNA-dependent RNA polymerase RDR6 and SGS3 (see Figure). The mechanism routing AGO7-cleaved *TAS3* precursor to RDR6/SGS3 and its subcellular organization were unknown. We have shown that AGO7 accumulates together with SGS3 and RDR6 in cytoplasmic siRNA bodies. These siRNA bodies are membrane associated and may represent sites of accumulation of mRNAs stalled during translation. We have established the functional relevance of AGO7 being targeted to the siRNA bodies for ta-siRNAs biogenesis. Our results shed light on the subcellular organization of post-transcriptional gene silencing, a field that is only emerging. In addition, we have shown that the siRNA bodies are dynamically and physically linked to P-bodies, another class of bodies specialized in RNA processing. We have shown that plants with compromised P-body function display enhanced silencing of transgenes and endogenous transcripts. Furthermore, P- and siRNA bodies act mostly antagonistically on cellular transcripts. Our results suggest that RNA quality control represents the highly specific (only dysfunctional transcripts are eliminated) front line defense pathway against defective nucleic acids. We further showed that the very potent, albeit less specific, siRNA-mediated silencing (siRNAs guide the degradation of both defective and homologous normal transcripts) is induced only when the capacity of the RNA quality control pathways is saturated.
Major associated publication: (Jouannet et al., 2012)

Quantitative analysis of lateral root morphogenesis
My lab in collaboration with the one of EHK Stelzer (Frankfurt University), has pioneered the use of light sheet fluorescence microscopy (LSFM) for live imaging of plant development in close-to-natural growth conditions, at high spatial (micrometer) and temporal (minutes) resolution and for extended periods of time (days). Because of low phototoxicity and bleaching, LSFM is the tool of choice for the description of a morphogenetic event as it provides detailed time-resolved information on the behavior of all individual cells over the entire duration of the morphogenetic process. We have obtained high-resolution 4D (3D + time) recordings of lateral root morphogenesis providing an unprecedented level of insights. We have developed a pipeline allowing the quantitative analysis of these recordings (the Virtual lateral roots). We have assessed the precise lineages, position and division patterns of all cells of the lateral root primordium (LRP). We combined these data with genetics, and established that the overlaying tissues place biomechanical constraints on the LRP during lateral root emergence from the parental root that impact its morphogenesis. We also observed that the LRP morphogenesis is not underpinned by a rigid initial organization of its founders, neither a rigid pattern of cell divisions. This suggests that the stereotyped LRP shape and its growth dynamics are emerging properties from a small set of initial conditions and basic division rules.

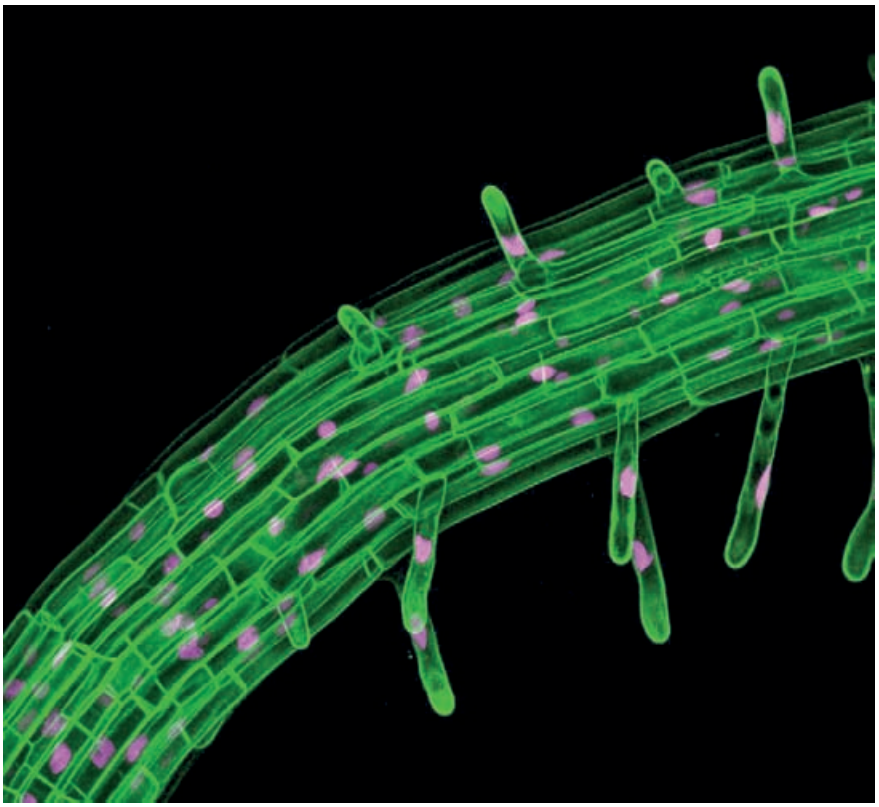


Figure 2
Fluorescence image of an Arabidopsis thaliana root. The cells nuclei are in magenta and the plasma membrane in green (by Fernan Federici – Cambridge University).

Planned research and new directions

The shape of multicellular organisms results from the combined action of their genes and the physical environment in which development takes place. However, developmental biologists have essentially focused on unraveling how genes control shape. Consequently, very little is known on the impact of the environment on morphogenesis.

I plan to build upon the tools we have developed to study quantitatively post-embryonic development *in planta* and investigate how the cell properties driving morphogenetic processes respond to changes in the environment. Using LSFM, we will elucidate how biochemical gradients and cellular biomechanics coordinate during morphogenesis and differentiation and how variations in the physical environment influence these processes. Our specifics aims are to first pursue our quantitative analysis of lateral root morphogenesis at cellular resolution by complementing it with a dynamic view on how cell differentiation occurs (e. g. formation of a new meristem and stem cells population). We will in parallel interfere with the biochemical and mechanical forces contributing to lateral root morphogenesis (by combination of genetics and micro-manipulation) to monitor dynamically their impact on LRP morphogenesis. Finally, we will quantify the impact of the environmental conditions. We will focus on temperature and osmolarity, two parameters that have well-known effects on plant cell properties and are easily controllable in the lab. Change of temperature has a direct influence on many biochemical processes linked to cell mechanics: cell wall rigidity, plasma membrane fluidity, cytoskeleton rigidity and dynamics as well as protein function. Osmolarity affects turgor pressure, the driving force of plant cell elongation and thus growth. In addition, temperature and osmolarity both have ecophysiological relevance. Plants are exposed to important diurnal and seasonal variations in temperature and water supply, and as the root grows through heterogeneous soil, its cells are exposed to high fluctuations of the water potential. Finally, the current context of climate change is characterised by even more extremes fluctuations in these variables. By quantifying the role of the environment on morphogenesis, our findings will significantly advance our understanding of how biological shapes arise and will have a profound impact in developmental biology and ecophysiology.

Selected publications since 2009

Number of peer-reviewed articles (2009-2014): 15, number of citations (2009-2013): 272, h-index (2009-2013): 7, total h-index: 12

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.

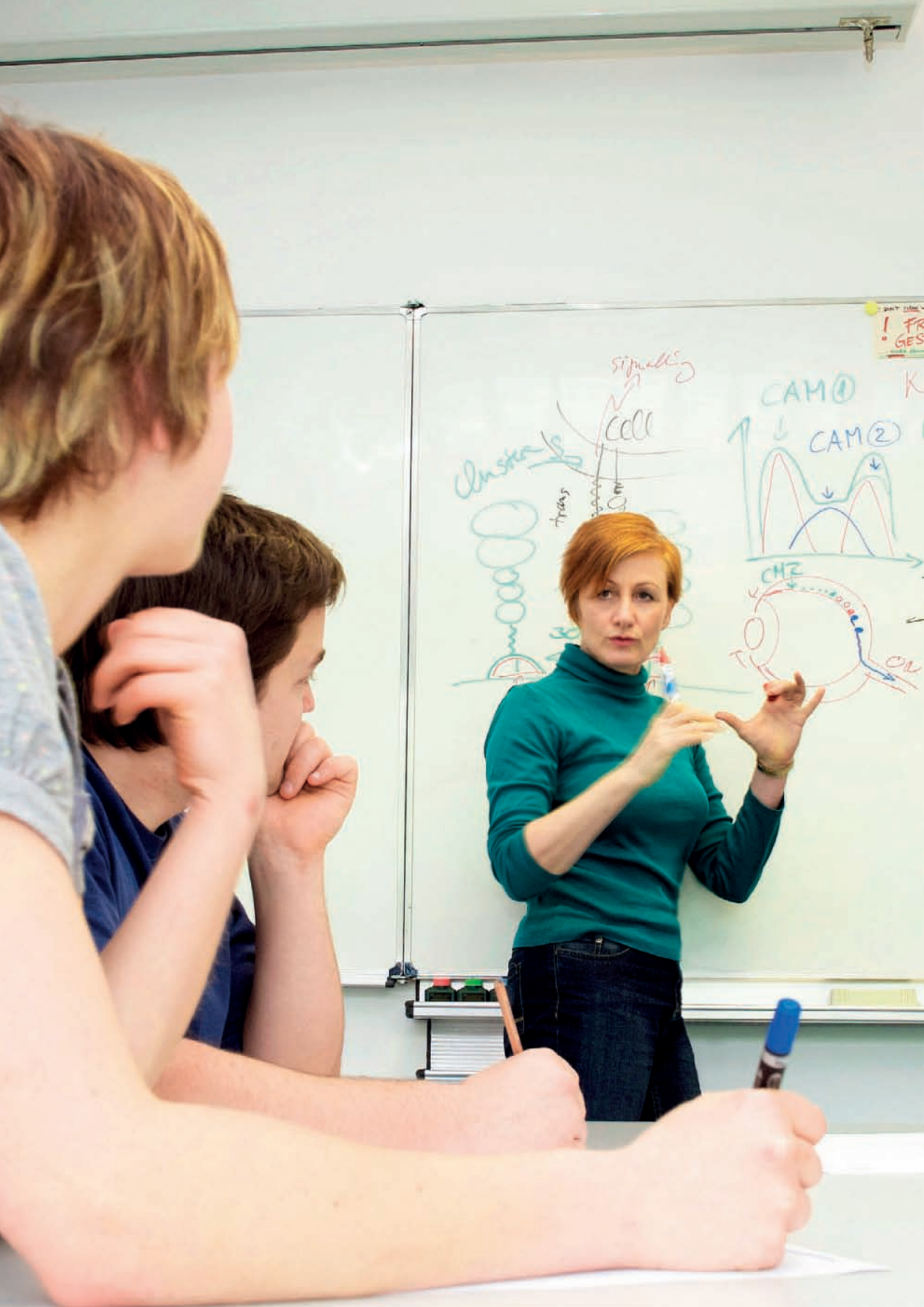
Lucas, M., Kenobi, K., Wangenheim, von, D., Voβ, U., Swarup, K., De Smet, I., Van Damme, D., Lawrence, T., Péret, B., Moscardi, E., Barbeau, D., Godin, G., Salt, D., Guyomarc'h, S., Stelzer, Ernst H. K., Maizel, A.*, Laplaze, L. and Bennett, M.J. (2013). Lateral root morphogenesis is dependent on the mechanical properties of the overlaying tissues. Proc. Natl. Acad. Sci. U.S.A. 110, 5229–5234.

Jouannet, V., Moreno, A.B., Elmayan, T., Vaucheret, H., Crespi, M.D., and Maizel, A.* (2012).
Cytoplasmic Arabidopsis AGO7 accumulates in membrane-associated siRNA bodies and is required for ta-siRNA biogenesis. Embo J. 31, 1704–1713.

Maizel, A.*, Wangenheim, von, D., Federici, F., Haseloff, J., and Stelzer, E.H.K. (2011). High resolution, live imaging of plant growth in near physiological bright conditions using light sheet fluorescence microscopy. Plant J. 68, 377–385.

Marin, E., Jouannet, V., Herz, A., Lokerse, A.S., Weijers, D., Vaucheret, H., Nussaume, L., Crespi, M.D., and Maizel, A.* (2010). miR390, Arabidopsis TAS3 tasiRNAs, and their AUXIN RESPONSE FACTOR targets define an autoregulatory network quantitatively regulating lateral root growth. Plant Cell 22, 1104–1117.

* corresponding author


CAM 1
CAM 2
CMZ

2.17 DEVELOPMENTAL NEUROBIOLOGY
PROF. DR. GABRIELE ELISABETH POLLERBERG

PROF. DR. GABRIELE ELISABETH POLLERBERG
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Fields of Interest
Cellular and molecular processes underlying growth and navigation of axons during development and regeneration of the vertebrate nervous system; cell adhesion molecules, signalling molecules, cytoskeletal molecules

Brief summary of work since 2009
We are studying the cellular and molecular processes underlying growth and navigation of axons during development and regeneration of the nervous system. Specifically, we analyse the roles of cell adhesion molecules of the immunoglobulin superfamily (IgSF-CAMs) and the intracellular signalling cascades they elicit. ALCAM is a small and unusual IgSF-CAM present in the plasma membrane of growing axons and their growth cones (i.e. the sensory structure at the axon tip). We could show that ALCAM is synthesized and degraded in the growth cone which fine-regulates its plasma membrane density and is crucial for axonal substrate preference. By use of ALCAM-biofunctionalized nano-patterns, we were could demonstrate that ALCAM *per se* is able to promote cell adhesion and axon growth (initial axon outgrowth as well as axon elongation). This triggered a study aiming at the application of nano-patterned ALCAM as a biomimetic implant-coat to enhance axonal regeneration. We could also show that ALCAM clustering activates the kinase Erk and now want to identify the triggers. Downstream targets of the intracellular signaling are microtubules and microtubule-associated protein (MAPs), and we could show that kinase Cdk5, its activator P35, and phosphatase PP2B play crucial roles in regulation of the phosphorylation state and thereby the MAP functions. Moreover, by analysing a MAP k.o. mouse, we could show the importance of MAPs for axonal navigation. Recently, we developed a technique to long-term image microtubules inside growth cones and are aiming at the quantitative analysis of microtubule dynamics during growth cone explorative behaviour and steering reactions.

Major contributions since 2009
During development and regeneration of the nervous system, neurons send out axons with a motile sensory structure at their tip, the growth cone. Interactions of proteins in the growth cone's plasma membrane with molecules in the environment trigger intracellular signal cascades that act on the cytoskeleton; this ensures appropriate steering reactions of the growth cone and ultimately the correct target-finding of the axon during nervous system development and regeneration. We study a subgroup of integral plasma membrane proteins of the growth cone, the cell adhesion molecules of the immunoglobulin superfamiliy (IgSF-CAMs), in particular ALCAM (previously also termed DM-GRASP). We could show that ALCAM plays an important role for the path-finding of axons, for example they fail to enter the optic nerve if ALCAM is blocked. We moreover demonstrated – for the first time for a CAM – that ALCAM is translated in growth cones (Thelen et al., 2012a). This

local translation is controlled by regulatory elements (CPEs) in ALCAM's mRNA untranslated region (3'UTR), depends on the kinase Erk; it is moreover a prerequisite for the ALCAM-induced enhancement of axon elongation as well as for the axonal preference of ALCAM-containing microlanes. We also showed that ALCAM's synthesis in the growth cone is counterbalanced by its endocytosis; both processes together fine-regulate the density of ALCAM in the plasma membrane and are moreover crucial for the capability of axons to prefer to grow on ALCAM.

To further elucidate the role of ALCAM spacing, we made use of nano-patterned substrates produced by the group of Prof. J. Spatz (PCI, Heidelberg) which allow for the presentation of ALCAM in highly defined distances (varying from 30-140 nm) as substrate for growing axons (Jährling et al., 2009; Thelen et al., 2012b). These studies revealed for the first time that ALCAM is able to promote cell attachment and axon growth correlating to the substrate ALCAM density in a roughly dose-dependent manner and without the assistance of other molecules. The 70 nm spacing of substrate ALCAM, however, unexpectedly turned out to be incompatible with efficient axon growth which induced us to model the spectrin-based cortical cytoskeleton structure/dimensions. This gave rise of the hypothesis that ALCAM is anchored to the cortical cytoskeleton which we could indeed prove by a variety of approaches recently. In addition, we also performed screens for novel intracellular (Y2H approach) and extracellular (cellular display) binding partners of ALCAM (Pollerberg et al., 2013). Among others, we could identify a guidance molecule as well as a cytokine-like molecule and are currently studying the functional role of these molecular interactions for axon growth. The findings obtained by using the nano-patterned ALCAM substrates also induced translational studies aiming at the development of a biomimetic implant presenting ALCAM in an optimized nano-spacing to enhance axonal regeneration; upon positive external evaluation, the University of Heidelberg supported an international patent application for such an implant and a second one is currently in preparation.

We also analyzed the CAM signaling elicited by inside the growth cone as well as the down-stream signaling targets, in particular microtubules and microtubule-associated proteins (MAPs) which regulate the dynamics of the microtubules. We could show that activation of ALCAM – by clustering this CAM in the plasma membrane – activates the kinase Erk (a series of other signaling components could be excluded). Moreover, MAP1B is regulated in its phosphorylation levels by kinase Cdk5, its activator P35, and phosphatase PP2B which together control the association of MAP1B to the microtubules and thereby their dynamic stability. In cooperation with the Almeida group (MPI of Psychiatry, Munic) it could be revealed that also stress activates Erk, GSK3, Cdk5, and P35 resulting in MAP hyperphosphorylation (Sotiropoulos et al., 2011). We are currently imaging microtubule dymanics using fluorescent fusion proteins which binding to their plus tips. We could to optimize various parameters so that high frequency time-lapse videos up to two hours can be obtained; thereby we are now able to long-term image microtubules in navigating axons of warm-blooded vertebrates for the first time worldwide. We are at the moment employing this optimized technique for a comprehensive quantitative study how microtubules inside the growth cone react to external stimuli and which signaling components are involved. To gain deeper insight into the role of MAPs for axonal navigation *in vivo*, we are also analysing k.o. mice and could find abnormalities with respect to several aspects of axonal growth, including axon elongation and navigation; the findings reveal for the first time a crucial role of the studied MAPs for these important axonal features underlying development and regeneration of the nervous system.

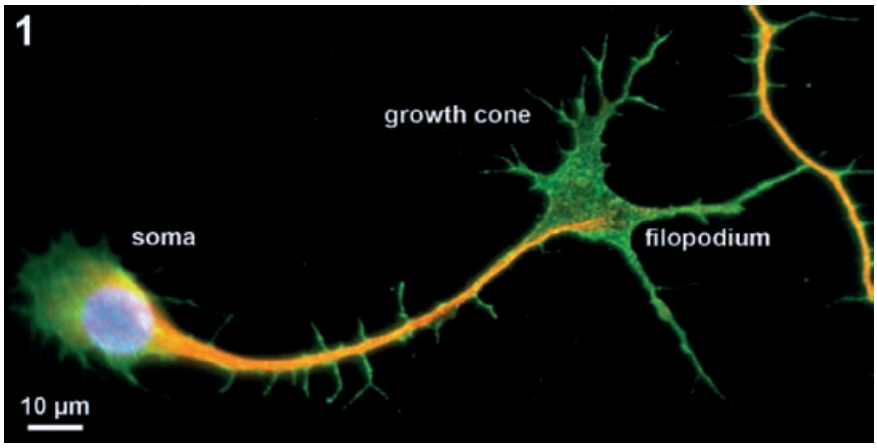

Figure 1
Neuron sending out an axon in vitro, with its growth cone contacting another axon.
Green: CAM-labelling visualizing the plasma membrane, red: microtubules, blue: nucleus.

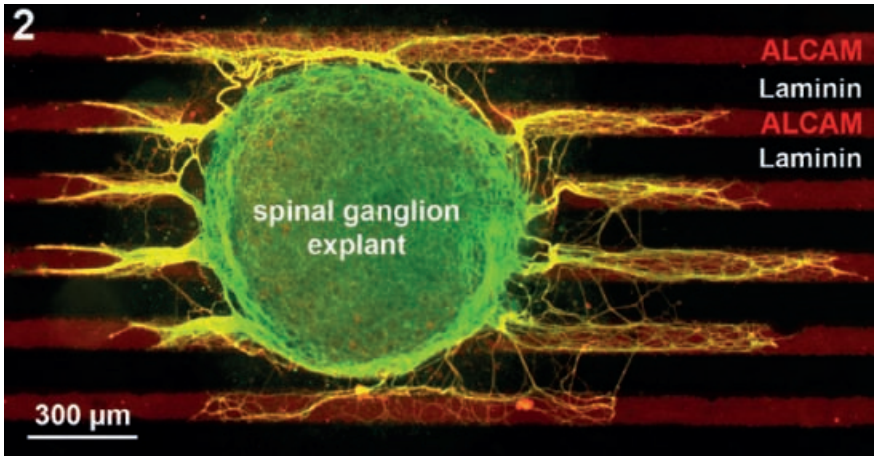

Figure 2
Spinal ganglion with its regenerating axons extending preferentially on ALCAM lanes.
Green: microtubules, red: ALCAM coated as micro-stripes on Laminin substrate.

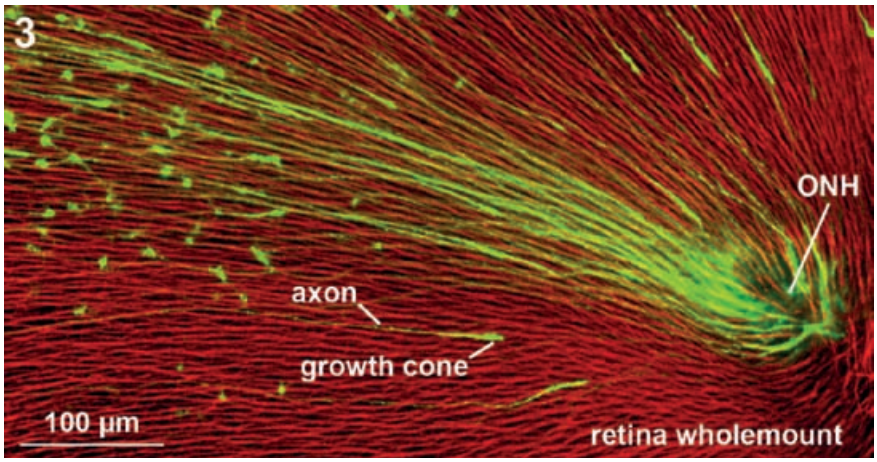

Figure 3
Retina ganglion cell (RGC) axons navigating to the optic nerve head (ONH).
Green: GFP-labelled RGC subpopulation, red: tubulin staining visualizing all RGC axons.

Planned research and new directions

On the basis of our previous findings and accumulated expertise, we will continue performing basic research on cellular and molecular level and study the roles of CAMs, signaling, and cytoskeleton in the growth cone. We will put, however, stronger emphasis on one aspect which turned out to be extremely motivating (from lab student to head of group): The potential application of our findings. We are eagerly aiming at a contribution to the improvement of the paradigms currently used to enhance axon regeneration. This could be achieved by optimisation of properties of CAMs presented in the plasma membrane and as a coated substrate. Moreover, also the tuning of components inside of the growth cone might contribute to a swift and well directed axon regeneration. For this, we will also further strengthen the already ongoing cooperations with clinicians, for example Prof. N. Weidner and Prof. A. Blesch (Heidelberg University Hospital). To get closer to the application situation with our experiments, we already supplemented our long-term model system, the embryonic avian retina, by a second one, the spinal ganglia of the embryonic and adult rodent. In detail, we will address the following questions by the approaches outlined below:

1. How does ALCAM exert its impact on axon formation, elongation, and navigation?
– Identification of the *in vivo* triggers of ALCAM's signaling and turnover in the growth cone
– Analysis and modification of ALCAM to optimize its axon promoting properties
– Study of the novel ALCAM interaction partners and impact on axon growth

2. What is the function of the CAM – cytoskeleton crosstalk in the growth cone?
– Identification of CAM-elicited intracellular signaling and impact on growth cone cytoskeleton
– Study of the physical interaction of CAM – cytoskeleton and impact on axonal functions
– Analysis of the impact of signaling and cytoskeletal components on the CAM properties

3. What is the role of the cytoskeletal components in the growth cone?
– Imaging of cytoskeletal dynamics during growth cone turning manoeuvres
– Quantitative analysis of the physical properties of the cytoskeleton upon external stimuli
– Study of the impact of more general factors such as age and stress on these parameters

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 5, Number of citations 2009-2013: 62
Thompson-Reuters h-index (2009-2013): 3; h-index (total): 20

Pollerberg G.E., Thelen K., Theiss M.O., Hochlehnert B.C. (2013) The role of cell adhesion molecules for navigating axons: Density matters. Mech Dev. 2012:10.1016 (Review)
Thelen K., Maier B., Faber M., Albrecht C., Fischer P., Pollerberg G.E. (2012a) Translation of cell adhesion molecule ALCAM in axonal growth cones: regulation and functional importance. J Cell Sci. Mar 15.;125 (Pt 4):1003-14.
Thelen K., Jährling S., Spatz J.P., Pollerberg G.E. (2012b) Depending on its nano-spacing, ALCAM promotes cell attachment and axon growth. PLoS ONE 7(12): e40493.
Sotiropoulos I., Catania C., Pinto L.G., Silva R., Pollerberg G.E., Takashima A., Sousa N., Almeida O.F. (2011). Stress acts cumulatively to precipitate Alzheimer's disease-like tau pathology and cognitive deficits. J Neurosci. 31(21):7840-7.
Jaehrling S., Thelen K., Wolfram T., Pollerberg G.E. (2009). Nanopatterns biofunctionalized with cell adhesion molecule DM-GRASP offered as cell substrate: spacing determines attachment and differentiation of neurons. Nano Lett. 9(12):4115-21.

2.18 MOLECULAR BIOLOGY OF CENTROSOMES AND CILIA
DR. GISLENE PEREIRA INDEPENDENT RESEARCH GROUP

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Fields of Interest
Cell cycle regulation, mitosis, mitotic checkpoints, asymmetric cell division, cytokinesis, centrosomes, ciliogenesis

Brief summary of work since 2009
Centrosomes of mammalian cells and spindle pole bodies (SPBs) of fungi are microtubules organising centres (MTOCs). Both MTOCs play a decisive role in the temporal and spatial organisation of the microtubules of the bipolar spindle, which segregates the sister chromatids to opposite poles of the cell during mitosis. Furthermore, specialised substructures of centrosomes, named centrioles, serve as 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 centrosome-associated components in cell cycle control and during the process of cilia formation. We are using *Saccharomyces cerevisiae* as a genetically tractable and versatile model system to investigate how SPB-associate signalling coordinates spindle orientation and cytokinesis with chromosome segregation. In addition, we are investigating how primary cilia formation is regulated in human cells.

Major contributions since 2009
Previously, others and we described the existence of the SPindle Orientation Checkpoint (SPOC) as a SPB-associated mitotic checkpoint in budding yeast and established that the SPOC was activated in response to errors in the orientation of the spindle in respect to the cell polarity axis (*reviewed in Caydasi et al. 2010 and 2012*). Any failure in this process can lead to chromosome instability – a factor directly associated with the development of several diseases including cancer. In addition, the orientation of the mitotic spindle with respect to a defined cell polarity axis is seminal for cell fate determination of asymmetrically dividing cells in a variety of organisms. Building on this work, my group identified novel SPOC components (*Caydasi et al. J Cell Biol 2010, Bertazzi et al. J Cell Biol 2011*), established by fluorescence resonance energy transfer (FRAP) analysis that SPOC activation changes the binding dynamics of components at SPBs (*Caydasi and Pereira Dev Cell 2009*) and developed quantitative analysis of SPOC signalling (*Caydasi et al. Mol Syst Biol 2012*). Our work helped define the current model of SPOC function.

My group also made important contributions to the molecular understanding of cytokinesis in yeast that is inhibited by the SPOC. The conserved nuclear Dbf2-related (NDR)-kinase complex Dbf2-Mob1 and Cdc14 phosphatase have an important yet ill-defined role in the coordination of mitotic exit with cytokinesis. Using a combination of genetic, microscopy and biochemical approaches, we showed that Dbf2 and Cdc14 play a critical role in

acto-myosin ring (AMR) contraction, cell separation, actin re-polarisation and vesicle trafficking (*Meitinger et al. J Cell Sci 2010*). We established that the conserved F-BAR-family protein Hof1 is a substrate of Dbf2-Mob1 and showed that phosphorylation promotes the relocation of Hof1 from septins to the AMR, where Hof1 controls AMR contraction and membrane ingression (*Meitinger et al. Genes Dev 2011 and Meitinger et al. Mol Biol Cell 2013*). In addition, we established novel substrates of the phosphatase Cdc14 involved in AMR contration (*Palani et al. J Cell Sci 2013*) and cell cleavage/ abscission (*Mancini Lombardi et al. Curr Biol 2013*). We proposed that these phosphorylation/dephosphorylation events under control of Dbf2-Mob1 and Cdc14, respectively, are key for the coordination of AMR contraction, membrane ingression and cell separation during cytokinesis (*rev. in Meitinger et al. 2012*). Recently, we established that a novel safeguard mechanism, under control of the protein Gps1, regulates the conserved Rho GTPases, Rho1 and Cdc42. We demonstrated that Gps1 has a dual function; it maintains Rho1 signaling at the bud neck to promote cell separation whereas it inhibits Cdc42 activation to block daughter cell regrowth at the same site that cytokinesis had occurred (*Meitinger et al. Plos Biol 2013*).

In addition, my group contributed to the molecular understanding of cilia biogenesis. Cilia are evolutionary conserved microtubule based organelles, which play essential functions in embryonic development as well as in tissue homeostasis in adulthood. Although several advances were made towards the identification of structural components of the cilia, the knowledge of ciliogenesis is still fragmented and lacks a molecular understanding on how components come together to initiate and maintain the cilium. To understand how cilia biogenesis is regulated, we performed a genome wide siRNA based screen to identify kinases involved in cilia formation. We linked the function of 30 novel kinases with the control of ciliogenesis. Of those, we characterised the function of the microtubule affinity regulating kinase 4 (MARK4) using a combination of biochemistry, fluorescence- and electron-microscopy analysis. We established that MARK4 localises at the basal body and promotes axoneme extension most likely through phosphorylation of the mother centriole appendage protein ODF2 (*Kuhns et al. J Cell Biol 2013*).

Furthermore, we investigated the molecular function of the distal appendage protein, Cep164, during ciliogenesis. We showed that Cep164 is essential to initiate ciliary membrane biogenesis at the mother centriole. We identified components of the Rab-family of GTPases, Rab8 and its activator Rabin8, to interact with Cep164 both in vivo and in vitro. Our work indicated that Cep164 recruits Rabin8 to the mother centriole, which in turns activates the GTPase Rab8 to initiate vesicle fusion events (*Schmidt et al. J Cell Biol 2012*).

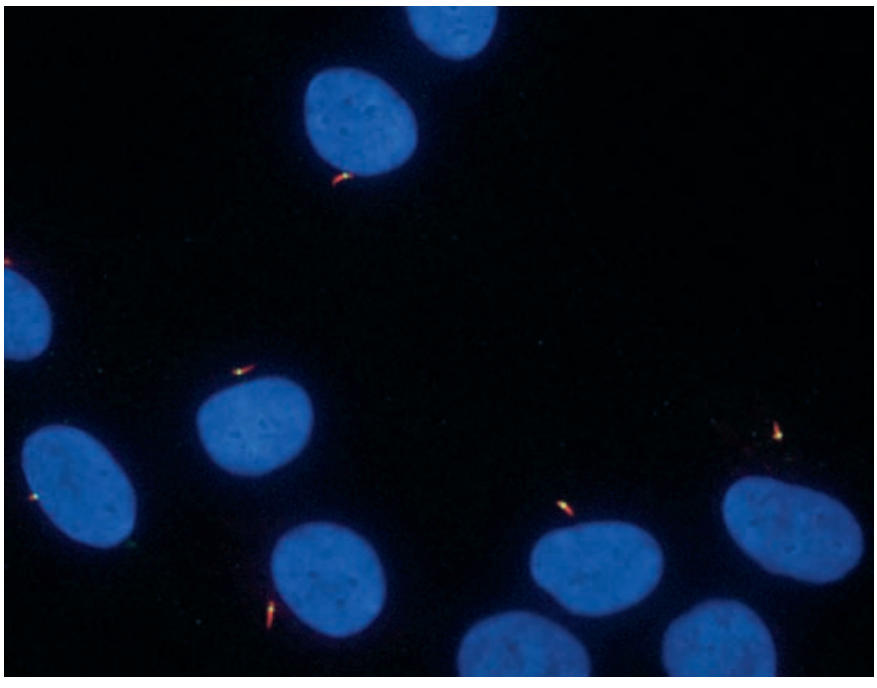

Figure 1
Ciliated human retina pigment epithelial (RPE) cells in culture. Ciliary axoneme is labelled in red, the basal body in green and the DNA in blue.

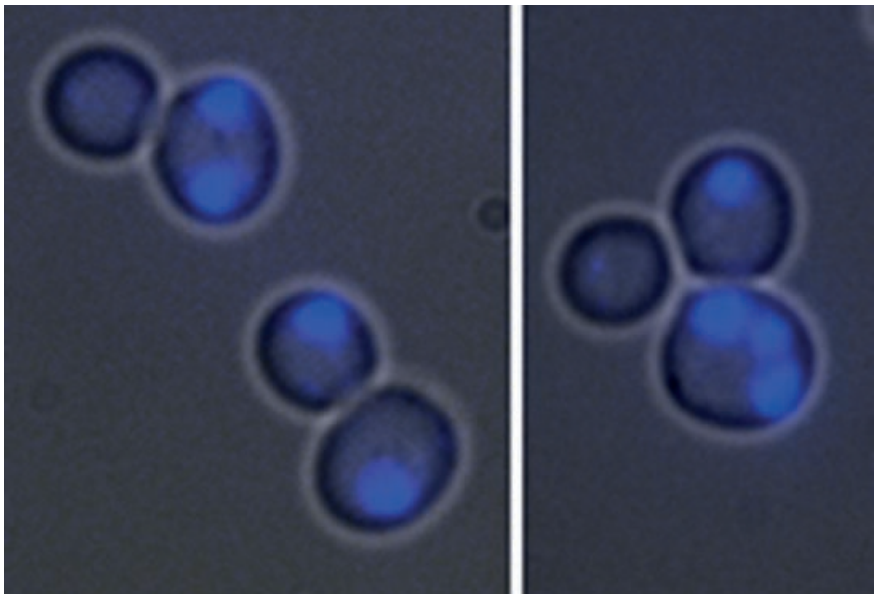

Figure 2
Budding yeast cells with showing normal chromosome Segregation (mother and daughter cells with blue labelled DNA masses) and chromosome mis-segregation (two or more Blue-labelled DNA masses in one cell compartment).

Planned research and new directions

The precise positioning of the cell division plane (which is dictated by the orientation of the mitotic spindle in respect to the cell polarity axis) and the presence of cilia are two factors critical for correct animal development and tissue morphogenesis. The centrosomes play a critical role in both processes and our major aims are to understand how spindle/centrosome positioning influence cell cycle progression in mitotically dividing cells and how centrosomes give rise to the basal body in quiescent cells.

In a short term, we will continue taking advantage of yeast genetics and available tools to further characterise the SPOC. In a mid to long-term, we plan to focus our studies mostly on the investigation of the role of centrosomes in cell cycle regulation and ciliogenesis in mammalian cells. Shortly, the planned research and future directions in the lab are:

– Investigation of compartment specific SPOC complex formation on a single cell basis in living cells using advance microscopy based methods.
– Analysis of the molecular link between SPOC and cell polarity establishment.
– Investigation of SPOC-like mechanisms in higher eukaryotes.
– Study the role of appendage components in cilia membrane biogenesis.
– Investigate how ciliogenesis is regulated by the novel protein kinases identified in our siRNA-based screen.

Selected publications since 2009

Number of peer-reviewed articles (2009-2014): 16; number of citations (2009-2013): 214. h-index (2009-2013): 9; total h-index: 17 (according to Thomson Reuters).

Caydasi, A.K., and Pereira, G. (2009). Spindle alignment regulates the dynamic association of checkpoint proteins with yeast spindle pole bodies. Dev Cell *16*, 146-156.
Meitinger, F., Boehm, M.E., Hofmann, A., Hub, B., Zentgraf, H., Lehmann, W.D., and Pereira, G. (2011). Phosphorylation-dependent regulation of the F-BAR protein Hof1 during cytokinesis. Genes Dev *25*, 875-888.
Schmidt, K.N., Kuhns, S., Neuner, A., Hub, B., Zentgraf, H., and Pereira, G. (2012). Cep164 mediates vesicular docking to the mother centriole during early steps of ciliogenesis. J Cell Biol *199*, 1083-1101.
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.
Meitinger, F., Richter, H., Heisel, S., Hub, B., Seufert, W., and Pereira, G. (2013). A safeguard mechanism regulates Rho GTPases to coordinate cytokinesis with the establishment of cell polarity. PLoS biology *11*, e1001495.

2.19 PLANT MOLECULAR PHYSIOLOGY
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Fields of Interest
Metabolic switches/adaptation in response to environmental challenges and developmental cues: e. g. sucrose & fructan metabolism, glutathione biosynthesis, secondary metabolism. Arabidopsis as model, but focus on crop plants (biotechnological potential)

Brief summary of work since 2009
Plants are sessile and make the best out of it. Consequently, there is a continuous challenge for smart trade-offs between development (i.e. growth, vegetative and/or sexual propagation) and adaptation to environmental stress (i.e. biotic and/or abiotic). Adding to dynamic reprogramming of gene expression, rapid post-translational regulatory mechanisms allow fine tuning of metabolic fluxes. Our research addresses metabolic adaptation processes at transcriptional and post-translational level, respectively, with a focus on the following topics: Post-translational regulation of sucrose hydrolysis, regulation of fructan metabolism (for details see report from S. Greiner), post-translational regulation of glutathione biosynthesis, and regulation of plant secondary metabolism. – Post-translational regulation of cell wall and vacuolar invertases by inhibitor proteins is currently being explored in Arabidopsis KO mutants for invertase inhibitors. During seed germination, complex formation (1:1) of a cell wall invertase with its corresponding inhibitor allows the fine tuning of sucrose hydrolysis and sugar signaling in response to hormonal cues (e. g. abscisic acid). – The redox-active tripeptide glutathione is the major cellular redox buffer, but may also modify proteins via glutathionylation. The enzyme γ-glutamylcysteine ligase (GCL) catalyzes the first step of glutathione biosynthesis and is subject to post-translational controls. We explore these regulatory mechanisms via structure-function studies. Furthermore, we have generated transgenic plants lacking these regulatory mechanisms and study their responses to abiotic and biotic stress. – Plant secondary metabolism depends on coordinate transcriptional regulation of complex metabolic pathways but also on appropriate precursor flux from primary metabolism. We attempt to decipher the underlying controls, which limit secondary metabolite accumulation.

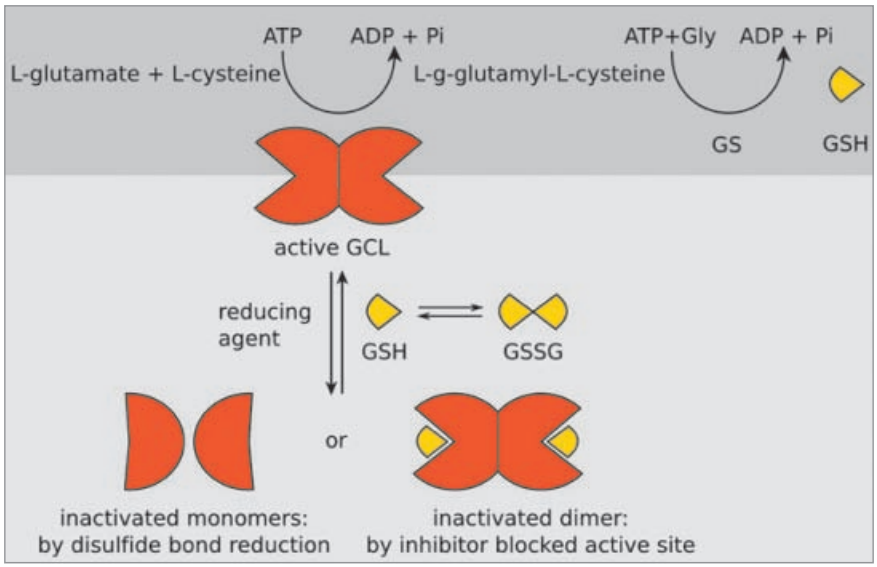


Figure 1
GSH synthesis in plants The two step synthesis of GSH by GCL and GSHS is shown together with the suggested posttranslational regulation of GCL: reducing agents inactivate GCL via disruption of the intramolecular disulfide bond CC2 in the dimer interface, leading to the disruption of the active GCL dimer. Alternatively, GSH binds specifically to the GCL active site and acts as a feedback inhibitor.

Major contributions since 2009

During the period of October 2010 till September 2013, I joined the Rectorate as Prorector for Research and Structure. During this time, I contributed to the Strategic Concept of Heidelberg University for the second phase of the Excellence Initiative. Therefore, the credit for the research done during this period goes to my then post docs Steffen Greiner, Sebastian Wolf and Tatjana Peskan-Berghöfer. Furthermore, the continued collaboration with Klaus Scheffzeck and Jochen Bogs (guest professors at COS and co-supervisors for PhD students) has been a strong support.

In plants, sucrose hydrolysis in the cell wall and/or the vacuole by cell wall and vacuolar invertases impacts on source-sink relationships and affects cell division and cell expansion by providing hexoses for metabolism, by impacting on osmoregulation, and via sugar signalling. After the seminal paper of Greiner et al. (1998), describing the first cloning of a plant invertase inhibitor, it took more than ten years until the first structure of a complex of cell wall invertase with its inhibitor was solved (Hothorn et al., 2010). Despite this progress at the structural level, the *in vivo* functions of invertase inhibitors have remained largely elusive. We have now generated KO-mutants for invertase inhibitors in Arabidopsis. This has led to the discovery of a molecular switch, consisting of a cell wall invertase isoform and its inhibitor, which plays a role in early seed germination. As expression of both, the target invertase and its inhibitor, is under differential hormonal control, and since apolastic pH and concentration of sucrose impinge on complex formation, we speculate that the dynamic regulation of complex formation allows adaptation of apoplastic sucrose hydrolysis to endogenous and environmental cues (Tao Su, 2014; Su et al., manuscript in preparation).

After solving the first structure of a plant γ-glutamylcysteine ligase in collaboration with the group of Klaus Scheffzeck at the EMBL (Hothorn et al., 2006), and after demonstrating that the reversible redox-mediated dimerization, which causes enzyme activation, is a unique feature of plants (Gromes et al., 2008), we realized that due to dual regulation (i.e. feedback control by glutathione and redox-regulation [Lenherr et al., 2014 submitted]), glutathione accumulation in planta is tightly controlled. In an attempt to overrule these controls, we have transformed tobacco with a bifunctional enzyme from *Streptococcus thermophilus*, which catalyzes the entire glutathione synthesis reaction. The StGCL-GS enzyme is completely redox-insensitive and largely insensitive to feedback inhibition. Transformed tobacco plants accumulated up to 20-fold more glutathione than wild type and displayed improved tolerance towards oxidative stress (Liedschulte et al. 2010). Surprisingly, StGCL-GS expressing plants were phenotypically not affected. This prompted us to explore their cellular redox status at cellular level with the GRX1-RoGFP2 sensor. Unexpectedly, the cytosolic redox potential of StGCL-GS expressing plants revealed a modest oxidative shift. Furthermore, these plants showed constitutively up-regulated expression of defense genes and higher levels of activated MAP kinases. Monitoring their

response to adapted and non-adapted pathovars from *Pseudomonas syringae*, revealed that due to the high glutathione content, transgenic plants were primed for defense (Curcin et al., 2014, *submitted*).

Our research on control mechanisms involved in the regulation of secondary metabolism has addressed two questions: Does precursor flow from primary metabolism limit the extent of secondary metabolite accumulation? How are the pathways for flavonoid and stilbene biosynthesis differentially regulated by different sets of R2R3MYB-type transcription factors? – In a collaborative study with the group of Zu Yuangang (Harbin, P.R.China), we have explored the control mechanisms which affect the expression of the enzymes in the plastidic MEP pathway in *Catharanthus roseus*. In this species, the MEP pathway provides the building blocks for the monoterpene moiety of the monoterpene indolealkaloids, a group of medically important secondary metabolites. Using specific inhibitors to enzymes of the MEP pathway, we could unravel novel aspects of MEP pathway regulation (Han et al., 2013). – The discovery of key amino acids in R2R3MYB transcriptions factors, which determine promoter target specificity (Heppel et al., 2013), and the identification of R2R3MYB transcription factors specifically regulating stilbene synthesis (Höll et al., 2013), are the fruit of a collaboration with Jochen Bogs (Bingen), who deserves most of the credit.

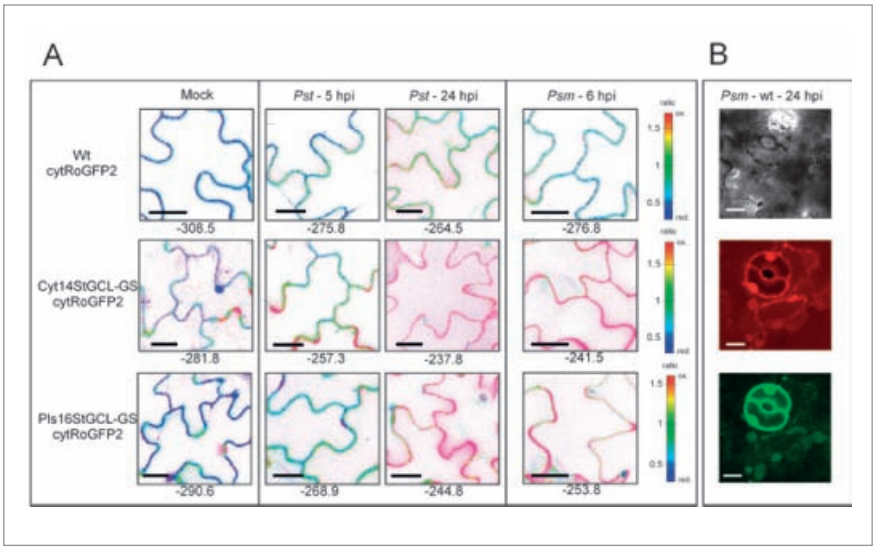


Figure 2
Dynamics of cellular redox state in epidermal cells of wild type and high glutathione lines in response to challenge with adapted (*Pst*) and non-adapted (*Psm*) pathovars (A), redox state of cyt-roGFP2 ranges from oxidized (ox, red) to reduced (red, blue). Wild type and transgenic lines were analyzed 6 (*Pst/Psm*) and 24 hours (*Pst*) post infection, respectively. (B), epidermis of Psm-infected plants 24 hours post infection, revealing loss of fluorescence except for strong signal detected in fully open stomata. From top to bottom: transmitted light microscopy image, in red excitation 405 nm/emission 500-530 nm, in green excitation 488 nm/emission 500-530 nm. Scale bar indicates 10 µm.

Planned research and new directions

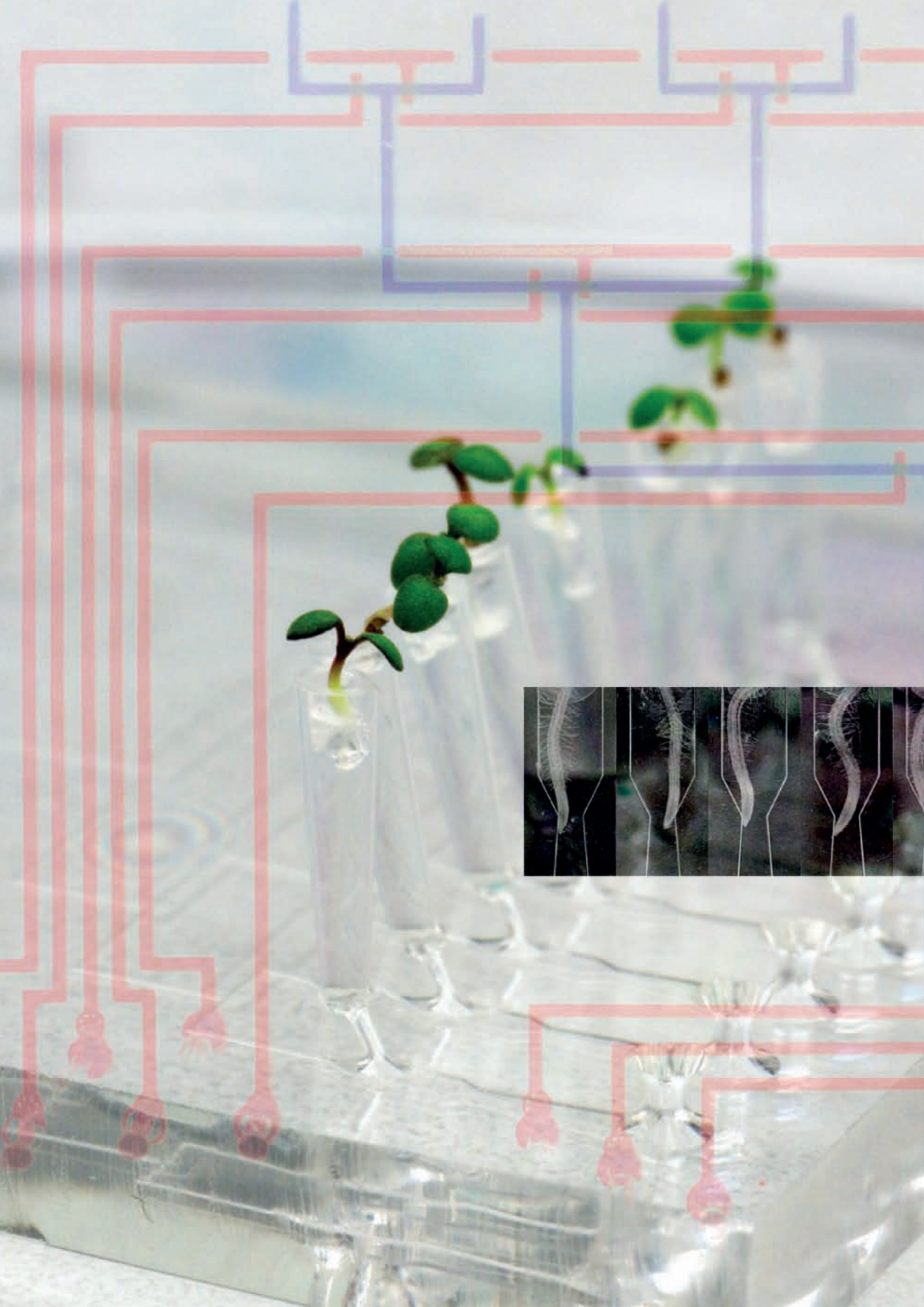
Being part of a DFG priority program on the »Dynamics of thiol-based redox switches in cellular physiology«, we will, in close collaboration with Rüdiger Hell and Markus Wirtz, explore the *in vivo* function of the thiol switch in the plant γ-glutamylcysteine ligase enzyme. First, we aim to decipher via structure-function relationship studies the mechanistic details of its operation, which involves formation of an intramolecular disulfide bridge in both monomers, followed by dimerization via a zipper-like interface. Second, we will complement a γ-glutamylcysteine ligase KO line with engineered redox-insensitive variants of the enzyme and monitor plant growth and stress adaptation to evaluate the physiological significance of this molecular switch. The results will show which role this thiol switch plays in the plants strategic decision making between growth and stress adaptation (perhaps in conjunction with other thiol switches, e. g. in APS reductase). Related to this project, we will explore in more detail the glutathione-based priming for defense against pathogens by studying the link between cellular redox control and the regulation of MAP kinase cascades.

A second line of research will focus on the *in vivo* function of the post-translational control of cell wall invertase by its proteinaceous inhibitor. Here we aim to decipher the cellular controls which regulate complex formation between enzyme and its inhibitor, including differential regulation of gene expression via plant hormones. *In vivo* studies will be complemented by in vitro studies with recombinant proteins, including variants generated by site-directed mutagenesis. The goal is to unravel the dynamic nature of this invertase activity switch with respect to its role in metabolism and sugar signaling during seed filling and seed germination.

A third line of research will address regulatory aspects of phenylpropanoid-based secondary metabolism. First, we will explore to what extent »refractory periods« limit the accumulation of flavonoids after multiple plant stimulation (UV-B). Refractory periods may result from limitations in the signaling pathway(s) and/or may be the consequence of limitations set by precursor flow from primary metabolism. The ultimate goal is to maximize secondary metabolite accumulation by driving the plant into a gauntlet of multiple stress exposures. Second, we will elucidate the transcription factor networks that regulate flavonoid and lignin biosynthesis, respectively, in *Miscanthus* species and hybrids. Here, the goal is to elucidate how environmental cues and genetic disposition in different genotypes interact to direct metabolic flux towards different endproducts.

Selected publications since 2009
Number of peer-reviewed articles 2009-2013: 12, number of citations 2009-2013: 88, h-index (2009-2013): 6, total h-index: 28 (according to Thomson Reuters).

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
Han M, Heppel SC, Su T, Bogs J, Zu Y, An Z, Rausch T (2013) Enzyme Inhibitor Studies Reveal Complex Control of Methyl-D-Erythritol 4-Phosphate (MEP) Pathway Enzyme Expression in *Catharanthus roseus*. PLoS ONE 8(5): e62467. DOI:10.1371/journal.pone.0062467
Hsu FC, Wirtz M, Heppel SC, Bogs J, Krämer U, Khan MS, Bub A, Hell R, Rausch T (2010) Generation of Se-fortified broccoli as functional food: Impact of Se fertilization on S metabolism. Plant, Cell & Environment 34(2):192-207
Liedschulte V, Wachter A, An Zhigang, Rausch T (2010) Exploiting plants for glutathione (GSH) production: Uncoupling GSH synthesis from cellular controls results in unprecedented GSH accumulation. Plant Biotech J 8(7): 807-820
Kusch U, Greiner S, Steininger H, Meyer A, Corbière-Divaille, Harms K, Rausch T (2009) Dissecting the regulation of fructan metabolism in chicory (Cichorium intybus) hairy roots. New Phytol 184: 127-140



PROJECT LEADER: DR. STEFFEN GREINER

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02/04/1969, Frankfurt/Main

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Fields of Interest
Sucrose and fructan metabolism,
Methane production in plants, Cell wall carbohydrates

Brief summary of work since 2009
About 15 % of all higher plants accumulate fructans. In plants, fructans perform multiple physiological functions, 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, in particular with respect to the postulated physiological functions (e. g. transient carbohydrate storage, membrane stabilization under stress exposure, role in osmotic regulation), and (ii) to provide relevant information for chicory breeding towards improved inulin content and composition.

Major contributions since 2009
The enzymes for fructan synthesis and degradation are evolutionarily related to cell wall and vacuolar invertases. Based on our experience in post-translational regulation of invertase inhibitors, we have addressed the question whether fructan-active enzymes (FAZYs) are also under control of inhibitor proteins. In a study on the commercially important chicory (*C. intybus*), we could demonstrate that unlike their invertase counterparts, FAZYs are not regulated by inhibitory proteins and are insensitive to the plants own invertase inhibitors (Kusch et al., 2009a). Furthermore, in a chicory hairy root culture system, the complex control of fructan synthesis and degradation in response to endogenous (hormonal, e. g. via abscisic acid) and environmental cues (e. g. N-nutrition, cold treatment, etc.) has been explored (Kusch et al., 2009b). For this industry-funded research project, transcriptome sequencing has provided a valuable source for future studies.

In another project we work on plant cell growth largely controlled by the balance between turgor pressure and the extensibility of the cell wall. Here we focus on homogalacturonan demethylesterification catalyzed by the ubiquitous enzyme pectin methylesterase (PME) controlled by endogenous PME inhibitors as a growth control module. In particular, we analyzed the N-terminal processing of the PME-enzyme and the impact of this regulation on subcellular localization and activity (Wolf et al., 2009)

In addition, we concentrate on methane (CH_4) formation in plants, a fact that has caused much controversy and debate within the scientific community over recent years. We demonstrated that CH4 formation occurs in plant cells under sterile conditions. While CH4 production is very low under normal growth conditions, these could be increased by two orders of magnitude when sodium azide, a compound known to disrupt electron transport flow at the cytochrome c oxidase (complex IV) in plant mitochondria, was added to the cell cultures. Thus, we provided new insight on non-microbial CH_4 formation from living plants particularly under abiotic stress conditions that might affect the electron transport flow at the cytochrome c oxidase in plant mitochondria (Wishkerman et al., 2011). Very recently we could show that S-methyl groups of organosulphur compounds are efficiently converted into methane in an *in vitro* system. This conversion seems to happen – though less efficiently – *in vivo* as well (Althoff et al., 2014)

Planned research and new directions

Future research will focus on the transcriptional control of FAZY gene expression, as recent work has shown that in wheat the expression of enzymes for fructan biosynthesis is controlled by a R2R3-MYB-type transcription factor. First chicory R2R3-MYB factor candidates have already been identified. Apart from transcriptional controls, post-transcriptional control by differential polysome-recruitment of FAZY mRNAs will also be addressed. Thus, 1-SST, the first enzyme for inulin synthesis, shows substantial differences between mRNA expression and 1-SST protein expression (the latter correlating with enzyme activity). Since in the chicory taproot, a gradient of O_2 builds up with significant hypoxia in the central part of this bulky organ, we hypothesize that hypoxia-mediated differential mRNA recruitment to polysomes may be the molecular basis for the observed discrepancy between 1-SST mRNA and protein. Again, results from this research will provide fundamentally new insight into fructan metabolism and may open new routes for biotechnology.

In the methane project we will try to set up an *in vitro* model system to study methane formation under aerobic conditions. This will hopefully lead to the identification of candidate precursor compounds for methane formation in live plants, which will then be tested in the *in vivo* system.

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 7, number of citations 2009-2013: 88, h-index (2009-2013): 6, total h-index: 16 (according to Thomson Reuters).

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. Nat Commun 5.

Wolf, S., Mravec, J., Greiner, S., Mouille, G., and Hofte, H. (2012). Plant cell wall homeostasis is mediated by brassinosteroid feedback signaling. Curr Biol 22, 1732-1737.

Wishkerman, A., Greiner, S., Ghyczy, M., Boros, M., Rausch, T., Lenhart, K., Keppler, F. (2011) Enhanced formation of methane in plant cell cultures by inhibition of cytochrome c oxidase. Plant Cell and Environment 34: 457-464

Kusch, U., Harms, K., Rausch, T., Greiner, S. (2009) Inhibitors of plant invertases do not affect the structurally related enzymes of fructan metabolism. New Phytol 181: 601-612

Wolf, S., Rausch, T., Greiner, S. (2009). The N-terminal pro region mediates retention of unprocessed type-I PME in the Golgi apparatus. Plant J 58, 361-375.

2.20 PLANT DEVELOPMENTAL BIOLOGY
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Fields of Interest
pH- and ion-homeostasis, proton-pumps, membrane transport, protein trafficking, turgor control, genetically encoded sensors

Brief summary of work since 2009
Compartmentation allows the simultaneous occurrence of biochemical processes in different reaction spaces and necessitates the exchange of material via membrane transport or vesicular trafficking. 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 not only energizes secondary active transport but also 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. Building on previous work in which we demonstrated that the subcellular distribution of the Arabidopsis V-ATPase is determined by the isoform of the membrane-integral subunit VHA-a, we were able to show that V-ATPase activity is strictly required for the dynamics and identity of the trans-Golgi network/early endosome, the central sorting hub for protein trafficking in higher plants. In contrast, plants lacking tonoplast V-ATPase show severely limited growth and disturbed ion homeostasis but are viable. We are beginning to see how the V-ATPase is integrated in the diverse cellular and metabolic networks and many tools including genetically encoded sensors for pH and Ca^{2+}, FRET-FLIM based protein-protein interaction studies and chemical genetics screens have been established that will allow us to dissect the underlying molecular mechanisms.

Major contributions since 2009

The TGN/EE – the major hub for protein sorting in plants

As a consequence of their sessile lifestyle, plants have to be able to rapidly adapt their functional interfaces and their repertoire of plasma membrane (PM) proteins is thus constantly adjusted to suit the plant's needs. By regulating the PM density of receptors for environmental and developmental signals as well as transporters for ions, nutrients, and hormones through the secretory and endocytic pathways, plants can effectively adapt to new environmental conditions. Despite the fact that the importance of regulated secretory and endocytic trafficking was becoming increasingly clear; our knowledge of the compartments and molecular machinery involved was still fragmentary. Based on the identification of the trans-Golgi network/early endosome (TGN/EE) as the central hub for trafficking in plants, we have made important contributions to charting the plant endomembrane system. We provided evidence that both endocytic and secretory cargo pass through the trans-Golgi network/early endosome (TGN/EE) and demonstrated that cargo in late endosomes/multivesicular bodies is destined for vacuolar degradation. Using spinning-disc confocal microscopy, we could show that TGN/EEs move independently and are only transiently associated with Golgi stacks (Viotti et al., 2010). Moreover, we found that multivesicular bodies/late endosomes (MVBs/LEs) originate from the TGN/EE via a maturation process that requires the action of the V-ATPase and the ESCRT complex for the formation of intraluminal vesicles (Scheuring et al., 2011).

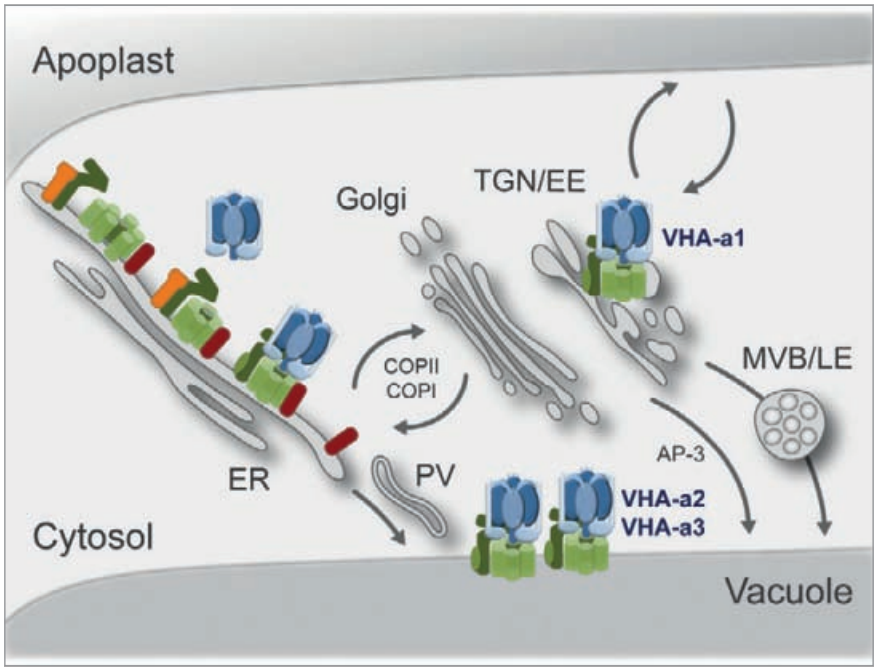


Figure 1
Assembly, trafficking and function of the plant V-ATPase. Assembly of the V0 subcomplex (green) takes place in the endoplasmic reticulum (ER) and requires dedicated assembyl factors (red). The ifinal destination oft he holocomplex is determined by the presence of the individual isoforms of subunit VHA-a. V- ATPase complexes containing VHA-a1 localize to the trans-Golgi network/early endosome (TGN/EE), whereas holoenzymes containing VHA-a2 or VHA-a3 are targeted to the vacuolar membrane. WHereas VHA-a1 complexes leave the ER via COPII-vesicles, VHA-a2 and VHA-a3 complexes are sorted into provacuoles (PV) that can fuse with pre-existing vacuoles. Function of the TGN/EE localized V-ATPase is essential for endocytic and secretory traffic. V-ATPase complexes in the tonoplast are required for membrane energization and vacuolar acidification and have been demonstrated to be necessary for vacuolar ion homeostasis.

Vacuoles – pumping up the plant volume

The productivity of higher plants, our major source of food and energy, is linked to their ability to buffer changes in the concentrations of essential and toxic ions. Transport across the tonoplast is energized not only by the V-ATPase but also by the vacuolar H+-pyrophosphatase (V-PPase), however, their functional relation and relative contributions to ion storage and detoxification remained unclear. By identifying an Arabidopsis mutant in which energization of vacuolar transport solely relies on the activity of the V-PPase we were able to elucidate the contribution of the V-ATPase. The *vha-a2 vha-a3* mutant, which lacks the two tonoplast-localized isoforms of VHA-a, was found to be viable despite a major metabolic shift to nitrate assimilation caused by its reduced vacuolar storage capacity Unexpectedly, salt tolerance and accumulation are not affected in the *vha-a2 vha-a3* double mutant whereas reduction of V-ATPase activity in the trans-Golgi network/early endosome (TGN/EE) leads to increased salt sensitivity (Krebs et al., 2010). This result pointed to an important and so far neglected role of the plant endosomal V-ATPase in pH-

and ion homeostasis that was confirmed in our analysis of a triple mutant lacking both V-ATPase and V-PPase ((Schumacher and Krebs, 2010), Kriegel et al., in preparation) and by in vivo pH-measurements in the TGN/EE using a genetically encoded pH-sensor (Scholl et al., in preparation). Despite the essential function of vacuoles in cell growth, storage, and detoxification, knowledge about the mechanisms underlying their biogenesis and associated protein trafficking pathways remains limited. By combining genetic and pharmacological approaches with live cell and electron microscopy we could recently show that biogenesis of vacuoles as well as trafficking of sterols and of the V-ATPase and V-PPase as the two major tonoplast proteins, occurs independently of endoplasmic reticulum (ER)–Golgi and post-Golgi trafficking. Instead, both pumps were found in provacuoles that, although structurally resembling autophagosomes, are formed independently of the core autophagy machinery (Viotti et al., 2013).

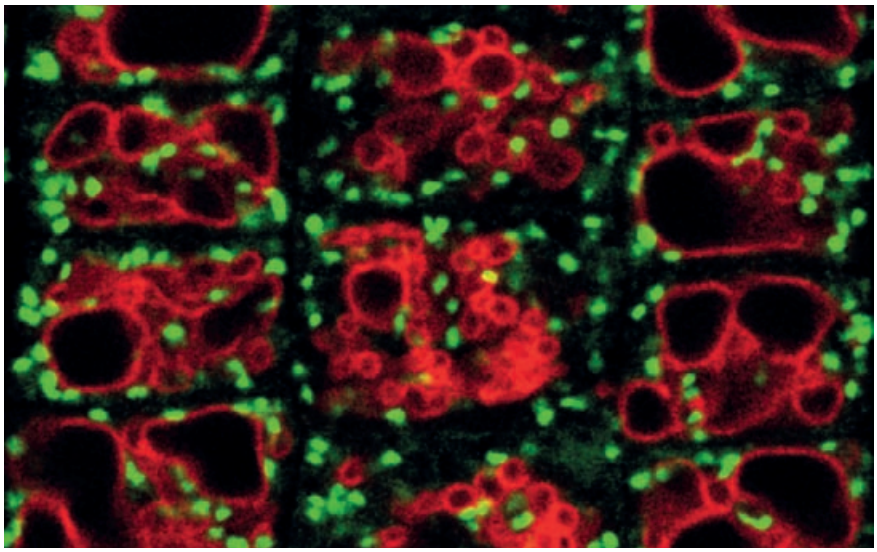


Figure 2
Cells of a transgenic line co-expressing VHA-a1-GFP and VHA-a3-mRFP illustrating the differential localization of the V-ATPase in Arabidopsis.

CAMeleons and GECOs – recording Ca^{2+}-signatures in plants

Temporally and spatially defined calcium signatures are integral parts of numerous plant signalling pathways. Monitoring calcium dynamics with high spatial and temporal resolution is therefore critically important to understand how this ubiquitous second messenger can control diverse cellular responses. Yellow cameleons (YCs) are fluorescence resonance energy transfer (FRET)-based genetically encoded Ca^{2+}-sensors that provide a powerful tool to monitor the spatio-temporal dynamics of Ca^{2+}- fluxes and we developed an advanced set of tools that allows live cell Ca^{2+}- imaging in different subcellular compartments. Using this toolkit, we identified temporally distinct responses to external ATP at the plasma membrane, in the cytosol and in the nucleus of neighbouring root cells (Krebs et al., 2011). However, in the course of our studies it became increasingly clear that the dynamic range of YC3.6 was not sufficient to detect Ca^{2+} changes that were faithfully and robustly reported by other methods an we thus switched to the so-called GECOs, a novel class of intensity based Ca^{2+} sensors with a very high dynamic range and available in different colours thus opening the door to multicompartment or multiparameter imaging (Krebs et al., unpublished).

Planned research and new directions
In the past years we have made several important contributions highlighting that the plant endomembrane system is characterized by the continuous generation and consumption of compartments that are connected by a dynamic flow of material. Based on these findings we are developing an integrated concept of membrane transport and trafficking and are currently focusing our efforts in the following areas:

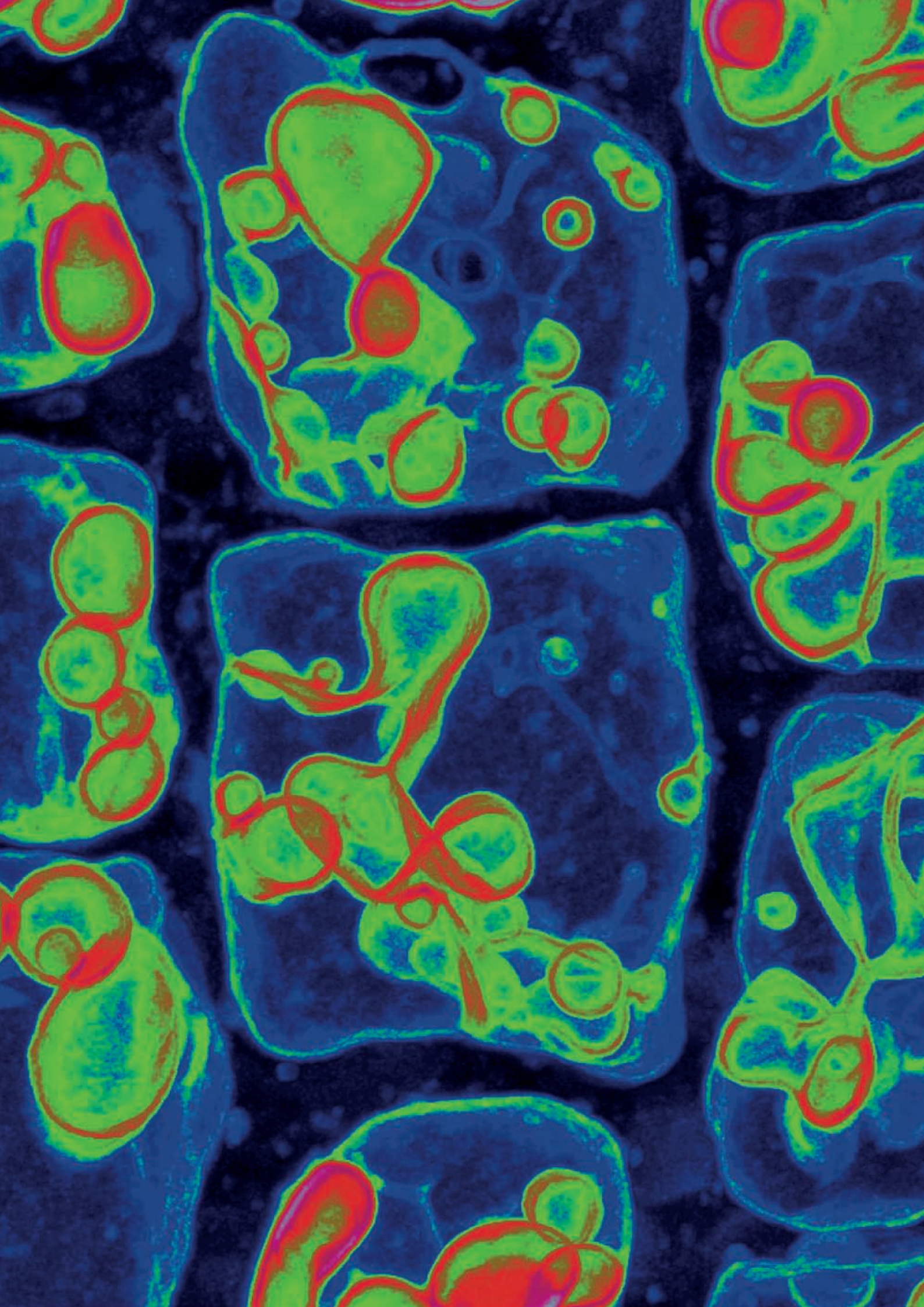
1. What are the molecular mechanisms underlying vacuole biogenesis?
The fact that the ER is a compartment enclosed by a continuous membrane makes it easy to overlook that it actually is an assembly of several, distinct membrane domains that execute diverse functions. Our findings have added vacuole biogenesis to this list and our goal is now to identify the lipids and proteins present in the »vacuole-associated membrane (VAM)«. We will make use of the fact that with V-ATPase and V-PPase, the two most abundant tonoplast proteins reach the tonoplast via the VAM-pathway and we will employ both unbiased genetic and biochemical approaches as well as targeted approaches to identify the underlying molecular mechanism.

2. Modelling of cellular pH- and Ca^{2+} -homeostasis
pH and Ca^{2+} are essential cellular parameters that integrate many growth and stress responses in plants. By combining our collection of genetic backgrounds lacking individual proton-pumps or proton-coupled transporters with the tools that we established for in vivo pH- and Ca^{2+}-measurements in all relevant compartments, we will be able to measure all the parameters required for mathematical model of pH- and Ca^{2+}-homeostasis. Using cell type-/tissue-specific promoters to drive our GECIs, we will be able to integrate our data into models for cell growth and stress responses based on transcript and protein profiling. Moreover, we have developed tools that allow us to interfere with pH-homeostasis and Ca^{2+}-signalling in an inducible manner so that the resulting models can be tested and refined.

3. Improving vacuolar storage capacity
Nitrate is the major nitrogen source for plants. When it is taken up in excess of immediate requirements, it is stored in the vacuole and is remobilized upon metabolic demand. The vacuolar nitrate storage and remobilization capacities are key factors controlling the NUE of crop plants. As nitrate transport is directly coupled to the tonoplast proton-gradient, we are pursuing several strategies to manipulate proton-pumping activities employing tissue-specific and inducible transgene expression as well as chemical genetic approaches.

Selected publications since 2009
Number of peer-reviewed articles 2009-2014: 16, number of citations 2009-2013: 554, h-index (2009-2014): 10, total h-index: 30 (according to Thomson Reuters).

Viotti, C., Krüger, F., Krebs, M., Neubert, C., Fink, F., Lupanga, U., Scheuring, D., Boutté, Y., Frescatada-Rosa, M., Wolfenstetter, S., Sauer, N., Hillmer, S., Grebe, M. and Schumacher, K. (2013). The Endoplasmic Reticulum Is the Main Membrane Source for Biogenesis of the Lytic Vacuole in Arabidopsis. Plant Cell *25*, 3434–3449
Krebs, M., Held, K., Binder, A., Hashimoto, K., Herder, Den, G., Parniske, M., Kudla, J., and Schumacher, K. (2011). FRET-based genetically encoded sensors allow high-resolution live cell imaging of Ca(2+) dynamics. Plant J *69*, 181–192.
Scheuring, D., Viotti, C., Krüger, F., Künzl, F., Sturm, S., Bubeck, J., Hillmer, S., Frigerio, L., Robinson, D.G., Pimpl, P. and Schumacher K (2011). Multivesicular bodies mature from the trans-Golgi network/early endosome in Arabidopsis. Plant Cell *23*, 3463–3481.
Viotti, C., Bubeck, J., Stierhof, Y.-D., Krebs, M., Langhans, M., van den Berg, W., van Dongen, W., Richter, S., Geldner, N., Takano, J., Jürgens, G., de Vries, SC., Robinson, DG. and Schumacher K. (2010). Endocytic and secretory traffic in Arabidopsis merge in the trans-Golgi network/early endosome, an independent and highly dynamic organelle. Plant Cell *22*, 1344–1357.
Krebs, M., Beyhl, D., Görlich, E., Al-Rasheid, K.A.S., Marten, I., Stierhof, Y.-D., Hedrich, R., and Schumacher, K. (2010). Arabidopsis V-ATPase activity at the tonoplast is required for efficient nutrient storage but not for sodium accumulation. Proc Natl Acad Sci USA *107*, 3251–3256.



PROJECT LEADER: DR. STEFAN HILLMER

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Fields of Interest
Membrane transport, protein trafficking, cell ultrastructure

Brief summary of work since 2009
My major emphasis lies in development and refining of techniques for specimen preparation in transmission electron microscopy. Teaching these methods to students and to users of the COS Electron Microscopy Facility as well as supporting the research projects of the Department of Plant Developmental Biology is an integral part of my lab.

Underlying goal and long term research interest has been to gain more insights into structure function relationships in the secretory system of plants cells, especially vacuole biogenesis and protein trafficking have been in the focus. For specimen preparation, aiming to analyse subcellular structures and localizing proteins on the ultrastructural level, cryomethods have replaced more and more the traditional chemical fixation regimes in our lab, since high pressure freezing due to its millisecond-fast and complete immobilization of the biological material is regarded being less prone to artefacts compared to the traditional chemical methods.

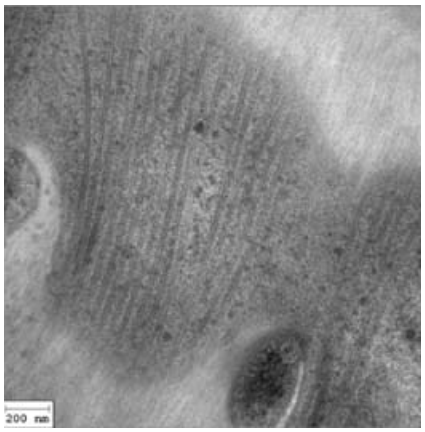
Major contributions since 2009
We have adapted protocolls for high pressure freezing and freeze substitution to plant material used in the labs of the Plant Cell and Developmental Biology Department allowing immunolocalizations at the ultrastructural level (Hillmer et al., 2012). The advances made over the years led to collaborations far beyond Heidelberg, since the number of laboratories employing these techniques is relatively small. This resulted in a series of publications that all deal with certain aspects of the secretory system of plants.

For plant supension cultures a formerly unknown cytosol to cell wall exocytosis has been described, that still awaits further characterization (Wang et al., 2010). Arabidopsis root tips have been used for several studies, most recently elucidating the relationship of the endoplasmic reticulum and the emerging vacuole in roots (Viotti et al., 2013). And in the framework of a more biotechnologically oriented EU project (EU Pharma-Planta; Framework IV) Arabidopsis seeds have been utilized for a immuno-gold study localizing antibody fragments expressed in plant seeds (Loos et al., 2011).

Planned research and new directions
The COS Electron Microscope Facility, which is up to now an integral part of the Department of Plant Developmental Biology, will fuse with the campus wide Electron Microscopy Core Facility (EMCF) and therefore undergo an organizational change during the next months. Aim is to bundle competences and equipment to allow researchers without experience and own equipment the integration of electron microscopical techniques in

their projects. In the context of building reconstruction at INF230 the high pressure freezer, the JEM 1400 transmission electron microscope, freeze substitution units and ultra microtomes also will have to move to another location. This offers the chance to bundle resources of the two electron micoscopy units in a single location which would be beneficial for staff and users.

Scientifically the focus will remain on improvement of sample preparation. If high pressure freezing is applied, it minimizes distortion related to slow action of chemical fixatives, nevertheless improvement of freeze substituion protocolls is still very much needed to increase selective contrast of membranes. Refinements already resulted in shorter freeze substitution times with minimal amounts of fixatives added to the substitution medium followed by embedding of the cellular material in Lowicryl HM20 at low temperatures and UV polymerization. This allowed immunolabelling studies that often complement microscopical studies in living cell at the confocal level. Even if this results in excellent structural preservation, selective membrane contrast is often less pronounced as it would be desired and has to be improved in the future. Especillay in the context of three-dimensional reconstruction of larger parts of a cell or a tissue, sectioning quality and beam stability are also important issues and will be addressed to improve sample preparation. Longterm goals are in addition a better integration of light and electron micoscopy aiming to improve the ability to find certain cellular/subcellular structures that are labelled with fluorescently tagged molecules and analyze these structures using the superior resolution of the electron microscope.



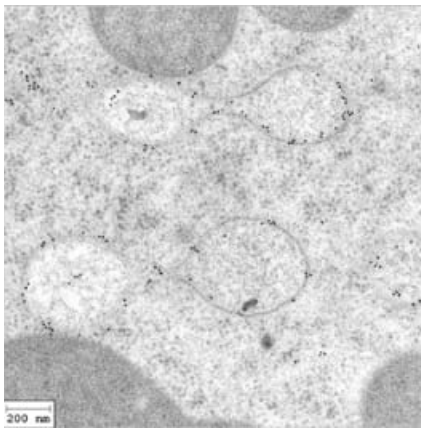


Figure 1
Tangentially sectioned leaf cell with microtubles parallel aligned to cellulose microfibrils of the cell wall. Material has been high pressure frozen, freeze substituted with osmium tetroxide and uranyl acetate and embedded in Epon.

Figure 2
Immuno-gold labeling with antibodies against vacuolar pyrophposphatase visualized with goat-anti-rabbit antibodies coupled to 10nm colloidal gold particles. Labeled structures are developing vacuoles in Arabidopsis root cells after high pressure freezing, freeze substitution and low temperature embedding.

Selected publications since 2009
Number of peer-reviewed articles 2009-2013: 20, number of citations 2009-2013: 276, h-index (2009-2013): 9, total h-index: 28 (according to Thomson Reuters).

Viotti, C., Krüger, F., Krebs, M., Neubert, C., Fink, F., Lupanga, U., Scheuring, D., Boutté, Y., Frescatada-Rosa, M., Wolfenstetter, S., et al. (2013). The endoplasmic reticulum is the main membrane source for biogenesis of the lytic vacuole in Arabidopsis. Plant Cell *25*, 3434–3449.
Hillmer, S., Viotti, C., and Robinson, D. G. (2012). An improved procedure for low-temperature embedding of high-pressure frozen and freeze-substituted plant tissues resulting in excellent structural preservation and contrast. Journal of Microscopy *247*, 43–47.
Loos, A., Van Droogenbroeck, B., Hillmer, S., Grass, J., Kunert, R., Cao, J., Robinson, D.G., Depicker, A., and Steinkellner, H. (2011). Production of monoclonal antibodies with a controlled N-glycosylation pattern in seeds of Arabidopsis thaliana. Plant Biotechnology Journal *9*, 179–192.
Scheuring, D., Viotti, C., Krüger, F., Künzl, F., Sturm, S., Bubeck, J., Hillmer, S., Frigerio, L., Robinson, D.G., Pimpl, P., et al. (2011). Multivesicular bodies mature from the trans-Golgi network/early endosome in Arabidopsis. Plant Cell *23*, 3463–3481.
Wang, J., Ding, Y., Wang, J., Hillmer, S., Miao, Y., Lo, S.W., Wang, X., Robinson, D.G., and Jiang, L. (2010). EXPO, an Exocyst-Positive Organelle Distinct from Multivesicular Endosomes and Autophagosomes, Mediates Cytosol to Cell Wall Exocytosis in Arabidopsis and Tobacco Cells. The Plant Cell *22*, 4009–4030.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2.21 CELL CHEMISTRY
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Fields of Interest
Protein glycosylation and glycomics, glycosyltransferase GT-C superfamily, ER protein homeostasis and stress response, cell adhesion and signaling

Brief summary of work since 2009
Protein O-mannosylation is a vital protein modification that is conserved among fungi, animals, and humans. We are using yeast and mammalian model systems to answer the key questions: i) How does the O-mannosylation machinery work? and ii) What is the functional role of O-mannosyl glycans?

Structure and dynamics of the protein O-mannosylation machinery.
O-mannosylation is initiated in the endoplasmic reticulum (ER) by a conserved family of protein O-mannosyltransferases (PMTs). During this period we demonstrated that yeast PMTs associate with the co- and posttranslational translocon complex and efficiently mannosylate proteins while they are entering the ER. In addition, the molecular characterization of PMTs defined protein domains involved in substrate binding and established a structural model of a major loop region facing the ER lumen.

The functional role of O-mannosylation in yeast and mammals.
The transcriptional response upon inhibition of O-mannosylation threw light on the question why O-mannosylation is crucial for the survival of yeast cells. Two vital processes, namely cell wall integrity and ER homeostasis are primarily affected when O-mannosylation is blocked.
In humans, defective O-mannosylation results in congenital muscular dystrophies. We showed that the severity of the clinical phenotype is inversely correlated with PMT activity. Further, in mammals we identified the cell adhesion glycoproteins T- and E-cadherin as novel O-mannosylated proteins indicating that O-mannosyl glycans contribute to far more cellular processes than initially expected.

Major contributions since 2009

Structure and dynamics of the protein O-mannosylation machinery
Protein translocation across the ER membrane and protein glycosylation are intimately connected, fundamental processes essential for growth and development of eukaryotes. In fungi, secretory and membrane proteins can be N-glycosylated, O-mannosylated or both. Protein translocation and N-glycosylation are highly coordinated processes which take place at the translocon-oligosaccharyltransferase (OST) complex. In analogy, it was assumed that PMTs also act at the translocon, however, in recent years it turned out that prolonged ER residence allows O-mannosylation of un-/misfolded proteins or slow folding intermediates by PMT complexes (Fig. 1; reviewed in Loibl and Strahl, 2013). Defining the mechanism of O-mannosylation is crucial to understand its diverse physiological roles for ER protein homeostasis. Thus, we reinvestigated O-mannosylation in the context of protein translocation in baker´s yeast. We showed the *in vivo* association of PMTs with OST and the translocon. The coordinated interplay between PMTs and OST was further demonstrated by a comprehensive mass spectrometry-(MS) based analysis of N-glycosylation site occupancy in pmtΔ mutants. In addition, we established a new microsomal translation/translocation/O-mannosylation system which revealed that PMTs efficiently mannosylate proteins during their translocation into microsomes. This system will allow us to unravel mechanistic differences between co- and post-translocational O-mannosylation in the future. (Loibl et al., 2014)

To fully understand the mechanism of protein O-mannosylation a more complete picture of the molecular architecture of PMTs in needed. PMTs are polytopic membrane proteins with two major hydrophilic loops (loops 1 and 5) facing the ER lumen (Fig. 2). The formation of dimeric PMT complexes is crucial for mannosyltransferase activity (reviewed in Loibl and Strahl, 2013). To further characterize PMT complexes, we developed a photoreactive probe based on an artificial mannosyl acceptor substrate. Photoaffinity labelling in combination with site directed mutagenesis identified the loop 1 region as binding site of the mannosyl acceptor (Lommel et al., 2011). Besides, we were aiming to get structural information on PMTs. Due to the limitations in the purification of the PMT

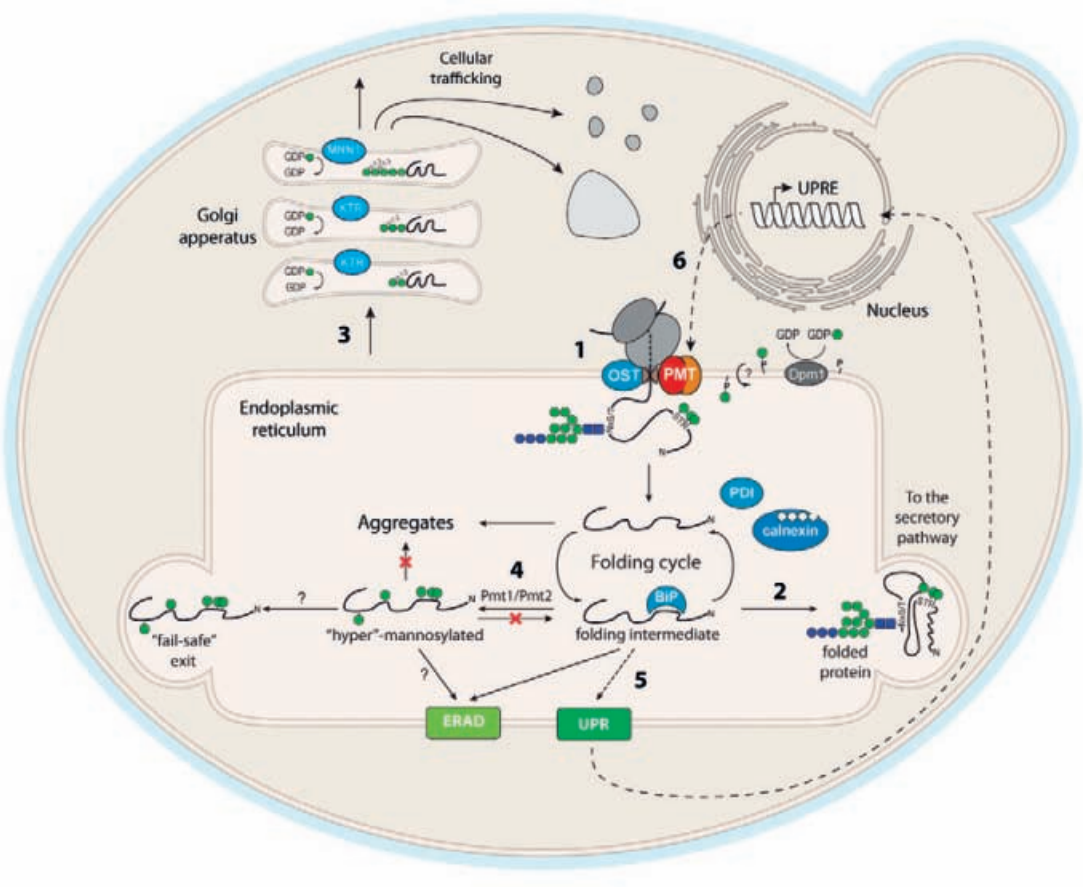

Figure 1
Protein O-mannosylation in yeast. (1-3) Bona vide glycosylation substrate proteins entering the ER are glycosylated by PMTs and/or OST. Once the proper conformation is reached proteins are exported and travel along the secretory pathway where O-mannosyl glycans are further elongated. Terminally misfolded proteins are removed via ER associated degradation (ERAD). (4-6) A fraction of proteins receive O-mannosyl glycans only in cases when they un-/misfolded. The function of those glycans is controversially discussed. O-mannosyl glycans might specifically target misfolded proteins for ERAD and/or increase solubility of the target proteins thereby allowing ER export. Misfolded proteins trigger the unfolded protein response (UPR) which enhances transcription of many UPR target genes among those are PMTs.

loop5 domain, we turned to stromal cell-derived factor2 (SDF2) from *Arabidopsis thaliana* which is highly homologous to this loop. We showed that *At*SDF2 is an ER protein that is a crucial target of the unfolded protein response (UPR) and most likely directly involved in the ER protein quality control. In collaboration with the laboratory of I. Sinning (BZH, Heidelberg University), we solved the *At*SDF2 3D crystal structure at 1.95 Å resolution (Schott et al., 2010) which allowed us to generate a structural model of the loop 5 domain of PMTs. This model provides the ground for targeted functional studies.

The functional role of O-mannosylation in yeast and mammals
In fungi, O-mannosylation is an essential and abundant protein modification. Many yeast cell wall proteins bear numerous O-mannosyl glycans and conditional lethal pmtΔ mutants show cell wall defects (reviewed in Loibl and Strahl, 2013). However, why O-mannosylation is crucial for the survival of yeast cells was still hardly understood. The genome-wide transcriptional response to a general inhibition of O-mannosylation in baker´s yeast identified major stress regulatory pathways – the cell wall integrity pathway and the UPR – that are activated when O-mannosylation is blocked. Moreover, we identified an overlapping/compensatory impact of N- and O-glycosylation on these processes. Genetic and biochemical analyses verified that O-mannosylation is not only crucial for the formation of an intact cell wall but also significantly impacts on ER protein homeostasis. Our results explain why a lack in O-mannosylation results in lethality of yeast even when cells are osmotically stabilized. (Arroyo et al., 2011)

In recent years protein O-mannosylation has become a focus of attention as a pathomechanism underlying severe congenital muscular dystrophies associated with neuronal migration defects (reviewed in Lommel and Strahl, 2009). During this period we reported novel patients with mutations in the human PMT-family member POMT1 and showed that the severity of the clinical phenotype of is inversely correlated with POMT activity (Judas et al., 2009 and Lommel et al., 2010). A key feature of these disorders is the lack of O-mannosyl glycans on α-dystroglycan resulting in abnormal basement membrane formation. However, other proteins bearing this modification were still largely unknown. We established antibodies specific for an O-mannosyl conjugated epitope which revealed that O-mannosyl glycans are present on early mouse embryos when α-dystroglycan is not yet expressed. To screen for novel O-mannosylated mammalian proteins, we set up a highly reliable method combining glycosidase treatment with LC-MS analyses. Whereby, cadherins which play important roles in cell adhesion were identified as novel O-mannosylated proteins. (Winterhalter et al., 2013 and Lommel et al., 2013)

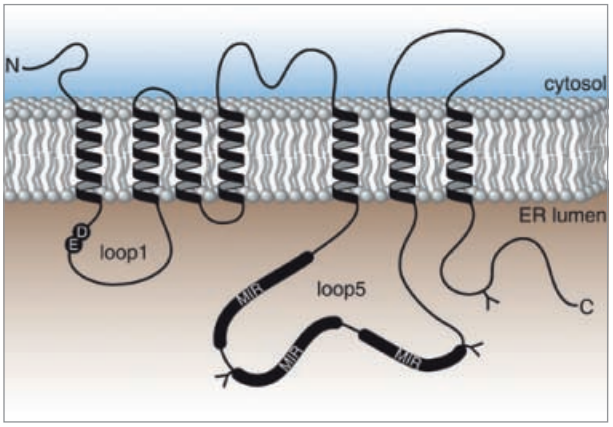

Figure 2
Topology model of PMT proteins. A conserved Asp-Glu (DE) motif in loop1 and N-glycosylation sites (Y) are indicated. In loop5, conserved MIR motifs are coloured in orange, blue, and green.

Planned research and new directions
Our previous work revealed that protein O-mannosylation plays a crucial role for ER protein homeostasis. However, due to its complexity, the molecular mechanisms and diverse functions of protein O-mannosylation in the ER are yet not well defined and remain challenging questions to be addressed in the future. Using baker´s yeast as model system we aim to: i) unravel the basic principles of protein O-mannosylation of bona vide substrates in the context of protein translocation; ii) dissect the role of protein O-mannosylation with respect to ER homeostasis during stress conditions; iii) decipher the molecular architecture of PMTs.

Considering the immense impact of cadherins on normal as well as pathogenic cell behaviour, our current results redefined the significance of O-mannosylation in mammals and human. In the future we want to focus more closely on how O-mannosylation impacts on cell adhesion, migration and signalling, especially in the context of tumor invasion and metastasis.

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 16, number of citations 2009-2013: 204, h-index (2009-2013): 7, total h-index: 25 (according to Thomson Reuters).

Loibl M, Wunderle L, Hutzler J, Schulz BL, Aebi M, Strahl S. (2014) Protein O-mannosyltransferases associate with the translocon to modify translocating polypeptide chains. J Biol Chem. 289:8599-611

Lommel M, Winterhalter PR, Willer T, Dahlhoff M, Schneider MR, Bartels MF, Renner-Müller I, Ruppert T, Wolf E, Strahl S. (2013) Protein O-mannosylation is crucial for E-cadherin-mediated cell adhesion. Proc Natl Acad Sci U S A. 110:21024-9

Arroyo J, Hutzler J, Bermejo C, Ragni E, García-Cantalejo J, Botias P, Piberger H, Schott A, Sanz AB and Strahl S. (2011) Functional and genomic analyzes of blocked protein O-mannosylation in baker´s yeast. Mol Microbiol. 79:1529-46

Schott A, Ravaud S, Keller S, Radzimanowski J, Viotti C, Hillmer S, Sinning I and Strahl S. (2010) Arabidopsis SDF2-like is a Crucial Component of the Unfolded Protein Response in the Endoplasmic Reticulum. J Biol Chem. 285:18113-21

Lommel M, Cirak S, Willer T, Hermann R, Uyanik G, van Bokhoven H, Körner C, Voit T, Barić I, Hehr U and Strahl S. (2010) Correlation of enzyme activity and clinical phenotype in POMT1 associated dystroglycanopathies. Neurology 74:157-64

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Fields of Interest
Vacuolar sugar partitioning, tonoplast glucose transporter, metabolic stress response

Brief summary of work since 2009
Vacuolar sugar compartmentation represents an essential cellular response to various external and internal factors. Recently, we identified some of the corresponding sugar transporters. While *VGT1* drives glucose import into isolated vacuoles from transgenic yeasts, ERD6-like 6 (*ERDL6*) mediates active glucose export from the plant vacuole. Knock-out of *ERDL6* leads to elevated Glc-levels, while *ERDL6* over-expression has the reverse effect. These changes in Glc-levels are restricted to the vacuole and affect seed germination as well as seed storage compounds in *erdl6* mutants. Transcriptome analyses revealed possible links to jasmonate and cellulose biosynthesis. In contrast to *ERDL6*, VGTs do not seem to affect Glc-homeostasis in leaves due to their predominant expression in pollen and roots. *VGT2* is strikingly co-regulated with several flavonoid biosynthesis genes and *vgt2* mutants have reduced levels of flavonoid glycosides.

Major contributions since 2009
In higher plants, sugars play important roles as nutrients and signal molecules. Hence, cells allocate sugars to different cellular compartments in adaptation to developmental cues and external conditions. In addition to transitory starch, vacuolar sugars represent a highly dynamic pool of instantly accessible metabolites serving as energy source, tags to modify proteins, secondary metabolites and hormones, building blocks for biosynthetic processes and compatible solutes for osmo-protection. Until recently, very little was known about the corresponding transport proteins. We identified the first vacuolar glucose transporter, *VGT1*, which drives Glc-uptake into vacuoles from transgenic yeasts. However, Arabidopsis mutant lines of *VGT1* and its homolog *VGT2* don't show changes in cellular Glc-levels, possibly because *VGT1* is predominantly expressed in pollen and *VGT2* in seedling roots, while both are weakly expressed in adult leaves. Interestingly, *VGT2* is strikingly co-expressed with a number of genes from the flavonoid synthesis pathway including its major regulators MYB12 and MYB111. Transient promoter assays revealed that MYB factors can directly bind to the *VGT2* promoter. Accordingly, *vgt* mutants have reduced levels of flavonoid glycosides.

ERDL6 belongs to a second sub-group of MST-like transporters which localize to the vacuole. Mutant plants lacking *ERDL6* have elevated cellular Glc-levels and non-aqueous fractionation revealed that Glc accumulates within the vacuole. Consistent with a possible Glc-export function, *ERDL6* expression is regulated during conditions that require the transport of carbohydrates across the tonoplast. Consequently, *erdl6* mutants show reduced Glc-export from the vacuole, particularly under stress conditions (heat, cold, wounding, drought/salt/osmo). Moreover, lack of *ERDL6* causes a delay of seed germination and significant elevation of the major seed storage reserves (sugars, proteins, lipids)

and total seed yield (Poschet et al., 2011). Recently, we could directly demonstrate vacuolar Glc-export activity of *ERDL6* by patch clamp (Klemens et al., 2014). In *erdl6* mutants, a number of jasmonate (JA) synthesis and signaling genes are up-regulated and the major regulators of the JA pathway, MYC2 and JAZ factors can directly target the *ERDL6* promoter. JA plays a role in wound response, during which ERD6 expression is strongly induced. Interestingly, wound-induction of *ERDL6* is markedly reduced in *myc2* mutants. In accordance, the Glc-release from the vacuole after wounding is significantly reduced in *erdl6* mutants.

Planned research and new directions

Our previous work revealed that vacuolar glucose transporters play important roles in adapting cellular sugar homeostasis to developmental cues and external conditions. Future work is required to further elucidate the physiological role of these transport proteins and to develop a clearer picture of how and when plants exploit different types of sugar transporters, i.e. active ERDL6/VGT transporters vs. the recently identified passive transporter SWEET16, or transport of glucose vs. sucrose. Therefore, we will further explore the role of ERDL6 during responses to stress conditions (osmotic/drought stress, freezing tolerance, light limitation) and during development (root development, pollen maturation /germination, cell wall composition) also with respect to its post-transcriptional regulation (ERDL6 phosphorylation was confirmed by LC-MS). One major aspect will be to unravel the links between vacuolar Glc-sequestration and secondary metabolism and hormone signaling. However, the remaining export activity in *erdl6*-KOs indicates that other ERD6 homologs contribute to vacuolar glucose partitioning. Thus, it will be important to address candidate functions by analyzing multiple *erdl*-KOs (enhancement of *erdl6*-KO effects), as well as expression in the *erdl6*-KO background (complementation of the *erdl6*-KO effects). Our work will help to further decipher the functional network of tonoplast transporters underlining the importance of vacuolar sugar partitioning in plant performance during stress adaptation and development.

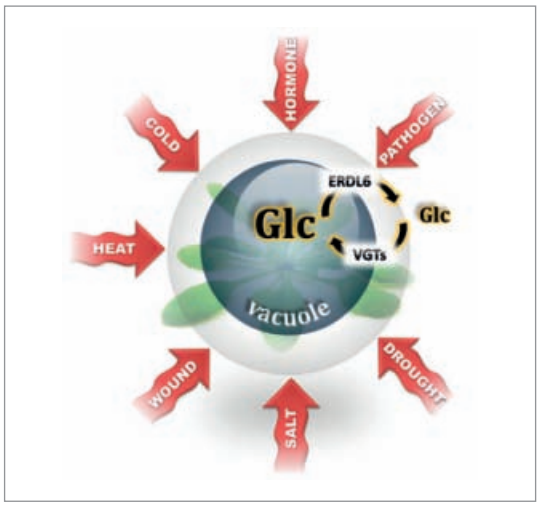

Figure 1
Internal and external factors affecting vacuolar glucose partitioning mediated by VGT-type importers and ERDL6-type exporters.

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 6, number of citations 2009-2013: 61, h-index (2009-2013): 5, total h-index: 15 (according to Thomson Reuters).

Poschet, G., Hannich, B., and Büttner, M. (2010). Identification and characterization of AtSTP14, a novel galactose transporter from Arabidopsis. Plant Cell Physiol. *51(9)*, 1571-1580.

Büttner, M. (2010). The Arabidopsis sugar transporter (AtSTP) family: an update. Plant Biol. *12*, 35-41.

Schulz, A., Beyhl, D., Marten, I., Wormit, A., Neuhaus, E., Poschet, G., Büttner, M., Schneider, S., Sauer, N., and Hedrich, R. (2011). Proton-driven sucrose symport and antiport is provided by the vacuolar transporters SUC4 and TMT1/2. Plant J. *68(1)*, 129-136.

Poschet, G., Hannich, B., Raab, S., Jungkunz, I., Klemens, P.A.W., Krueger, S., Wic, S., Neuhaus, H.E., and Büttner, M. (2011). A Novel Arabidopsis Vacuolar Glucose Exporter Is Involved in Cellular Sugar Homeostasis and Affects the Composition of Seed Storage Compounds. Plant Physiol. *157(4)*, 1664-1676.

Klemens, P.A., Patzke, K., Trentmann, O., Poschet, G., Büttner, M., Schulz, A., Marten, I., Hedrich, R., and Neuhaus, H.E. (2014). Overexpression of a proton-coupled vacuolar glucose exporter impairs freezing tolerance and seed germination. New Phytol. *202(1)*, 188-197.

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Fields of Interest
Glycobiology, developmental biology, protein glycosylation, cell-cell and cell-cell-matrix interactions, cellular migration

Brief summary of work since 2009
Protein O-mannosylation has an extensive impact on the development of the mammalian organism. In humans, mutations in genes involved in the biosynthesis of O-mannosyl glycans, result in a heterogeneous group of congenital muscular dystrophies (CMDs). In mice, impaired O-mannosylation leads to early embryo lethality. Using patient-derived cells, our recent work has provided insights in the mechanisms underlying CMDs. Furthermore, by blocking O-mannosylation in mouse embryos, we established a role of O-mannosyl glycans in cell adhesion of the preimplantation embryo and identified E-cadherin as a novel target of protein O-mannosylation.

Major contributions since 2011

ISPD loss-of-function mutations impair enzymatic activity in CMD patients.
Mutations in six genes encoding for known or suspected glycosyltransferases involved in the biosynthesis of O-mannosyl glycans, have been implicated in CMDs. Nevertheless, approximately 65 % of the CMD cases have no mutation in these genes, suggesting additional genes associated with CMD. Recently, Willer et al., identified recessive mutations in *ISPD* (encoding isoprenoid synthase domain containing) as a novel cause of CMD. (Willer et al., 2013) We supported this study by determining POMT enzymatic activity in patient-derived cells which was markedly reduced. Since no glycosyltransferases activity is proposed for *ISPD*, our results provide first insights in how mutations give rise to CMD. Impaired *in vitro* mannosyltransferase activity in ISPD deficient cells suggest that this protein is directly involved in the O-mannosyl transfer reaction in the ER.

Protein O-mannosylation is crucial for E-cadherin-mediated cell adhesion.
The common pathogenic denominator in CMD patients and O-mannosylation deficient mouse models is the loss of functional glycosylation of α-dystroglycan (α-DG) causing aberrant basement membrane formation. However, other biological functions of O-mannosylation and other proteins bearing this modification are largely unknown. We recently reported that targeted disruption of *Pomt2* in mice leads to embryo lethality during preimplantation development. O-mannosylation deficient embryos failed to proceed from morula to blastula stage due to defects in the formation of adherens junctions. (Lommel et al., 2013) An identical phenotype was observed when O-mannosylation was pharmacologically blocked using a POMT-specific inhibitor established during this study. Using mass spectrometry, we demonstrated that O-mannosyl glycans are present on E-cadherin, the major cell-adhesion molecule of blastomeres. Furthermore, inhibitor treatment of MDCK cells lead to a gradual decline in cadherin-mediate aggregation accompanied with a decreased O-mannosylation of E-cadherin. These results strongly indicate that protein

O-mannosylation is crucial for cadherin function. Considering the fundamental role of cadherins in several biological processes our study clearly redefines the biological significance of protein O-mannosylation.

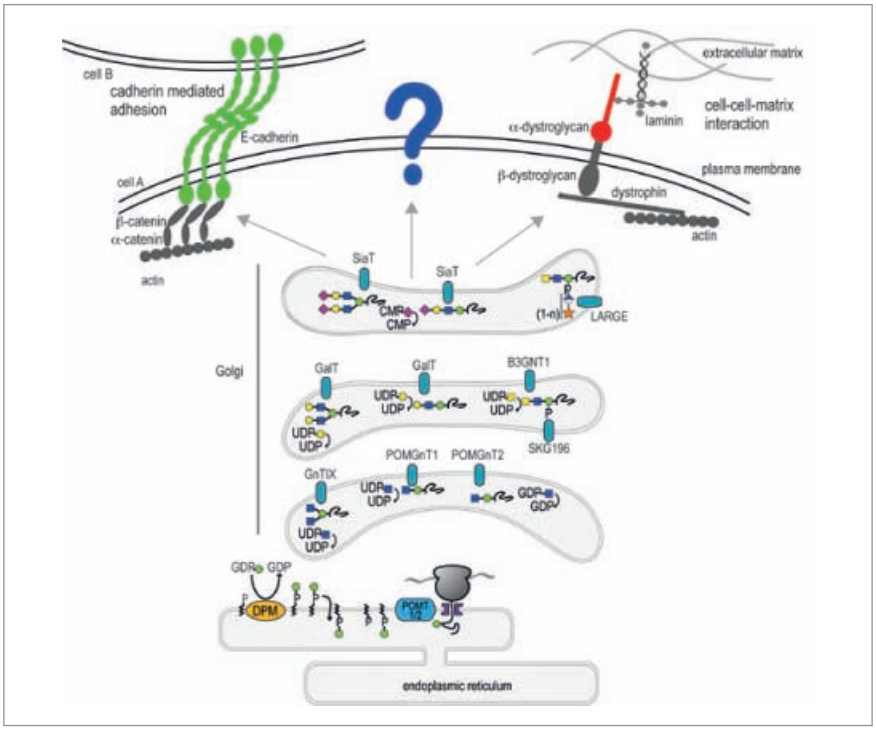

Figure 1
Biosynthesis and biological functions of O-mannosyl glycans. Protein O-mannosylation is initiated in the ER by the transfer of a single mannose from dolichol phosphate ß-D-mannose. Extension of the O-linked mannose residue takes place in the Golgi apparatus where a variety of O-mannosidically linked glycan structures are formed. These glycans are essential for cadherin-mediated cell adhesion as well as for dystroglycan mediated cell-cell-matrix interactions. Further biological functions of protein O-mannosylation remain to be identified.

Planned research and new directions

We recently demonstrated the presence of O-mannosyl glycans on E-cadherin. However, their molecular function remains to be established. Although impaired cadherin-mediated adhesion was observed upon disturbed O-mannosylation, alternative mechanisms arising from so far unknown POMT substrates may give rise to this phenotype. To address this issue we aim to study the direct impact of O-mannosyl glycans on E-cadherin function.

Although mouse models of O-mannosylation helped in establishing the role of these glycans for α-DG function and cadherin-mediated adhesion, embryo lethality hampered the identification of other functions of this protein modification. Since zygotic gene activation in fish occurs at much later stages of development, O-mannosylation deficient embryos will presumably overcome early embryo lethality. In the future, we aim to employ fish models of impaired O-mannosylation to further elucidate the pathomechanisms that give rise to CMD as well as to reveal novel biological function of O-mannosyl glycans.

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 7, number of citations 2009-2013: 136, h-index (2009-2013): 5, total h-index: 8 (according to Thomson Reuters).

Lommel M, Winterhalter PR, Willer T, Dahlhoff M, Schneider MR, Bartels MF, Renner-Müller I, Ruppert T, Wolf E, Strahl S. (2013) Protein O-mannosylation is crucial for E-cadherin-mediated cell adhesion. Proc Natl Acad Sci U S A. 110:21024-9

Willer T, Lee H, Lommel M, Yoshida-Moriguchi T, de Bernabe DB, Venzke D, Cirak S, Schachter H, Vajsar J, Voit T, Muntoni F, Loder AS, Dobyns WB, Winder TL, Strahl S, Mathews KD, Nelson SF, Moore SA and Campbell K.P. (2012) ISPD loss-of-function mutations disrupt dystroglycan O-mannosylation and cause Walker-Warburg syndrome. Nat Genet. 44:575

Lommel M, Cirak S, Willer T, Hermann R, Uyanik G, van Bokhoven H, Körner C, Voit T, Barić I, Hehr U and Strahl S. (2010) Correlation of enzyme activity and clinical phenotype in POMT1 associated dystroglycanopathies. Neurology 74, 157-64.

2.22 DEVELOPMENTAL BIOLOGY/ PHYSIOLOGY
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Fields of Interest
Molecular and developmental genetics, eye evolution, development and differentiation, neuronal networks, transcriptional networks

Brief summary of work since 2009
The vertebrate eye, a lateral out-pocketing of the brain, represents an ideal model for studying the development, growth and regeneration of the brain. We initially addressed early development (patterning and morphogenesis) and now shifted our focus towards post-embryonic stages (growth and regeneration). We identified cells in the ciliary marginal zone of the retina as retinal stem cells. To study the behavior of stem cells during development, growth and regeneration we developed a new toolkit, termed Gaudi. We can now follow any cell and all of its descendants by stochastic clonal labeling. We apply the Gaudi toolkit to address the impact of extrinsic and intrinsic factors on fate decisions of stem cells and combine it with long-term life-imaging to gain mechanistic insights. The Gaudi toolkit allowed expanding our scope from the eye to intestine and skin, where we are applying the same unique tools for addressing related questions in the context of organ homeostasis and cancer. In combination with our expertise in imaging we aim at following, manipulating and understanding the decisions of stem cells in their »natural« environment, the growing and regenerating organ. Our clonal analyses resemble population genetics: single, genetically distinct, cells in the pool of a population (organ). In a population genomics approach we tackle the genetic bases for the adaptation of populations. We have initiated the identification of an unstructured medaka population in Japan. In an international collaboration we are currently establishing population genomics resource (at KIT) to ultimately address genome-environment interactions.

Major contributions since 2009
Stem cells have the capacity to both self-renew and generate post-mitotic cells. Long-term tracking of individual clones in their natural environment constitutes the ultimate way to validate post-embryonic stem cells. We identified retinal stem cells (RSCs) taking advantage of the temporo/spatial organization of the fish retina and followed the complete offspring of a single cell during postnatal life. RSCs generate two tissues of the adult fish retina, the neural retina (NR) and the retinal-pigmented epithelium (RPE). Despite their common embryonic origin and tight coordination during continuous organ growth, we showed that NR and RPE are maintained by dedicated RSCs that contribute in a fate-restricted manner to either one or the other tissue. We showed that in the NR, RSCs are multipotent and generate all neuron types and glia. (Centanin et al., 2011).

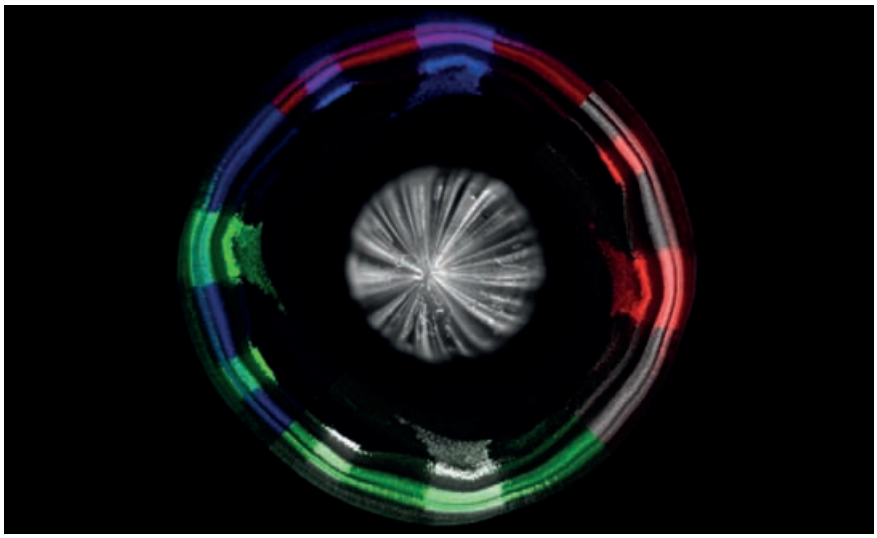

Figure 1
Rainbow retina. Permanent labeling of individual stem cells and all of their descendants in different colors reveals the composition of the retina. Clonally related cells within an ArCoS (Arched Continuous Stripes) establish the continuously growing fish retina.

To analyse whether the potency of NSCs changes with development we established a toolkit (Gaudi) for inducible clonal labelling and non-invasive fate tracking. We addressed post-embryonic stem cells in different tissues and functionally differentiated transient retinal progenitor cells (RPCS) from RSCs. Clonal induction demonstrated that post-embryonic retinal NSCs are constitutively multipotent and give rise to the complete spectrum of retinal cell types. Intriguingly, long-term clonal analysis uncovered a preference for asymmetric cell divisions. In response to external stimuli like injuries, NSCs in the retina maintained this preference, while regeneration is mediated by the transient expansion of RPCs. Our comprehensive analysis of individual post- embryonic NSCs in their physiological environment establishes the teleost retina as an ideal model for studying adult stem cell biology at single cell resolution (Centanin et al., 2014, in press).

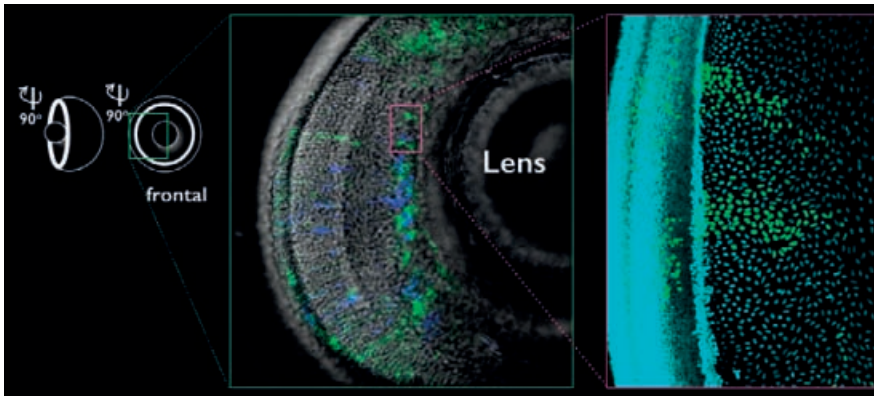

Figure 2
Individual clones in the rental stem cell niche. Individual retinal stem cells have been labelled by stochastic activation of a fluorescent protein (green frame). With time these cells expand both, symmetrically within the stem cell niche as well as asymmetrically to give rise to all retinal cell types (Centanin et al. 2011, Centanin et al. 2014, in press).

Cell and tissue specification are triggered by the precisely controlled expression of a unique combination of developmental genes. Synexpression groups (SGs) are batteries of spatio-temporally co-expressed genes that act in shared biological processes. Although several SGs have been described, the regulatory mechanisms that orchestrate their common complex expression pattern remained to be elucidated. In a pilot analysis on 560 medaka genes we established a resource for deciphering developmental gene regulatory networks. SGs share common cis-regulatory motifs that are arranged in various combinations. In contrast to previous assumptions that these genes are scattered throughout the genome, we discovered that genes of one SG frequently cluster genomically in a synexpression locus providing redundant and combinatorial regulatory information. Given that synexpression loci display a striking conserved synteny, we speculate that they also show a sequential onset of gene expression similar to the spatio-temporal co-linearity described for Hox gene clusters (Ramialison et al., 2012)

After the move from EMBL to Heidelberg University the lab massively invested into developing new technologies, from instruments to organisms. This was possible due to excellent and highly motivated Master students who were tackling technological challenges to address their biological questions. They were concerned with the »transregulatory screen«, golden gate cloning for fish, clonal transactivation systems, imaging and image analysis tools, TALEN and CRISPR/CAS targeted mutagenesis as well as homologous recombination. Due to space constraints I can only highlight a few (already published).

In collaboration with the Stelzer lab we have adapted the DSLM for the recording of large, nontransparent specimens, such as developing flies and fish by structured illumination. The decreased contrast due to massive light scattering was overcome by a combination of digital scanned laser light-sheet fluorescence microscopy with structured-illumination microscopy. That way the scattered background was discriminated from the fluorescence signal massively enhancing the contrast of in-focus structures. »We reconstructed cell positions over time ... and created a fly digital embryo« (Keller et al., 2010, http://www.digital-embryo.org).

To reliably introduce transgenes for monitoring or influencing cell fate decisions we were inserting efficient site specific recombination tools in the medaka genome. This allowed in particular to control copy number and genomic insertion site and thus standardized potential position effects. We identified »neutral« regions within the medaka genome by random transposon integration of a PhiC31-based site and reporter. The highly efficient PhiC31-integration of transgenes at a neutral genomic site facilitated the direct comparison of different transgenes. The landing site design enables a variety of applications such as reporter and enhancer switch in addition to the integration of any insert. (Kirchmaier, Höckendorf et al., 2013).

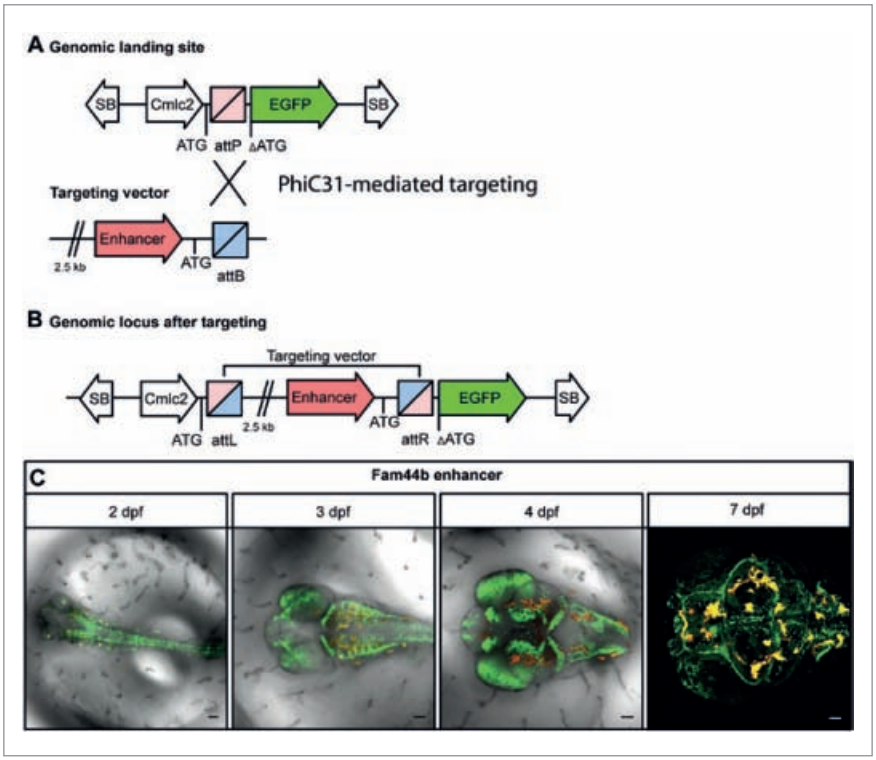

Figure 3
The PhiC31 toolkit. Single genomic landing site in a neutral locus of the medaka genome facilitate highly efficient site specific recombination with a targeting vector (A). Upon targeting of the landing site, the green fluorescent protein used as landing site sensor is coming under the control of an enhancer element provided through the targeting vector (B) resulting in an enhancer dependent expression pattern (C).

Importantly, our tool allowed assaying enhancer activity in injected embryos without requiring germline transmission in a site-specific manner, thus speeding up large-scale analyses of regulatory elements e. g. the encode study we participated in (The ENCODE consortium., 2012).

Planned research and new directions

The animal body has evolved a perfect homeostasis to maintain multi-potent adult stem cells with life-long proliferative potential as a continuous resource for replenishing cell populations in all kinds of tissues. This homeostatic control is tight enough to (in most cases) prevent their uncontrolled proliferation and exit to tumourigenesis. We plan to address the proliferative and differentiation potential of stem cells in the *in vivo* context combining advanced genetics, long term imaging in teleost model systems with image analysis and modelling approaches. RSCs in the ciliary marginal zone maintain their pluri-potency throughout life, without showing any higher incidence for retinal tumours. Our analyses on regeneration have indicated that stem cells don't show a regenerative response and rather maintain their asymmetric mode of cell division, while, conversely, retinal progenitor cells transiently expand to compensate and maintain functional homeostasis. We aim at *in vivo* studying adult neural stem cells in the ciliary marginal zone of teleost fish. Tanking advantage of the live-long maintenance of a stem cell niche in the fish retina, the short time span from niche exit to terminal differentiation in a genetically fully accessible system we aim to in vivo:

- understand fate decisions of retinal stem cells
  - statistical lineage analysis
  - following lineage by microscopy
  - establishment of RSC fate decisions model
- control fate decisions of retinal stem cells
  - functional challenge of fate decisions
  - testing and validation of fate decision model
- understand and modulate fate decisions of tumour (stem) cells
  - tumorigenic potential of retinal stem/progenitor cells
  - shift of symmetries and fate decisions
- application of technologies to inducible tumours in fish

Following these three global aims we plan addressing how the proliferation and differentiation paths are triggered and controlled to contribute to a well balanced ratio of terminally differentiated cells descending from a single multi-potent stem cell in the mature retina. Can that balance be challenged to make the stem cells start developing tumours and can we apply the technology developed for following and manipulating stem cells to follow and understand induced tumourigenesis in fish?

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 29, number of citations 2009-2013: 1080, h-index (2009-2013): 9, total h-index: 40 (according to Thomson Reuters).

Kirchmaier S, Höckendorf B, Möller EK, Bornhorst D, Spitz F, Wittbrodt J. (2013). Efficient site-specific transgenesis and enhancer activity tests in medaka using PhiC31 integrase. Development. 140(20):4287-95.

ENCODE Project Consortium (2012). An integrated encyclopedia of DNA elements in the human genome. Nature. 489(7414):57-74.

Ramialison M, Reinhardt R, Henrich T, Wittbrodt B, Kellner T, Lowy CM, Wittbrodt J. (2012). Cis-regulatory properties of medaka synexpression groups. Development. 139(5):917-28.

Centanin L, Hoeckendorf B, Wittbrodt J. (2011). Fate restriction and multipotency in retinal stem cells. Cell Stem Cell. 9(6):553-62.

Keller PJ, Schmidt AD, Santella A, Khairy K, Bao Z, Wittbrodt J, Stelzer EH. (2010). Fast, high-contrast imaging of animal development with scanned light sheet-based structured-illumination microscopy. Nat Methods.7(8):637-42.

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Fields of Interest
Neural Development, Cell Biology, Asymmetric Cell Division, Cell Lineage, In Vivo Time-Lapse Imaging

Brief summary of work since 2009
Generation of neuronal diversity in the vertebrate neuroepithelium requires coordinated regulation of cell polarity, cell division, and cell fate determination. In my group we aim to understand the complex networks integrating these processes *in vivo*. We take advantage of the easily accessible retinal neuroepithelium of the developing zebrafish embryo to track individual progenitor cell divisions and watch the occurrence of specific neural fates whilst elucidating the underlying cellular and molecular processes. We initially focussed on the identification of recurrent lineage patterns and molecular interactions during the occurrence of neural lineages *in vivo*. We found reproducible asymmetric cell divisions essential for the orderly generation of specific retinal subtypes (Figure 1). Building on this model, we are developing an integrated research program combining *live* imaging with transgenesis, cell biology, microarray analysis and CRISPR/Cas genome editing to investigate the molecular/cellular interactions linking cell division to asymmetric cell fate specification. Current investigations focus on the F-actin binding protein Anillin – essential for cytokinesis progression and midbody formation in animal cells – as important linking factor for these processes *in vivo* (Figure 2). Integrated mid-, long-term projects aim to understand Anillin function in relation to dynamics of the F-actin cytoskeleton, cell polarity cues and signalling pathways such as the Notch and Hippo pathway.

Major contributions since 2009
Lineage-dependent specification of neuronal subtypes (Jusuf et al., 2011; Schuhmacher et al., 2011, Jusuf, *Albadri et. al., 2012).* This work uncovered lineage-restricted expression of neuronal subtype biasing factors occurring within reproducible lineage patterns – an amacrine cell tends to arise from a ganglion cell progenitor through two consecutive rounds of asymmetric cell division (Figure 1). Our data suggest that lineage-specific developmental programs might underlie temporally ordered neuron subtype specification *in vivo*, essential for the correct assembly of early retinal networks. By the expression of a number of recently developed transgenes in the lab we are now using this lineage as *in vivo* model to visualize cell fate outcome (Figure 2) and study the underlying mechanisms of asymmetric cell division.

The role of Anillin in asymmetric inheritance and daughter cell fate (Paolini, Duchemin et al., unpublished data). Unequal segregation of apical plasma membrane proteins, such as the apical Par-complex protein Par3, upon cytokinesis has been linked to daughter cell developmental fate in vertebrate neuroepithelia. More recently, inheritance of the post-mitotic cytokinesis midbody remnants, previously regarded as »piece of cellular junk« has been linked to asymmetric fate outcomes in animal cells. Such studies drew our attention on Anillin function during neurogenesis. We firstly established a stable transgenic line for

the visualization of Anillin dynamics and midbody inheritance during retinal progenitor cell division (Figure 2). In addition to finding a direct role for Anillin in neurogenesis – *Anillin* knockdown favours symmetrical division – our work so far provides evidences suggesting that Anillin regulates F-actin and Pard3 asymmetric inheritance at one daughter's apical domain, predictive of daughter cell fate.

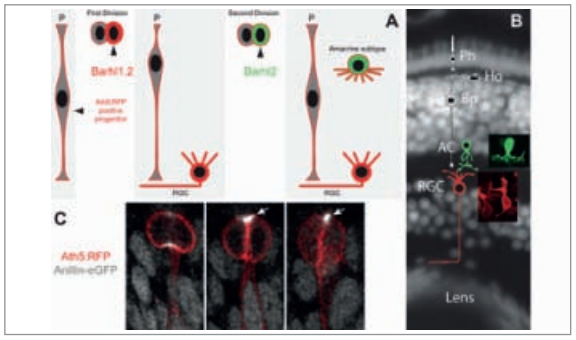

Figure (A) Barhl2-expressing amacrine subtypes are generated from retinal ganglion cells (RGCs) progenitors (Ath5:RFP positive P) through two consecutive asymmetric cell divisions. (B) A cartoon depicts main neuronal cells composing the retinal circuits. Retinal ganglion cells (RGCs) and amacrine cells (AC) can be visualized post-mitotically by UAS-driven expression of RFP and GFP from RCG and Am specific promoters driving GAL4 (insets, Albadri et al., unpublished data). DAPI (in grey) highlights the three nuclear retinal layers. Ph: photoreceptor cell, Ho: horizontal cell, Bp: bipolar cell. (C) Expression of an Anillin-eGFP fusion product in the retina of a stable *anillin:anillin-eGFP;ath5:gap43-RFP* double transgenic embryo highlights Anillin localization (in grey) in the cell nuclei during interphase and in the apically (top) localized midbody at the end of cytokinesis. Most Ath5:RFP positive progenitors asymmetrically distribute the Anillin spot towards one daughter cell apical domain at the end of cytokinesis (white arrow).

RFP and GFP from RCG and Ac specific promoters driving GAL4 (Albadri et al., unpublished data). DAPI (in grey) highlights the three nuclear retinal layers. Ph: photoreceptor cell, Ho: horizontal cell, Bp: bipolar cell.

Planned research and new directions

The established transgenic UAS/GAL4 lines, CRISPR/Cas-mutants, and *in vivo* cell biology approaches will serve us as foundation for developing long term projects aimed at understanding the role of Anillin, midbody inheritance, and their implications for asymmetric daughter cell fate *in vivo*. Current goals aim at understanding, molecularly, how Anillin regulation of the F-actin and midbody inheritance can be linked to unequal distribution and/or activity of key signalling pathways (e. g. the Notch and Hippo pathway) components between sibling cells. Links have already been made between midbody inheritance, fate and cancer and neurodegenerative disorders in human – our studies might therefore additionally provide significant insights into the underlying molecular mechanisms in the context of actin cytoskeleton, cell polarity cues and cell-cell mediated signals within the neuroepithelium.

The ability to precisely control protein activity in time and space in combination with visualization of cell-lineage outcome is an indispensable tool for fully addressing molecular interactions *in vivo*. We therefore started to test optogenetic tools for the control of protein activity through light activated cytoplasmic re-localization at single cell level and in a temporal-dependent manner. This study is in collaboration with the Synthetic Biology group of Barbara Di Ventura (Bioquant, Heidelberg).

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 7, number of citations 2009-2013: 42, h-index (2009-2013): 3, total h-index: 7 (according to Thomson Reuters).

Randlett, O., Poggi, L., Zolessi, F.R., and Harris, W.A. (2011). The Oriented Emergence of Axons from Retinal Ganglion Cells Is Directed by Laminin Contact In Vivo. Neuron *70*, 266-280.

Schuhmacher, L.-N., Albadri, S., Ramialison, M., and Poggi, L. (2011). Evolutionary relationships and diversification of barhl genes within retinal cell lineages. Bmc Evolutionary Biology *11*.

Jusuf, P.R., Albadri, S., Paolini, A., Currie, P.D., Argenton, F., Higashijima, S.-i., Harris, W.A., and Poggi, L. (2012). Biasing Amacrine Subtypes in the Atoh7 Lineage through Expression of Barhl2. Journal of Neuroscience *32*, 13929-13944.

Jusuf, P., Harris, W.A., and Poggi, L. (2013). Imaging retinal progenitor lineages in developing zebrafish embryos. Cold Spring Harb Protoc *2013*.

Scholpp, S., Poggi, L., and Zigman, M. (2013). Brain on the stage – Spotlight on nervous system development in zebrafish: EMBO practical course, KIT, Sept. 2013. Neural Development *8*.

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Fields of Interest
Stem cells, heterogeneity, clonal relations, developmental biology, genetics

Brief summary of work since 2009

Fish display a unique feature among vertebrates, which is their life-long growth by addition of newly generated cells. They contain permanently active stem cells that maintain growth in different tissues and organs. Fish therefore constitute a proper model to address the life-long activity of stem cells in their natural, intact environment. During the last years of my Post-Doc I generated a series of transgenic lines that allow labeling single cells and following lineages in vivo – the so-called Gaudí toolkit. Using these lines, as well as classical experimental embryology approaches, I focused on the study of retinal stem cells (RSCs) in medaka. Using the highly stereotyped spatio-temporal organization of the fish retina, we showed the full proliferation and differentiation potential of hundreds of individual RSCs in their natural niche. One of the emerging principles from our dataset is that although RSCs are very robust and homogeneous in their differentiation potential (every single RSC generates all the main retinal cell types), they are very heterogeneous regarding proliferation. Since I started my group, we have been studying how heterogeneities arise and are maintained, with a strong focus on the physiology of the niche (see A.c, Planned research).

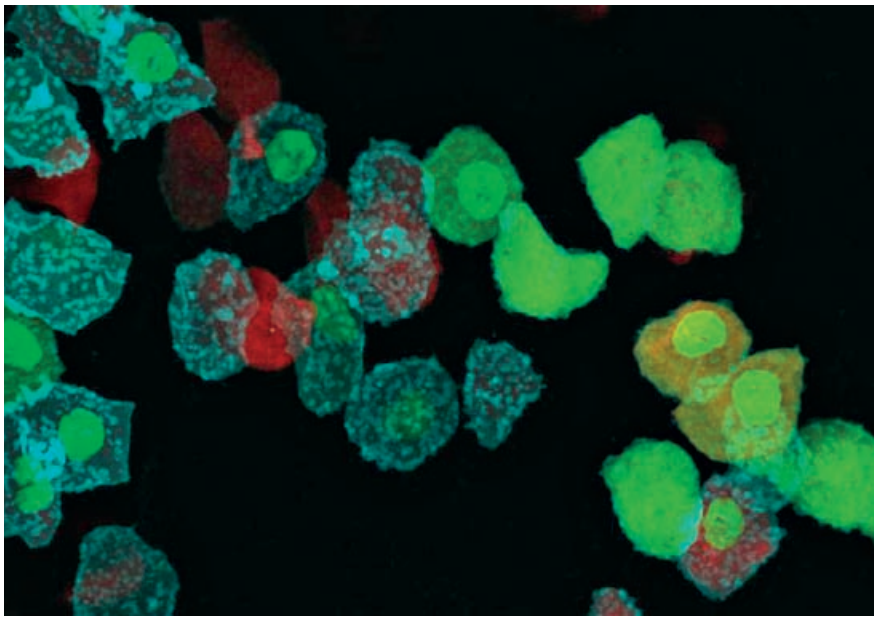

Figure 1
The Gaudí tool allows labeling of single stem cells and their progeny during embryonic and post-embryonic stages of medaka.

Major contributions since 2009

Understanding stem cells and their behavior is of key importance not just for bio-medical research but also for the environmental impact on organismal adaptation like growth or its arrest. These analyses should ideally be performed by following single stem cells in the living organism. Using the medaka retina as a model for constantly active stem cells, we have shown:

– RSCs generate two tissues of the adult fish retina, the neural retina (NR) and the retinal-pigmented epithelium (RPE). Despite their common embryonic origin and tight coordination during continuous organ growth, we proved that NR and RPE are maintained by dedicated RSCs that contribute in a fate-restricted manner to either one or the other tissue.
– We identified Rx2 as a marker for post-embryonic RSCs. Intriguingly, Rx2 is expressed by stem cells in the lineage-independent NR and RPE. Our data emphasize that single-cell labeling is a mandatory pre-requisite for lineage analysis. The population of Rx2+ RSCs generates the NR and the RPE, but an individual Rx2+ RSC contributes to either one or the other tissue.
– Retinal stem cells constitute a resource that never runs out – they constantly give rise to progenitors and to differentiated retinal cell types. Each individual stem cell ultimately lives up to its full potential and generates every major cell type in the neural retina. There is no stochastic decision regarding the cell types generated, nor heterogeneity in the progeny generated by different RSCs in the neural retina. This tissue of the CNS is thus maintained and grows by the activity of few universalists with multiple capacities rather than by a multitude of highly focused specialists.
– Our data strongly indicate that NSCs in the fish retina preferentially follow asymmetric divisions that result in permanent active clones during life. The preferential mode of asymmetric divisions by RSCs is fixed: it is maintained even in aged fish, and is not affected by external stimuli like injury responses.

Planned research and new directions

The study of individual stem cells in their natural environment has revealed a number of features that would have been unexpected from in vitro studies. We have observed a very robust, homogeneous behavior in terms of the cell types generated by individual stem cells within a population. However, proliferative responses vary a lot: different clones will have different expansion rates, which altogether constitute a huge dynamic range. My lab is interested in how heterogeneities are developed and maintained, focusing on the interaction of individual stem cells with their niche. We are developing a number of fluorescent sensors that will allow assessing the current molecular and metabolic state of individual stem cells, which will be combined with the lineage tools that we have already generated. Iterative imaging of growing clones will reveal how the initial state of a stem cell defines it future mitotic behavior, with a major focus on the physiology of the niche and the interdependence of the stem cells within.

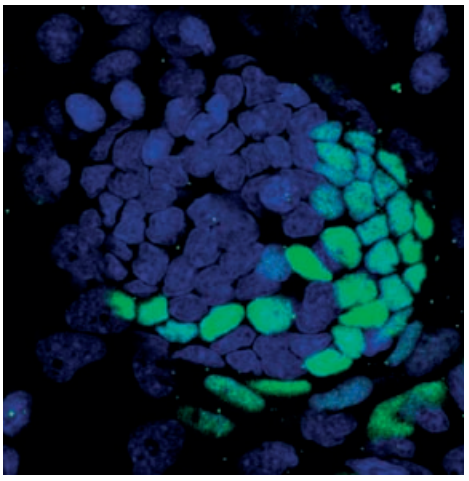

Figure 2
A clone of support cells in a neuromast of the posterior lateral line. Iterative imaging allows following clone progression in real time.

Selected publications since 2009

Number of peer-reviewed articles 2009-2014: 7; number of citations 2009-2013: 856; h-index (2009-2013): 4, total h-index: 7.

Centanin, L., and Wittbrodt, J. (2014). Retinal neurogenesis. Development *141*, 241–244.
Schaafhausen, M.K., Yang, W.-J., Centanin, L., Wittbrodt, J., Bosserhoff, A., Fischer, A., Schartl, M., and Meierjohann, S. (2013). Tumor angiogenesis is caused by single melanoma cells in a manner dependent on reactive oxygen species and NF-κB. J Cell Sci *126*, 3862–3872.
Centanin, L. (2013). The Potential of Single Cells Within Multi-Cellular Organisms. What do Fish Models Have to Offer? Cell News, DGZ *04/2012*.
ENCODE Project Consortium, Dunham, I., Kundaje, A., Aldred, S.F., Collins, P.J., Davis, C.A., Doyle, F., Epstein, C.B., Frietze, S., Harrow, J., et al. (2012). An integrated encyclopedia of DNA elements in the human genome. Nature *489*, 57–74.
Centanin, L., Hoeckendorf, B., and Wittbrodt, J. (2011). Fate restriction and multipotency in retinal stem cells. Cell Stem Cell *9*, 553–562.

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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 2009
Tremendous efforts have been undertaken to reduce the extent of environmental contamination and to improve the quality of both inland and marine waters. For this end, a huge body of regulations has been built up, and most environmental compartments are now subject to regular survey and control. However, despite massive improvement in water quality, fish populations still show massive deficits not only in the whole of western and central Europe, but also in North America and parts of Asia. The reasons being manifold, one approach to address these deficits is based on the assumption that at least part of the fish decline is due to contamination by a complex combination of specifically acting anthropogenic trace contaminants such as endocrine disruptors, pharmaceuticals and others. Since, however, the current surveillance programs are mainly based on acute toxicity testing, the combinatory effects of highly sublethal toxicant concentrations are likely to be overseen. Therefore, there is an urgent need for the development of more subtle procedures to elucidate the specific effects even of minute toxicant concentrations. Moreover, since both novel national and EU-wide regulations require the emphasis of alternative test methods, such specific test systems need to be integrated into alternatives – in the case of fish testing, into fish cell culture systems and embryo toxicity testing. The major goal of the Aquatic Ecology and Toxicology Group at the COS is to develop an integrated battery testing based on fish cells and embryos that allows the sensitive detection of a variety of specific endpoints.

Major contributions since 2009
Since earlier efforts to establish permanent fish cell lines as alternative test systems did not prove successful due to the limited sensitivity of the cell lines, major emphasis was put on the development of an acute fish embryo test as the basis for more specific toxicity testing schemes. According to the 2010 new EU regulation on animal welfare in scientific research, zebrafish embryos are not regarded protected, and are, therefore, potential candidates for the development of alternative test methods (Strähle et al. 2012). Likewise, in the sense of the 3R principle, transfer from adult to embryonic stages is at least a step towards refinement of animal testing, if not replacement. Thus, based on earlier work by Prof. Nagel (Technical University Dresden), the group submitted a draft proposal for a zebrafish embryo toxicity test to the OECD Test Guideline Program. After 6 years of R&D as well as extended validation studies, the fish embryo test was adopted as the first alternative test method in ecotoxicology within the OECD Test Guideline program in spring 2013.

As a major critique during the finalization of the international version (ISO) of the embryo-based test protocol for the routine toxicity screening in whole effluents, oxygen depletion

was speculated as a major reason of mortality in embryo testing. However, as demonstrated by Strecker et al. (2011), oxygen requirements by zebrafish embryos are far too small to be relevant in routine testing. Given the potential nature of the fish chorion as a barrier for anthropogenic contaminants, protocols for dechorionation with reproducibly high rates of survival of the embryos were developed (Henn et al. 2011, Kais et al. 2013). In order to more comprehensively describe the domain of applicability of the zebrafish embryo test, extensive testing programs were conducted into the toxicity of specific classes of chemical compounds (e. g., Böttcher et al. 2011, Grim et al. 2010, Holbech et al. 2010, Lammer et al. 2009, Opitz et al. 2009, Otte et al. 2010, Schmidt et al. 2011, Wetterauer et al. 2011, Zielcke et al. 2010).

With respect to more specific modes of toxic action, the following endpoints could be incorporated into fish embryo testing: estrogen-related endocrine disruption (induction of the synthesis of vitellogenin, zona pellucida protein and estradiol receptor β in four-day-old zebrafish embryos; Baumann et al. 2013, OECD 2010); thyroid disruption (Schmidt et al. 2011, 2012); genotoxicity (Böttcher et al. 2010, 2011, Faßbender et al. 2012, 2013a, b, Osterauer et al. 2011, Rocha et al. 2009, Schweizer et al. 2011); teratorgenicity (Weigt et al. 2009, 2010a, b, 2011a, b, 2012); sediment toxicity (Otte et al. 2013, Rocha et al. 2011, Wölz et al. 2010a, b,); cytochrome P450 induction (Otte et al. 2010, Weigt et al. 2009, 2010a, b, 2011a, b, 2012).

Within collaborations in international consortia, major contribution could be made to the development of OECD Test Guidelines no. 229, 230, 234 and 236, as well as to the OECD Fish Testing Strategy (OECD 2012). The discussion about the role of fish embryos within alternative testing frameworks was significantly stimulated by major reviews (Scholz et al. 2013) and position papers (Strähle et al. 2012). Likewise, the Heidelberg Ecotoxicology Group significantly contributed to the discussions about testing strategies for endocrine disruptors and fish embryos (Embry et al. 2010, Halder et al. 2010, Knacker et al. 2010, OECD 2010).

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, 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 (cf. Keiter et al. 2010) cover more specific modes of teratogenicity (e. g. Strecker et al. 2013), neurotoxicity and immunotoxicity.

Selected publications since 2009

Number of peer-reviewed articles 2009-2013: 62, number of citations 2009-2013: 441, h-index (2009-2013): 11, total h-index: 33 (according to Thomson Reuters).

Braunbeck, T., Johnson, R., Wolf, J. (2010) Guidance document on the diagnosis of endocrine-related histopathology in fish gonads. OECD Series on Testing and Assessment no. 123. OECD, Paris, ENV/JM/MONO(2010)14; 124 p.

Strähle, U., Scholz, S., Geisler, R., Greiner, P., Hollert, H., Rastegar, S., Schumacher, A., Selderslaghs, I., Weiss, C., Witters, H. and Braunbeck, T. (2011). Zebrafish embryos as an alternative to animal experiments. – A commentary on the definition of the onset of protected life stages in animal welfare regulations. Reprod. Toxicol. 33: 128-132.

Holbech, H., Kinnberg, K.L., Brande-Lavridsen, N., Bjerregaard, P., Petersen, G.I., Norrgren, L., Orn, S., Braunbeck, T., Baumann, L., Bomke, et al. (2012). Comparison of zebrafish (Danio rerio) and fathead minnow (Pimephales promelas) as test species in the Fish Sexual Development Test (FSDT). Comp. Biochem. Physiol. 155 C,407-415.

Braunbeck, T. and Walter-Rohde, S. (2013). Fish Embryo Acute Toxicity Test with Zebrafish (ZFET). ECVAM DB-ALM, http://ecvam-dbalm.jrc.ec.europa.eu.

Scholz, S., Sela, E., Blaha, L., Braunbeck, T., Galay-Burgos, M., García-Franco, M., Guinea, J., Klüver, N., Schirmer, K., Tanneberger, K, et al. (2013). A European perspective on alternatives to animal testing for environmental hazard identification and risk assessment. Regul. Toxicol. Parmacol. 67, 506-530.

2.23 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 2009
Communication between the extracellular matrix and the cell interior is essential for all organisms as intrinsic and extrinsic cues have to be integrated to coordinate development, morphogenesis, and behaviour. This applies in particular to plants, the growth and shape of which is governed by deposition and remodelling of the cell wall, a rigid, yet dynamic, extracellular network. To control growth, but also to respond to extrinsic perturbations, cell wall state is constantly monitored and information must be conveyed to the cell interior in order to fine-tune the physico-chemical properties of the wall for optimal responses. However, very little is known about this cell wall-mediated signalling in plants. Using targeted modification of the major cell wall component pectin, we have identified a novel feedback signalling pathway connecting cell wall surveillance with brassinosteroid (BR) hormone signalling. In case of cell wall perturbation, BR signalling is activated and orchestrates a response protecting against the loss of cell integrity by compensatory upregulation of cell wall biosynthesis and remodelling enzymes. Accordingly, correct BR hormone levels and signalling strength were found to be essential for maintaining cell wall integrity. In, addition we have identified the first component of the pathway connecting the cell wall and BR signalling in CNU2, a receptor-like protein located at the plasma membrane. CNU2 interacts with the BR receptor complex and is sufficient to trigger signalling activation. We currently investigate whether CNU2 is able to interact also with the cell wall or whether it receives information from yet unidentified upstream components.

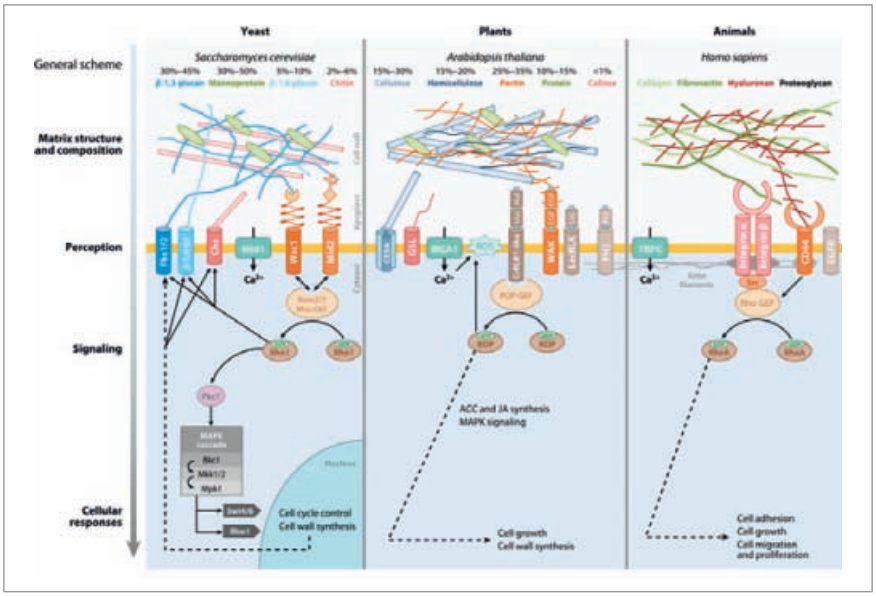

Figure 1
Schematic illustration of the differences and similarities found in cell wall/extracellular matrix integrity pathways across kingdoms. Reproduced from (Wolf et al., 2012a).

Major contributions since 2009

Plant growth is largely controlled by synthesis, deposition and modification of the polysaccharide-rich cell wall. Thus, coordination of growth and development with external cues poses unique challenges to plants: Cell wall extensibility has to be balanced with appropriate resilience to maintain cell integrity during growth, the relative amounts of the individual cell wall components have to be controlled, the cells must account for extrinsic perturbations, and lastly, cell expansion must be coordinated among neighbouring cells. Plants thus possess dedicated cell wall signalling pathways which sense the state of the wall, transduce information to the cytosol and, if necessary, implement a compensatory response, for example through, but not limited to, changes in gene expression (Wolf et al., 2012a).

Recently, we have shown that interference with modification of the major cell wall component pectin triggers the activation of the well-characterized brassinosteroid (BR) hormone signalling pathway, which in turn orchestrates a compensatory response involving cell wall remodelling (Wolf et al., 2012b). In the absence of BR-mediated feedback signalling, altered pectin modification severely compromises cellular integrity, ultimately resulting in cell rupture, demonstrating the relevance of cell wall surveillance and feedback signalling.

Having established the existence of a connection between pectin surveillance and hormone signalling, we have recently begun to identify the molecular components of this novel pathway. Through the use of a forward genetic screen we have isolated a mutant (cnu2) in a receptor-like protein essential for the cell wall-induced activation of the BR pathway. In turn, this receptor-like protein is sufficient to induce brassinosteroid signalling activation in the absence of cell wall stress, as constitutive overexpression results in a phenotype identical to plants expressing the brassinosteroid receptor BRI1 under the same promoter. Using genetic, biochemical, and transcriptomic approaches, we demonstrated that CNU2 is not itself part of the BR pathway, but instead conditionally activates hormone signalling when triggered by cell wall-related cues. Furthermore, our data suggest that CNU2 directly interacts with components of the BR receptor complex, providing a mechanism for signalling integration (Wolf et al., in revision). We now focus on elucidating how cell wall changes are perceived, on determining how exactly BR signalling is activated, and on characterization of the downstream responses with respect to changes in mechanical properties that are caused by cell wall signalling.

In contrast to the main load-bearing cell wall component cellulose, which is synthesized by membrane-localized cellulose synthase complexes, hemicellulose and pectin, the main cell wall matrix polysaccharides, are synthesized in the Golgi apparatus and transported to the cell wall in secretory vesicles. Traditionally, research has focused on cell wall biosynthesis, however, carbohydrate depositioning and cell expansion are not strongly coupled (Wolf et al., 2012a). Instead, reorientation and modification of existing polymers is a characteristic feature of plant growth. A well-studied example of polysaccharide modification is the selective de-methylesterification of pectic homogalacturonan (HG). HG is secreted to the cell wall space highly substituted with methyl groups, which can later be removed by the ubiquitous enzyme pectin methylesterase (PME). Depending on the pattern of methylesterification, and thus, the mode of action and activity of PMEs, plant cell wall properties change dramatically. Notably, pectin de methylesterified by PME (pectate) can form Ca^{2+}-mediated crosslinks (»egg-boxes«) in the wall and controlling the amount of these crosslinks is thought to be essential for the cell's ability to undergo turgor-driven mechanical deformation (Wolf and Greiner, 2012).

The findings outlined above indicate that PME activity has to be tightly controlled. This is especially pertinent given the possibility that pectins and PMEs might be transported in the same secretory vesicles. Indeed, we have observed tight regulation of specific PME isoforms at the transcriptional and post-transcriptional level, which plays an important role in controlling growth transitions (Pelletier et al., 2010; Wolf et al., 2012b). In addition, we have identified proteolytic processing as an alternative way in which PME activity can be regulated. Most PMEs are synthesized as pre-pro-proteins with a signal peptide and an (inhibitory) pro-domain, however, only the mature protein is found in the apoplast. Using cell biological tools in combination with genetics and site-directed mutagenesis we have shown that PMEs are processed at two conserved basic amino acid motifs, in a proteolytic event that at least partially involves Golgi-localized site-1 protease (S1P). Interestingly, unprocessed PME is retained in the Golgi and prevented from being transported to the apoplast, representing a possible mechanism for preventing premature PME activity on pectin en route to the cell wall (Wolf et al., 2009b).

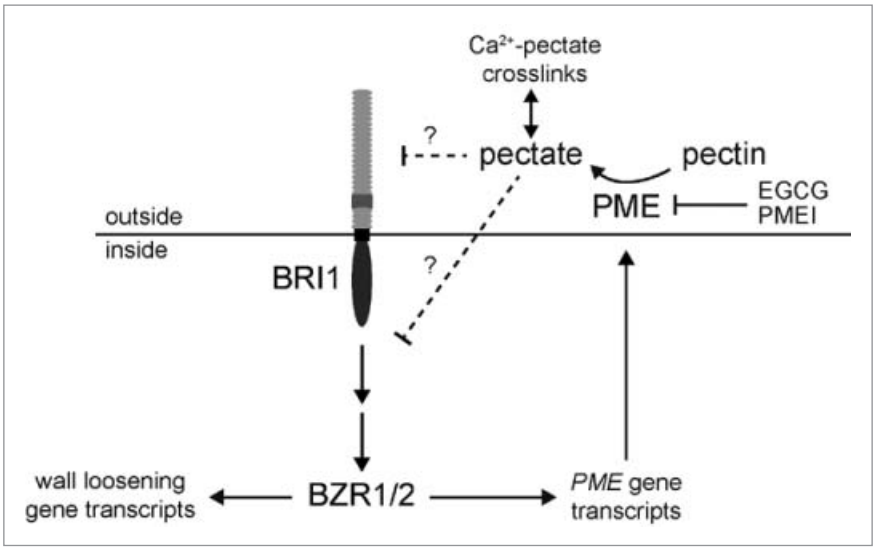

Figure 2
Plant cell wall homeostasis is mediated by brassinosteroid feedback signalling. Interference with pectin de-methylesterification producing pectate elicits activation of BR signalling, which in turn prevents loss of cell wall integrity by compensatory upregulation of cell wall remodelling genes. Reproduced from (Wolf et al., 2012b).

Planned research and new directions
The long-term goal of the group (established in October 2013) is to unravel how the cell wall is remodelled in response to developmental and extrinsic cues, and to determine how these cues are perceived. Thereby, we would like to contribute to answering one of the fundamental questions of plant morphogenesis: How do plant cell walls combine extreme tensile strength with remarkable extensibility to enable development adapted to environmental conditions? Using the newly discovered link connecting cell wall surveillance and brassinosteroid signalling as a stepping stone, we will concentrate on the following objectives:

i) To characterize the molecular events in cell wall perception and signalling
Specifically, we will study how BR signalling is activated in response to cues from the cell wall. We have already obtained evidence for the involvement of multiple components of the BR receptor complex and their kinase activities. In addition, we would like to understand how changes in the cell wall are perceived and ultimately determine the actual ligand for the signalling pathway mediating the response to changes in pectin modification.

ii) To unravel the role of Cell wall feed-back signalling in the maintenance of cellular mechanics and growth control
Using loss-of-function mutants in cell wall signalling components, we will study the role of this signalling module in normal development, i.e. in the absence of cell wall stress application. Activation of cell wall signalling and, consequently, BR signalling has dramatic effects on cell wall properties and on cell morphology (unpublished results). We plan to first describe in detail changes in cell wall properties elicited by BR-mediated feedback signalling and correlate this to changes in cell morphology and directional growth. Through a transcriptomic approach we already have a list of candidate genes possibly involved in the downstream effects of cell wall signalling and we intend to use gain-of-function and loss-of-function mutants to study their roles in compensating for cell wall alterations.

iii) To identify novel cell wall signalling components
Taking advantage of several unbiased screening approaches we have initiated, we want to identify novel components of cell wall signalling. In the first phase we will concentrate on the putative pectin surveillance pathway we identified, focusing on whether additional putative extracellular components can be isolated. Later, we also want to explore other pathways, possibly connecting different cell wall polymers or deduced properties, e. g. membrane stretching and mechanical stress, with intracellular signalling.

Selected publications since 2009
Number of peer-reviewed articles 2009-2013: 9, number of citations 2009-2013: 452, h-index (2009-2013): 7, total h-index: 11 (according to Thomson Reuters).

Wolf, S., Hematy, K., and Hofte, H. (2012a). Growth control and cell wall signaling in plants. Annu Rev Plant Biol 63, 381-407.
Wolf, S., Mravec, J., Greiner, S., Mouille, G., and Hofte, H. (2012b). Plant cell wall homeostasis is mediated by brassinosteroid feedback signaling. Curr Biol 22, 1732-1737.
Pelletier, S., Van Orden, J., Wolf, S., Vissenberg, K., Delacourt, J., Ndong, Y.A., Pelloux, J., Bischoff, V., Urbain, A., Mouille, G., et al. (2010). A role for pectin de-methylesterification in a developmentally regulated growth acceleration in dark-grown Arabidopsis hypocotyls. New Phytol 188, 726-739.
Wolf, S., Mouille, G., and Pelloux, J. (2009a). Homogalacturonan methyl-esterification and plant development. Mol Plant 2, 851-860.
Wolf, S., Rausch, T., and Greiner, S. (2009b). The N-terminal pro region mediates retention of unprocessed type-I PME in the Golgi apparatus. Plant J 58, 361-375.


Setup
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FACILITIES

3

3.1 NIKON IMAGING CENTER
LIGHT MICROSCOPY FACILITY

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

Brief summary of work since 2009
The Nikon Imaging Center at the University of Heidelberg (NIC) located in the Bioquant supports around 100 research groups on campus in light microscopy applications. It was initiated in 2004 by Dr. Holstein (COS) as a partnership with the company Nikon (as defined by a contract between the University of Heidelberg with Nikon 2005) to provide cutting edge imaging equipment and expertise. Most of the instruments are sponsored by Nikon, additional instrumentation has been added by equipment grants of CellNetworks (DFG funded) to provide a comprehensive choice of light microscopy techniques (see Table 1). A team of 3 postdoctoral scientists headed by Dr. Ulrike Engel trains researcher on equipment and gives guidance in assay development and data analysis. Once they have completed a training, researchers gain access to instrumentation 24/7 through an online booking system.

Table 1 gives an overview over current instrumentation available at the NIC. The main focus lies on live microscopy and especially on live confocal 3D imaging of cells or organisms containing fluorescent markers. Applications range from single cell or cell free systems to small organisms. For example, we have successfully imaged single microtubules to investigate their dynamics (Marx et al. 2013), imaged neuronal development over 4 days by 2-photon microscopy (Beretta et al. 2013, Dross et al. 2014), elucidated components necessary for cell division in yeast (Figure 1, Kammerer et al. 2010) and mammalian cells (Zhu et al. 2013) by time lapse microscopy. Live imaging is complemented by studies on fixed immunostained specimen by confocal microscopy, which many developmental studies depend on (Hassel et al. 2010, Shaw et al. 2010 Boy et al. 2010, Herrmann et al. 2012, Thelen et al. 2012). In addition we use laser light to manipulate the sample, e. g. by use of photoswitchable proteins (Kammerer et al. 2010), or cell ablation (Beretta et al. 2013). Equipment for FRAP studies to assay protein mobility (Brandt et al. 2009) and FRET (Kim et al. 2010) is also available. Superresolution microscopy was added to the NIC with a structured illumination microscope, which improves resolution by a factor 2 over conventional microscopy and was used to study actin morphology in neurons (Marx et al. 2013).

Table 1: Microscopes available in the Nikon Imaging Center

Type	Number of instruments	Short description
Inverted wide field fluorescence microscope	2	Automated inverted microscope for time lapse acquisition with perfect focus system (Nikon Ti), 7 channels (multiple fluorescent proteins, FRET, Fura). Cameras include EMCC (Andor Xion) and dual sCMOS (2 Andor sNEO on TuCAM).
TIRF	1	Total internal reflection fluorescence (TIRF) microscope with triggered acquisition and single molecule sensitivity. Laser lines: 488 nm, 561 nm, 640 nm
Wide field FRET-FLIM	1	Fluorescence Life Time Imaging Microscopy (FLIM) in the frequency domain on a sensitized CCD camera. Speed at 1000x1000 pixel resolution up to 1 fps. 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 (Ti) with perfect focus and and multipoint acquisition. Laser lines: 405, 488, 514, 561 nm. On A1R resonant mode and simultaneous photactivation and imaging as well as spectral detector are available.
Spinning disc confocal micro-scopes	2	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 micro-scope on intravital microscospe	1	2-photon system (LaVision Biotec) on upright fix-stage microscope (Nikon FN-1) for physiological deep-in-tissue observation. The 2 photon systems allows for fast multi-beam scanning (camera-based) at 60 fps or single beam detection on non-descanned highy sensitive PMT ports. Exciation of UV-dyes (fura) up to red fluorescent proteins (e. g. mCherry).
Structured Illumina-tion for superreso-lution	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 (37°C, 5 % CO_2) enclosed microcope with multipoint acquisition for fluorescence and brightfield observation over up to 100 hours (Nikon Biostation), 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 multipoint and stitching.
Macroscope	1	Monozoom macroscope for fluorescence imaging and differential interference contrast for manipulation and imaging of embryos. Can be used in confocal mode.

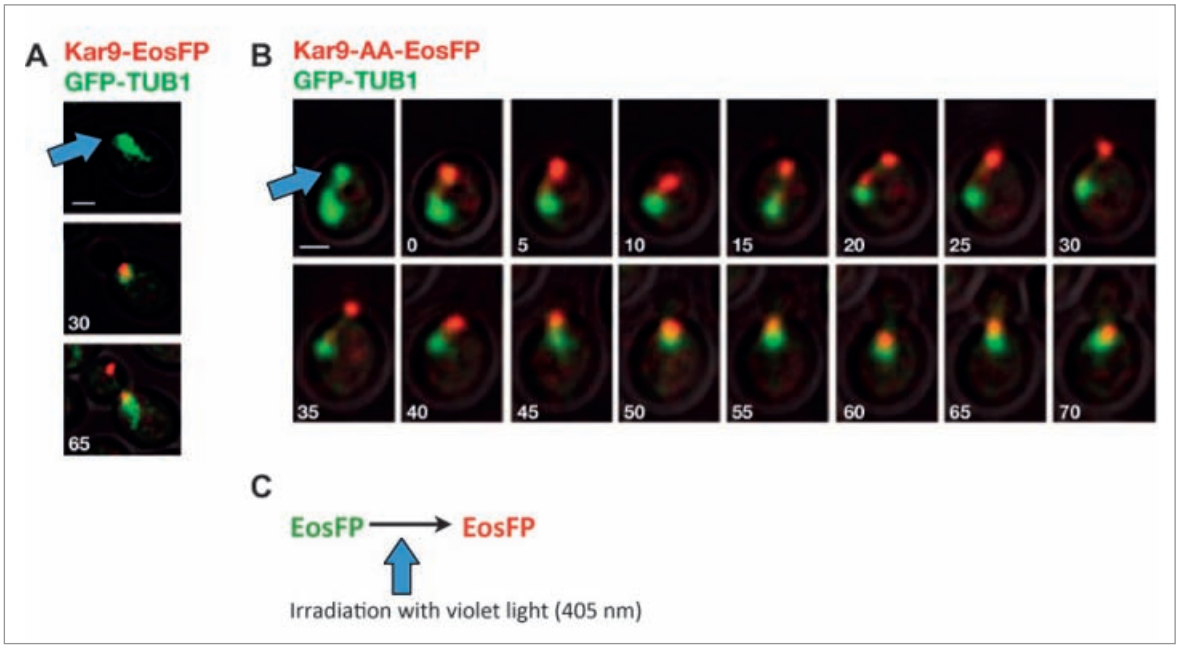


Figure 1
Role and distribution of Kar9 (APC homologue) in yeast mitosis. A photoconvertible fluorescent protein (EosFP) was used to highlight wildtype (A) or a mutated form of Kar9. Photoconverted protein in the mother cell translocates into the in wildtype (A) but not in the phosphorylation mutant (B). (C) EosFP is converted by 405 nm laser excitation in a diffraction limited point (indicated by a blue arrow. Figure modified from Kammerer *et al.* (2010).

Major contributions

A 2-photon microscope was acquired in 2008 for imaging applications that required high penetration depth. Dr. Dross supports users at this instrument. Together with the Carl group, he established longterm imaging in the zebra fish brain, that allowed to follow neuronal migration and axonal projections over 4 days Fig. 1 (Beretta *et al.*, 2013; Dross *et al.*, 2014). Ablation of single cells or small clusters by laser illumination was then used to demonstrate their role in axon guidance. In the same study, photoconversion allowed to highlight specific cells to study their migration path (Beretta *et al.*, 2013).

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. For this we combined fast confocal microscopy (by spinning disk confocal microscopy) with deconvolution (Romero-Brey *et al.* 2013). Spinning disk microscopy was applied on cytoskeletal dynamics in Dictyostelium (Clarke *et al.*, 2006; Gerisch *et al.*, 2009; Clarke *et al.*, 2010a; Clarke *et al.*, 2010b; Heinrich *et al.*, 2014) and in many studies on viral organization and assembly (Feederle *et al.*, 2009a; Feederle *et al.*, 2009b; Stolp *et al.*, 2009; Welsch *et al.*, 2009; Backes *et al.*, 2010; Reiss *et al.*, 2011; Baldauf *et al.*, 2012; Romero-Brey *et al.* 2013; Meier *et al.*, 2013).

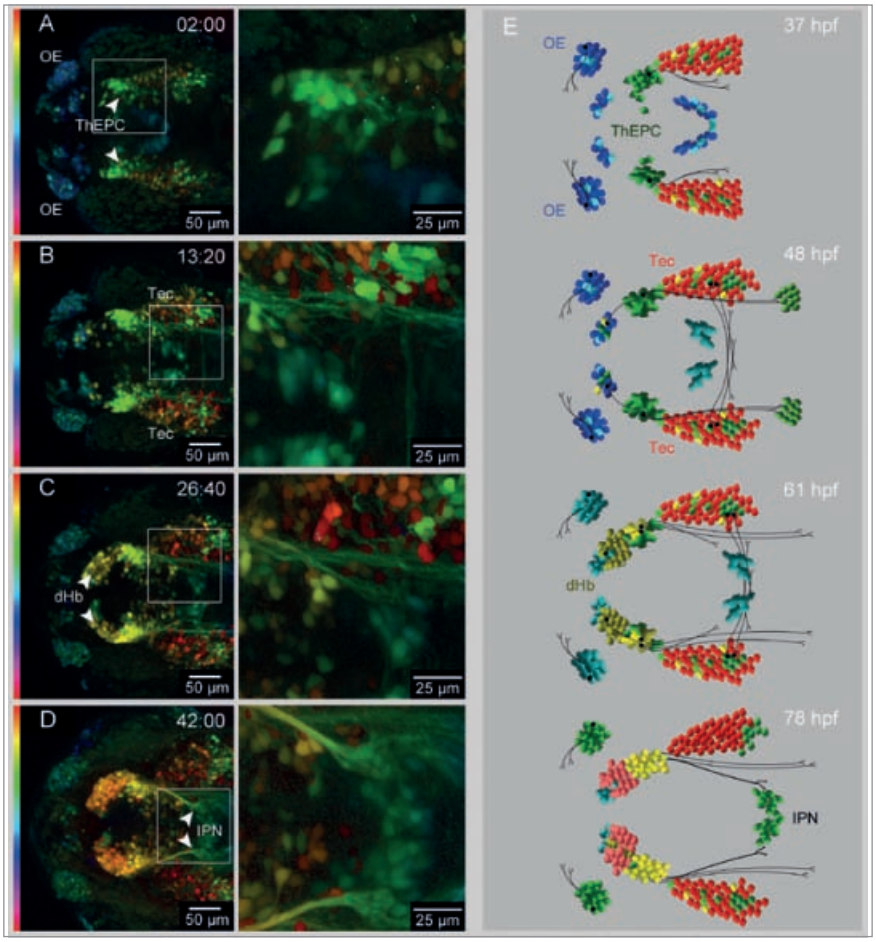


Figure 2
Long-term 2-photon *in-vivo* recording of zebrafish brain development. (A-D) Dorsal overview, anterior to the left of *Et(-1.0otpa:mmGFP)* hd1 transgenic embryos. Recorded between 37 hpf and 78 hpf with corresponding magnification of different brain areas (middle) and (E) summary of the main events during brain development. 300µm image stacks were acquired every 40 minutes, of which depth-color-coded projections of 4 different developmental stages are shown. Hb, habenula; IPN, interpeduncular nucleus; OE, olfactory epithelium; Tec.

Calcium dynamics are associated with many signalling processes. We use Fura-2 imaging and genetic encoded sensor to monitor calcium fluctuations. This application is a typical example for transfer and growth of expertise in the NIC. Fura-2 calcium imaging was initially established in collaboration with the Frings group (COS) in a cell culture system. Since then it was applied to calcium oszillations in primary fish cells (Schweizer *et al.*, 2011) and in two independent studies in human T-cells (Hubner *et al.*, 2013; Schwarz *et al.*, 2013).

In 2009 a HMLS funded project allowed us to improve image analysis capacities. With a own dedicated server for image deconvolution, computation was accelerated and a web access for deconvolution provided (Huygens remote manager). Dr. Bankhead provided custom analysis pipelines in ImageJ for a number of projects (Abraham *et al.*, 2012; Hofmann *et al.*, 2012; Ruggieri *et al.*, 2012; Beretta *et al.*, 2013; Marx *et al.*, 2013; Dross *et al.*, 2014) and training on image analysis in courses.

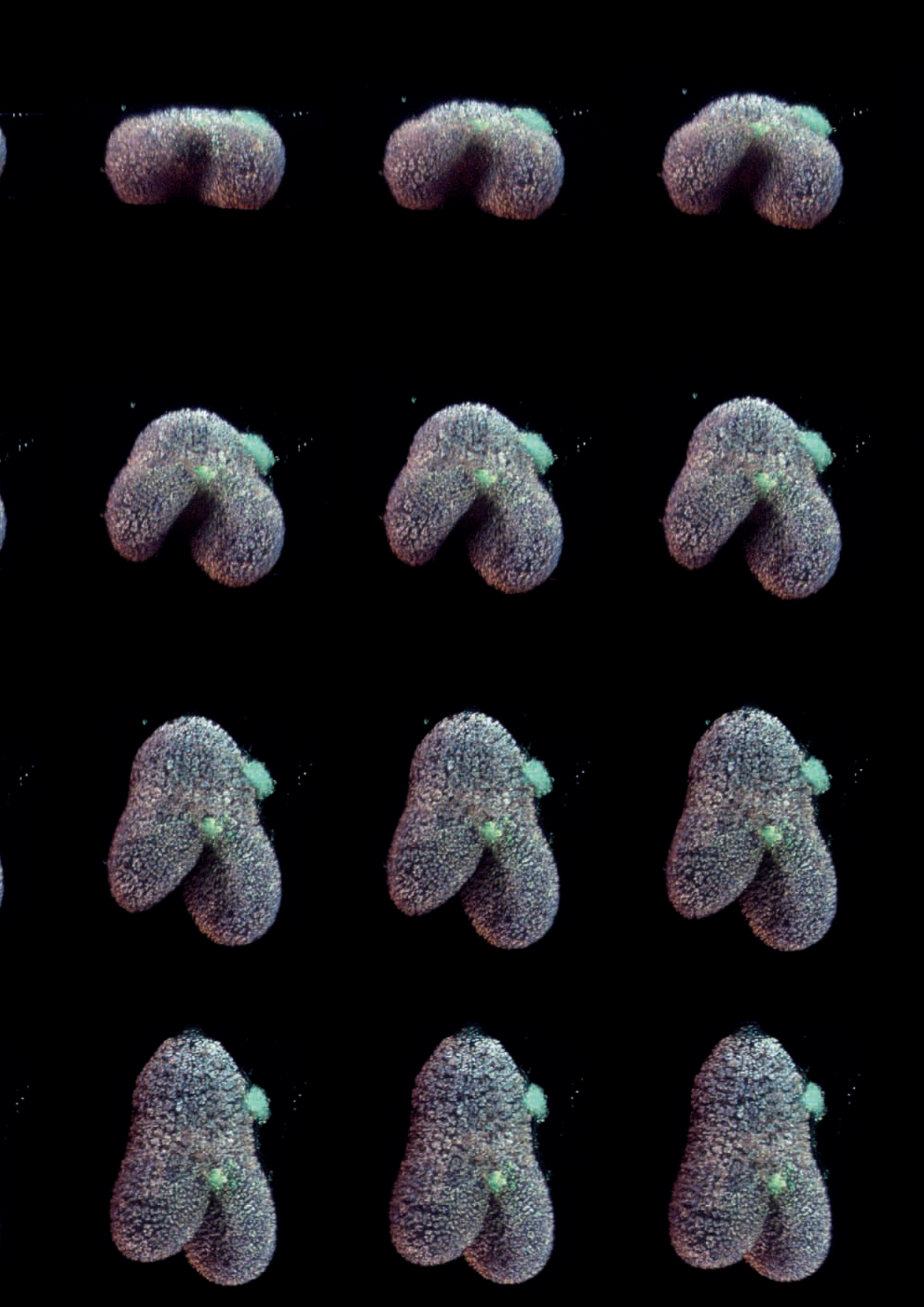
Planned research and new directions

We are currently working on automating high content imaging. We successfully run time lapses in 96-well plates over 3 days to follow the cell cycle with the fluorescent reporter FUCCI. Acquisition can be guided by image analysis: during a low resolution scan of the 96 well plate positions of interesting cells can be identified for high content imaging. Another focus is the imaging of custom developed artificial substrates to challenge the cells with a defined biophysical stimuli (Yoshikawa et al. 2011) or microfluidic chambers for chemical stimulation (Holstein group).

In the framework of the collaborative research center 873 (Maintenance and Differentiation of Stem Cells in Development and Disease) we will start a closer collaboration with several COS groups. This includes 2-Photon microscopy, FRET by fluorescence life time imaging microscopy (FLIM) and clonal analysis of cells over > 1 week (Cetanin group, COS).

Selected publications since 2009

Beretta, C.A., Dross, N., Bankhead, P., and Carl, M. 2013. The ventral habenulae of zebrafish develop in prosomere 2 dependent on Tcf7l2 function. Neural Dev 8, 19.
Brandt, D.T., C. Baarlink, T.M. Kitzing, E. Kremmer, J. Ivaska, P. Nollau, and R. Grosse. 2009. SCAI acts as a suppressor of cancer cell invasion through the transcriptional control of beta1-integrin. Nat Cell Biol. 11:557-568.
Kammerer, D., L. Stevermann, and D. Liakopoulos. 2010. Ubiquitylation regulates interactions of astral microtubules with the cleavage apparatus. Curr Biol. 20:1233-1243.
Yoshikawa, H.Y., Rossetti, F.F., Kaufmann, S., Kaindl, T., Madsen, J., Engel, U., Lewis, A.L., Armes, S.P., and Tanaka, M. (2011). Quantitative evaluation of mechanosensing of cells on dynamically tunable hydrogels. Journal of the American Chemical Society 133, 1367-1374.
Zhu, M., F. Settele, S. Kotak, L. Sanchez-Pulido, L. Ehret, C.P. Ponting, P. Gonczy, and I. Hoffmann. 2013. MISP is a novel Plk1 substrate required for proper spindle orientation and mitotic progression. J Cell Biol. 200:773-787.



3.2 DEEP SEQUENCING CORE FACILITY

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

Personnel and techniques involved
The DeepSeqLab is run by scientific (Dr. Gabriele Petersen) and technical staff (David Ibberson, M. Sci. Claudia Müller) and is currently located in the Bioquant building (INF 267). The facility offers the ability to prepare for users, libraries for Illumina Sequencing. In addition to facilitate library preparation it houses several instruments which are also available for use by the community (QuBit fluorometer, and Covaris S2 Sonification System, in addition access can be granted for the Agilent BioAnalyzer 2100 please contact us beforehand).

Service
The DeepSeqLab encourages interaction with its users, and provides assistance/support for the initial planning stages of your sequencing experiments. As the »bread and butter« services we offer users the following options:

Libraries
The DeepSeqLab can perform libraries for the following:
– RNA-Seq (as of June 2014 all libraries are strand specific)
– Small RNAseq
– Chip-Seq
– gDNA-Seq (de novo, resequencing)
  insert size: 150-600 bp or 2.5 – 5.0 kbp (mate pair)
  including genome capture
– methyl-Seq

In addition to the above mentioned libraries, the DeepSeqLab provides help and advice on »non-standard« applications, and will gladly provide assistance where it can. We are also open to new ideas and where possible try to incorporate them within our resumé.

Sequencing
The DeepSeqLab has access to a jointly owned HiSeq 2000 situated within the Genomics Core Facility of EMBL. This enables the following sequencing formats:
– 50 SE and PE, (Usually 150-200 million reads obtained)
– 100 SE and PE (please note 100 SE sequencing is a rarely used application and thus has long waiting times) (Usually 150-200 million reads obtained).

In addition there is access to the MiSeq at GeneCore with the following sequencing formats (all Paired End): 36, 75, 150, 250, 300 (Usually 12-18 million reads obtained).

Facility Growing from Strength to Strength
Thanks to our users the DeepSeqLab has grown from a humble beginning to a widely used facility year upon year.

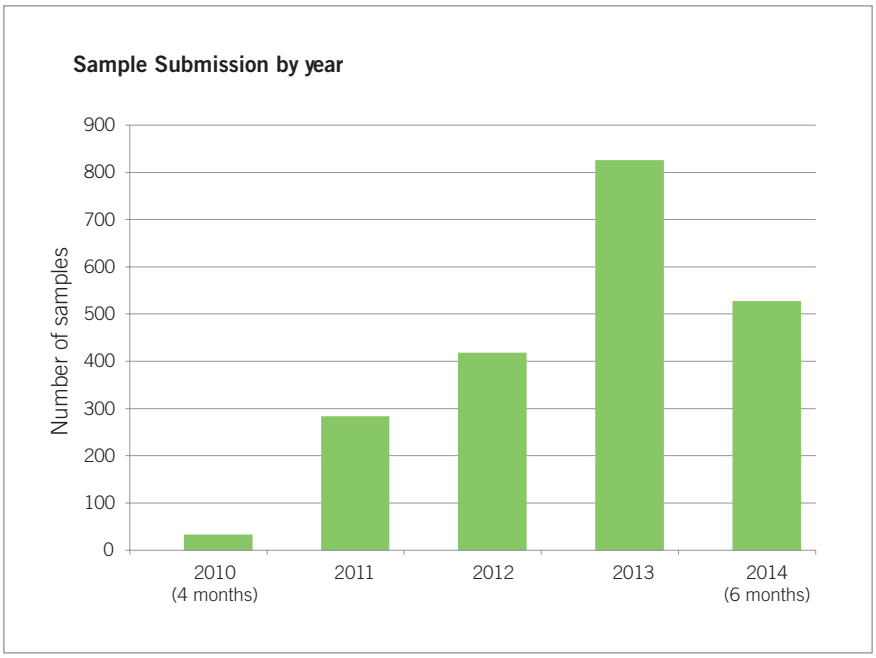


Figure 1
Graphic showing increase in sample submission.

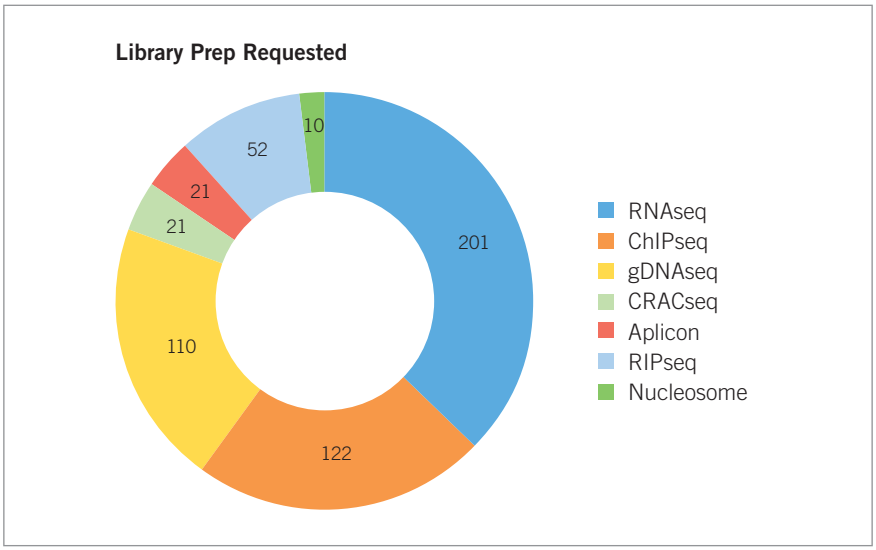


Figure 2
Graphic showing most requested library preps.

Who has access to the service?
The following clientele can enroll as user of the facility:
– Members of the University of Heidelberg, 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)

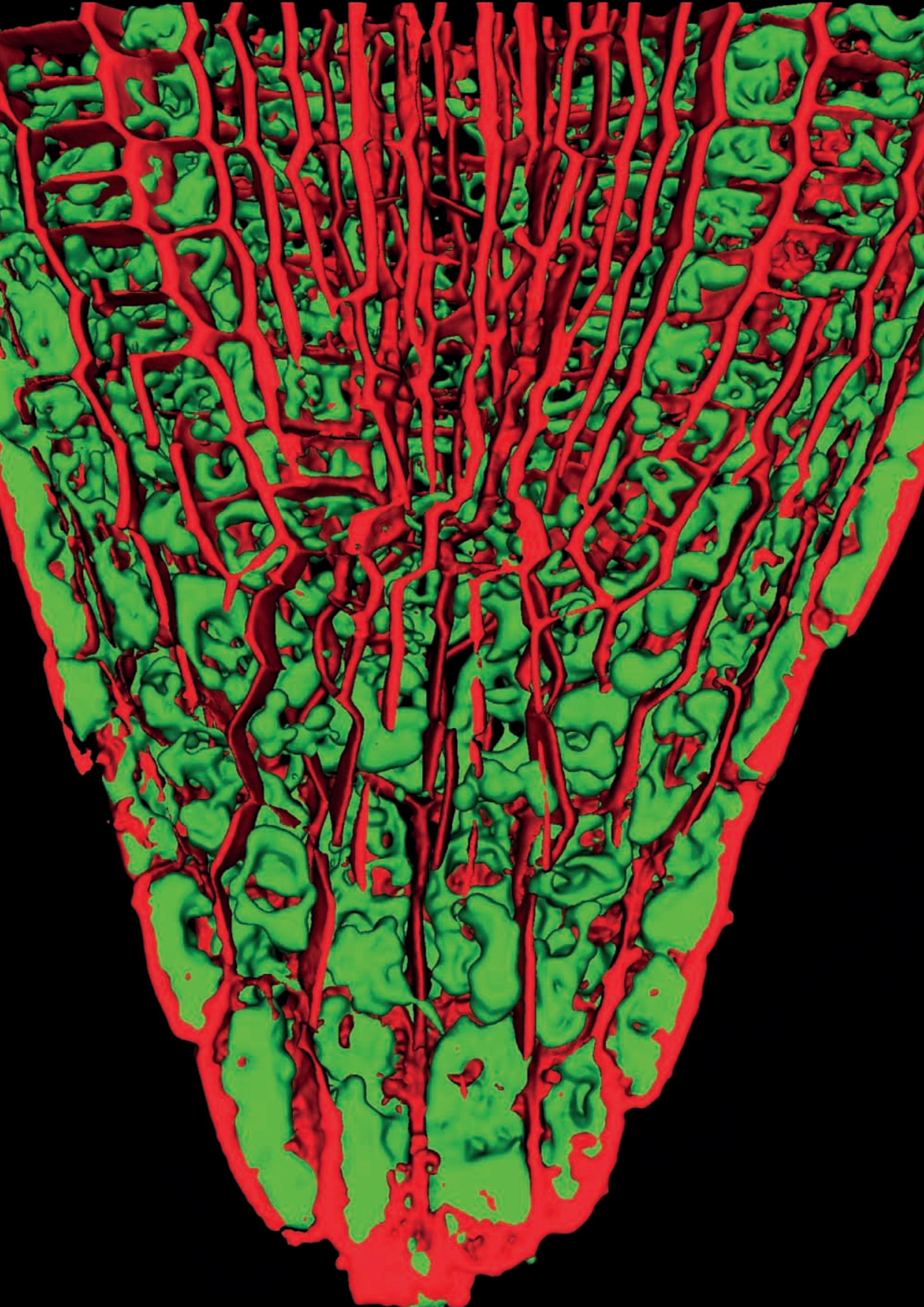
How to approach DeepSeqLab
Scientists interested in using the facility should contact us by mail:
deepseqlab@bioquant.uni-heidelberg.de

Fees
Users have to pay for all consumables, special EDV. Prices vary depending on whether or not a library prep is included, clustering is for paired end or not and last not least the read length. A detailed pricing list can be obtained upon request.

References
The following papers have recently been published using data generated by the facility:

Abeer Fadda, Valentin Färber, Dorothea Droll, Christine Clayton (2013) The roles of 3'-exoribonucleases and the exosome in trypanosome mRNA degradation. RNA *19*, 937–947.

Kathrin Leppek, Johanna Schott, Sonja Reitter, Fabian Poetz, Ming C. Hammond, Georg Stoecklin (2013) Roquin Promotes Constutitive mRNA Decay via a Conserved Class of Stem-Loop Recognition Motifs. Cell *153*, 869-881.



3.3 METABOLOMICS CORE TECHNOLOGY PLATFORM

Metabolomics
Core
Technology
Platform

Introduction

The Metabolomics Core Technology Platform (MCTP) was initiated by the Heidelberg Molecular Life Sciences Research Council in 2010 by funding of an LC-MS instrument and finally established by the Institutional Strategy of the University in the Excellence Initiative II program together with a junior research group »Plant Defense Metabolism« (Dr. Emmanuel Gaquerel). (MCTP) mission is to provide developmental and analytical services across the Heidelberg Molecular Life Sciences to improve the technical and scientific portfolio in Heidelberg. It is located in the Department of Molecular Biology of Plants within the Centre for Organismal Studies Heidelberg. It is directed by R. Hell and technically managed by M. Wirtz.

Personnel

A staff scientist (Dr. Gernot Poschet) was hired on 01.01.2013 and a staff technician (Dipl.-Ing (FH) Mrs. E.-M. Käshammer-Lorenz) on 01.10.2013.

Name	Nationality	Status at COS	Funding
Poschet, Gernot	German	Postdoctoral fellow	Excellence Initiative II
Käshammer-Lorenz, Eva-Maria (50 %)	German	Research technician	Excellence Initiative II

Service

MCTP is represented online and linked to the CellNetworks core facilities and COS webpages. According to its mission MCTP assists researchers in the development of innovative and tailor-made solutions to metabolomics challenges in their projects. Generally targeted (mostly liquid chromatography) and untargeted analyses (usually mass spectrometry based) are offered. Upon contact, the projects of the users are discussed with respect to biological system, statistical variability, sample preparation and strategies for metabolite analysis. Sample extraction, development of methods, measurements and initial data analysis are provided by the staff of MCTP. The facility is not available for large-scale routine or applied measurements or external professional services. MCTP is essentially fully booked and meets its profile already very well. Not only the untargeted metabolomics approaches, but unexpectedly also the requests for targeted analyses of metabolites by conventional liquid chromatography are quite high. These are currently met using equipment from COS and a new UPLC/multiple detector combination recently funded by combined CellNetworks and HMLS equipment programs. However, the requests are so massive that more instrumentation for liquid chromatography is urgently needed. In summary, the implementation of the MCTP facility in the 18 months of its existence went quite satisfying and the demand exceeds the existing workforce and instrumentation already by far.

Financing

Basic funding and resources are provided by Excellence Initiative II (ZUK 1.2. Metabolomics Platform). The budget cut of Excellence Initiative II enforced a redirection of planned measures. Emphasis was given on the funding of the junior group (about 2/3 of the platform's budget; 1/3 for MCTP). Therefore, MCTP abstained from the acquisition of an urgently needed LC-MS/MS QTOF instrument to balance the budget.

MCTP currently operates with equipment provided by Heidelberg Molecular Life Sciences Research Council, CellNetworks and research groups of COS (Departments of Plant Molecular Biology and Plant Molecular Physiology). These groups also provide rooms for the MCTP and maintenance of analytical systems. An operating procedure according to DFG recommendations, ordering and internal payment schemes (in accordance with the campus Mass Spectrometry Commission) were established.

The limitations of MCTP are now to hire more manpower (scientific and technical) and to enhance the analytical portfolio. The latter refers first to the originally requested LC-MS/MS QTOF instrument. Second, an additional ion chromatography system is necessary to meet the demands by users. The required funds cannot be created by MCTP itself, since user fees are based on net cost prices. Without additional funding the MCTP facility can neither improve scientifically nor serve more users. The requests for this are clearly there, and it will be a challenge for MCTP to find further funding during 2014.

Projects

Users so far are evenly distributed across Heidelberg Molecular Life Sciences campus: COS 35 %, Medical Faculty 20 %, Centre for Molecular Biology Heidelberg (ZMBH) 18 %, National Cancer Research Centre (DKFZ) 15 % and other institutes 12 % (e. g. Biochemistry, Pharmacy) (Fig. 1).

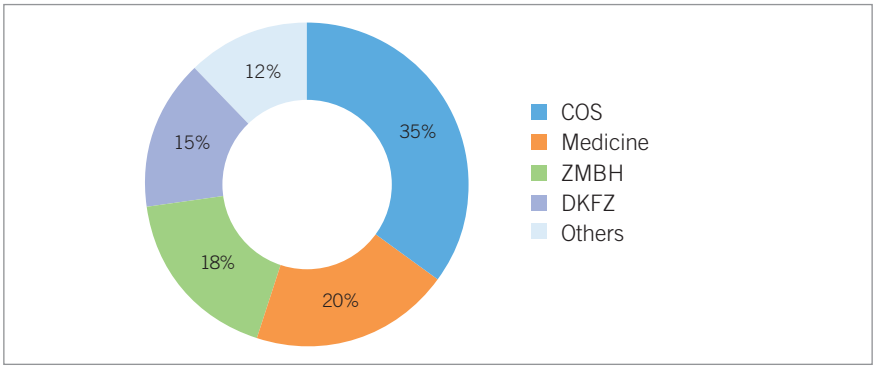


Figure 1
Distribution of analyses across HMLS users.

The requests for specific targeted metabolite analyses are very diverse across Heidelberg Molecular Life Sciences users covering all major classes of primary metabolism:

Amino acids 24 %, sugars 22 %, anions and organic acids 12 %, thiols 11 %, adenosines 8 %, cations 7 % and additional metabolites 16 % (e. g. urea, branched keto-acids) (Fig. 2). In total, 94 compounds can be identified and quantified at present. This number is constantly increasing, since many new projects raise specific demands for metabolites for which new protocols are developed.

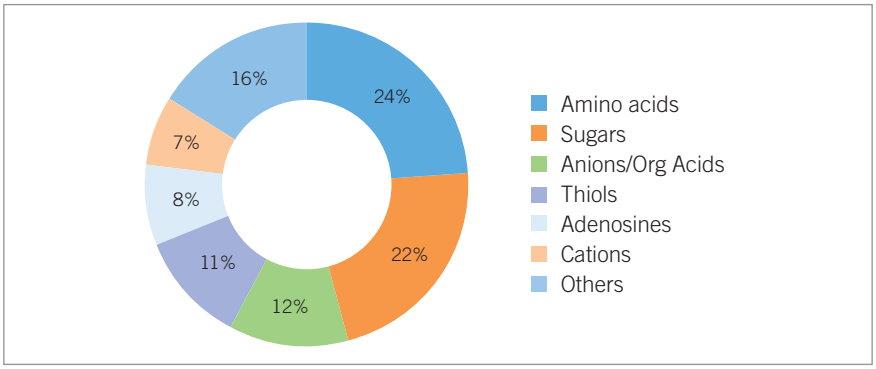


Figure 2
Distribution of targeted analyses between compound classes.

Publications

The Metabolomics Core Technology Platform is currently acknowledged in the articles below. Since many projects are in progress this number is expected to rise strongly during the next two years.

Demetriades, C., Doumpas, N, Teleman, A.A. (2014). Regulation of TORC1 in response to amino acid atarvation via lysosomal recruitment of TSC2. Cell 156 (4): 786-799.

Krüßel, L., Junemann, J., Wirtz, M., Birke, H., Thornton, J.D., Browning, L.W., Poschet, G., Hell, R., Balk, J., Braun, H.-P. Hildebrandt, T.M. (2014). The mitochondrial sulfur dioxygenase ETHE1 is required for amino acid catabolism during carbohydrate starvation and embryo development in *Arabidopsis thaliana*. Plant Physiology (in press).

Photos of equipment

Figure 3
H-class UPLC system coupled to PDA-, FLR- and QDA-detector (Waters). Used for targeted analyses.

Figure 4
I-class UPLC system coupled to Xevo qTOF (Waters). Used for untargeted metabolomics.

3.4 BOTANICAL GARDEN

History

The Heidelberg University 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 – including a famous orchid collection at that time – was lost due to combat activities. The collections of the Garden were highly enlarged by the tireless collecting activities of Werner Rauh – director of the Garden from 1960 to 1982 – who was interested particularly in bromeliads and succulents, especially Madagascar plants, and Karlheinz Senghas – custodian of the Garden from 1960 to 1993 – who built up a collection of ca. 3,000 tropical orchid species. These historic collections are still the core of the living specimens of the Heidelberg Botanic Garden. The collection of 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« originating from the early 19th century includes the private collections of Gottlieb Wilhelm Bischoff – the first Professor of Botany in Heidelberg – and of his famous successor Wilhelm Hofmeister. Important parts of the »new herbarium« are 50,000 specimens, especially cacti, bromeliads, orchids and tropical ferns, collected between 1950 and 1980 by Werner Rauh, Karlheinz Senghas and Werner Hagemann. Also approximately 25,000 vouchers have been contributed by the research activities of its current director, Marcus Koch, during the last 10 years. The Herbarium HEID encompasses ca. 2,300 type specimens.

Mission, Objectives & Vision

The Mission of the Heidelberg Botanic Garden is the conservation and development of its collections and its usage to promote the discovery, understanding, responsible use and enjoyment of the plant kingdom. The living collection with ca. 15,000 accessions and the 350,000 specimens in the Herbarium are among the most important plant biodiversity archives in Germany today and are actively used in scientific research programmes of internationally recognized excellence. 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 and its teaching and outreach programmes. The Garden's vision is to be widely acknowledged as an outstanding plant collection in Germany, valued by stakeholders as major scientific research facility and a centre for innovative public engagement with plant science via its collections and expertise.

General collection management and development policy – Living Collection

The collections – kept in greenhouses or outdoor gardens – meet research, educational, cultural, or conservation needs and can be divided into specialized and non-specialized collections. Our specialized collections are of a size and significance that merits national and international recognition, ideal suited to research: orchids (ca. 3,600 accessions), bromeliads (ca. 2,250 accessions), and succulent plants (3,600 accessions). Smaller non-specialized collections contribute to the diversity of the collections in general and are used primarily 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). Duplicates are generally avoided, whenever possible. The documentation of all accessions is recorded in the database »Gartenbank« (currently ca. 15,000 accessions) and all plants have a collection label, containing at least a collection number and a full scientific name. Specimens that are in conflict with the collection's criteria or unnecessary duplicates are continuously removed from the collection and 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 since 2007.

Figure 1
Succulents living collection.

Major Achievements & Activities during 2009 – 2013

Living collection development
The Heidelberg core collections (orchids, bromeliads, succulents) are currently under the special auspices of the Adminstrative District Gouvernmental Department at Karlsruhe. Consolidation of these core historic collections (plus modern additions) was an important part of our work during the last five years and is still an ongoing process. This process involved the inventory clearance of ca. 1,000 accessions following the guidelines established in our policy. However, valuable additions to the collection were also made, e. g. bromeliad accessions of direct wild origin. Regarding scientific curation ca. 700 new determinations or verifications, often by world leading experts of certain taxa, were possible. The documentation of ca. 1,000 historic accessions (»provenance research«) was significantly improved, especially in the context of the Werner Rauh Heritage Project (see below).

Herbarium collection development
Since 2003 a new collection has focussed on the mustard family (Brassicaceae) and in the period covered by this report the collection accessed several thousands new Brassicaceae vouchers and ca. 2750 seed accessions. In total ca. 25,000 vouchers were accessionized and currently ca. 120,000 of ca. 350,000 vouchers are recorded in the online accessible database »Gartenbank«. During the five last years 7,000 specimens were digitized. In 2009 1,221 digitized type specimens of African plants were also included in Aluka's African Plant Inititative (API) and Global Plant Initiative (GPI) online databases. In 2009 it was possible to install a professional quarantine facility for incoming specimens.

Figure 2
Spirit collection in the herbarium.

Material transfer for scientific purposes
As a member of the International Plant Exchange Network (IPEN) the Garden supplies material for research programmes conforming the Convention on Biological Diversity (CBD), in the period covered by this report material from ca. 1,440 accessions. This impressive number reflects the high scientific quality of our collections. These activities also led to a substantial number of external publications.

Support for Local Research Programmes
The Botanic Garden supports Heidelberg based research programmes with provision of plant material, test areas and horticultural expertise. The Scientific Plant Cultivation Service (SPCS) of the Garden cultivated in period under report yearly between 2,250 and 4,000 individual plants from a great variety of wild species, rarely cultivated elsewhere. This also led to a substantial number of SPCS based publications.

The Herbarium as a Research Facility
The Herbarium HEID is regularly visited by international scientists within their research activities and the Herbarium loans specimens for external research programmes. Moreover, professional loans from other international Herbaria via HEID are essential prerequisites for successful Heidelberg based evolutionary and biodiversity research.

Werner Rauh Heritage Project
The Werner Rauh Heritage Project is funded by Klaus Tschira Stiftung since late 2009 and will continue to mid of 2016: The project is based on a relational online database to store the heterogenous information found in field books and diaries with the aim to document the Rauh-collection data (25,000 herbarium specimens as well as 10,000 accessions of living plants) retrospectively; a leading-edge digitization and deep indexing project of its kind.

Academic Teaching
The Botanic Garden plays an important role in the academic teaching of COS Heidelberg: The Garden provided each year plant material for ca. 40 course days with ca. 250 students and every year academic courses with 700 students took place on ca. 40 days in the Garden's collections. The Herbarium is also integrated in teaching: The »Student's Herbarium« of plants (herbarium collection Marcus Koch), collected during field excursions during the last ten years incorporated more than 10,000 fully digitalized specimens.

Theses
The Garden provides plant material, experimental plants or test areas for a variety of theses. The numbers of Garden related theses submitted in the period of this report: 8 PhD theses, 1 diploma thesis, 4 master theses, 13 bachelor theses, and 13 state examination theses.

Public Education Programme
The Green School of the Botanic Garden represents a comprehensive outreach programme that has reached between 4,000 and 5,500 children and adults participating per year. The activities for children and teenagers contribute significantly to the programme of the Heidelberg Young University, which was introduced in 2007. In 2010 and 2011 the Heidelberg Rotary Clubs hosted two benefit concerts in support of the Botanic Garden, making it possible to convert a historic greenhouse into a multifunctional room for outreach, communication and education purposes (e. g. the Green School).

Figure 3
Teaching in the collections.

Visitors and Public Events
The Botanic Garden (gardens and greenhouses) are free and open to the public, the gardens being open 24 hours and ca. 2,000 m2 greenhouses at six days per week. Each year more than 50,000 visitors enjoy all the Heidelberg Botanic Garden has to offer. Importantly, hospital patients from the campus value the garden as a respite and an escape from their wards. In addition to 50,000 visitors per year ca. 10,000 attended several public events hosted by the Garden. The regular »GardenFests« were also an excellent public outreach opportunity for scientists belonging to the Centre for Organismal Studies (COS) Heidelberg and the Heidelberg Center for the Environment (HCE) of Heidelberg University. These events took place during the annual German-wide »Weeks of Botanical Gardens«, an official project of the United Nations Decade on Biodiversity. Besides the permanent presentations of the Gardens collections along with information boards and brochures, the Garden presented during the last five years three exhibitions developed by the Association of Botanic Gardens: »Darwin's Garden – Explore Evolution«, »Bionics in Botanic Gardens«, »Water for all of us«.

3.5 ZOOLOGICAL COLLECTION

Due to the necessary extensive renovation of the 50 year old building INF 230, the »Zoological Collection of Heidelberg University« is currently not accessible to the public.

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 the Zoological Collection is currently safely stored. With the completion of the renovation – expected in 2019 – 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 outsourced to the Senckenberg Research Institute and Natural History Museum in Frankfurt many years ago, there are still some remarkable specimens of the Zoological collection that are of general public interest. Many 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.

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. 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, which was extincted in 1936 (Figure 1).

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

APPENDIX

A

A.1 COS FUNDING

Finances COS Heidelberg

Funding of COS research and teaching is based on funding provided by the State Baden-Württemberg (Internal Funding via Heidelberg University) and increasing third party funds raised by COS research group leaders. The ratio between this external funding and the government sources increased from 1.37 in 2009 to 1.6 in the year 2013. From the government sources approximately 82 % are staff appropriations (2013), meaning that funds for equipment acquisition and other running costs are extremely scarce. Minor increment since 2009 is based on increase in standard wages, while disposable funds even decreased.

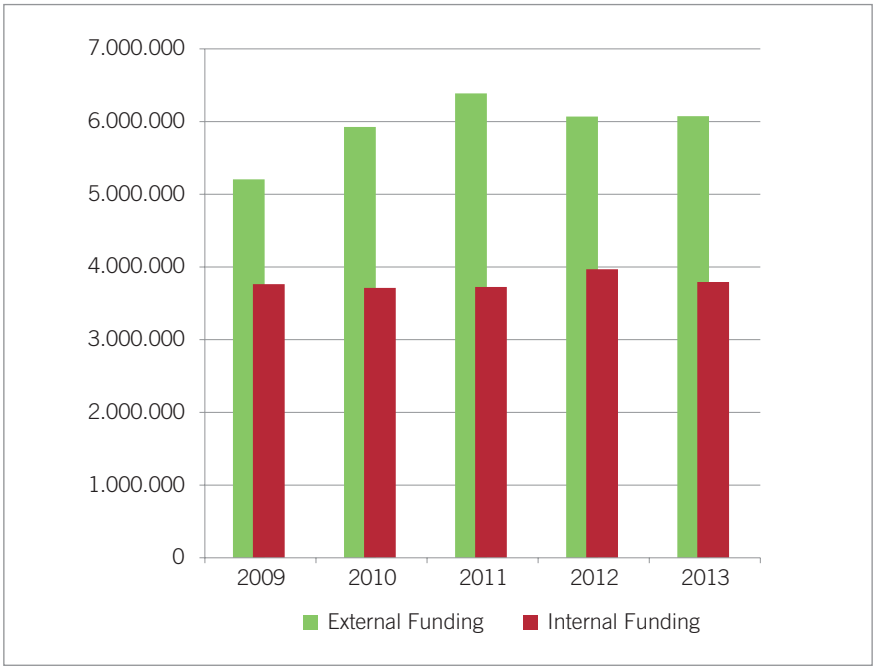


Figure 1
COS global finances: Government sources versus external funding in € p.a. from 2009 till 2013. Government sources without individual temporary commitments (Berufungszusagen) and funds from open positions (Mittelschöpfung). External funds without LGFG and DAAD grants. Numbers according to budget booklet of Heidelberg University (2009-2012) and SAP expenses (2013).

On the other hand third party funding increased significantly since 2009 (pre-COS). Main sources for third party funding are SFBs, DFG, BMBF, EU, Excellence Initiative, Foundations and others like industry. For further details please refer to figure2 below. As the compilation is based on expenses, recent increase in third party funding is not fully represented yet. For instance the number of independent research group leaders increased from 3 to 9 in the year 2013, but this will get fully operative at the level of expenses only in 2014. Similarly, there was a general holdup of funds in 2013 due to major reconstruction within COS.

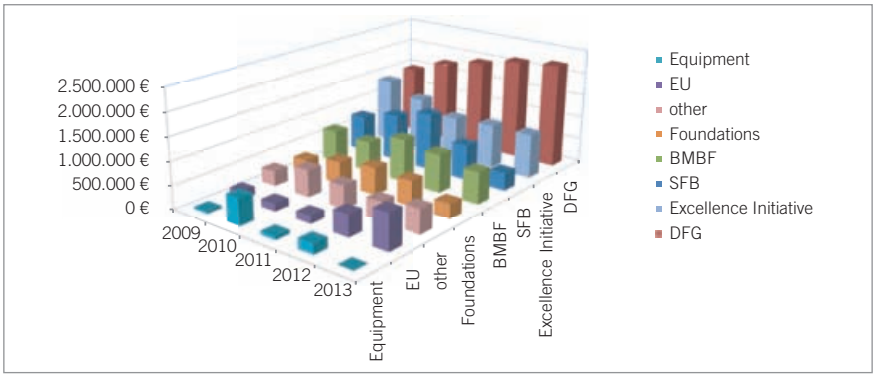


Figure 2
Detailed analysis of external funds 2009-2013. 2013 partially estimated (other: scholarships).

A.2 COS TEACHING

Teaching at COS Heidelberg
The teaching at COS Heidelberg reflects the aim of our centre to study organismic biology beyond the borders of organizational levels. Our research groups cover various fields of organismic biology, ranging from fundamental molecular and cellular biology to development, physiology, evolution and ecology. This platform allows us to design organismic teaching concepts and to implement them into Bachelor-, Teaching Degree-, Master- and PhD Bioscience programs of the University of Heidelberg.

Our aim is to transfer knowledge in organismal teaching concepts, which combine and compare biological mechanisms of plants and animals. This approach furthermore allows students to discover the intersecting mechanisms of plants and animals directly and thus to integrate the learning contents more efficiently. In practical terms this is reflected by the successful establishment of courses on general biology, physiology and development spanning the different realms of multicellular organisms. The aim is to provide a complete overview of the whole biological systems at various scales, starting atfrom the molecular and cellular level and finally reachingto whole organisms and their complex interactions with the environment.

The second key aspect of teaching at COS Heidelberg is a research-based approach of learning. Our aim is to link research and teaching as early as possible during the studies and therefore not only to allow the students to gain insights into their future scientific work but also to increase their motivation. Such a research-based approach on the one hand requires a design of authentic scientific questions and an introduction of current research topics and techniques into teaching. On the other hand, it involves a specific course design, which allows the students to develop their own questions and hypotheses, to design, perform and evaluate their own experiments as well as to present and discuss them in a social learning context. Current research topics are the basis for a high variety of courses and lab practicals at COS, beginning at the stage of Bachelor studies and encompassing projects from all research and independent junior groups. This environment has proven highly successful in motivating students to actively seek out their individual career paths based on personal interest for the research areas encountered.

Furthermore, we keep our teaching up-to-date and use state-of-the art teaching approaches. This implies the introduction of measureable learning objectives as well as new e-learning techniques like MobileQuiz. These new learning approaches help us to improve our teaching continuously and to create a transparent teaching-learning situation, which helps the students to take active parts in learning activities and therefore increases their performance.

Since COS Heidelberg plays a major role in the teaching of Bachelor-, Teaching Degree-, Master- as well as PhD Biosciences programs of the University of Heidelberg, we are dedicated to implement the organismal and research-based teaching approaches into all levels of scientific education. For that reason, we established a teaching team consisting of Prof. Dr. Ingrid Lohmann as well as two lecturers Dr. Roland Gromes and Dr. Natalie Keib to design and implement the organismic and research-based teaching concepts together with our lecturers at COS Heidelberg. The financing for this project is provided by the »innovation and quality funds« (IQF), which were established by the »Ministerium für Wissenschaft, Forschung und Kunst« and the »Landeshochschulen« to support the quality improvement of the academia and the implementation of innovative projects.

In the following, we would like to give a brief overview of the Bachelor-, Teaching Degree-, Master- and PhD Biosciences programs of the University of Heidelberg and discuss the teaching at COS Heidelberg in more detail.

The Bachelor of Biosciences program at the University of Heidelberg is composed of six semesters. From the 1st to the 4th semester, students participate in obligatory basic modules including seminars, lectures and practical courses. Here, they obtain a fundamental bioscientific knowledge including biochemistry, genetics, molecular biology, cell

biology, microbiology, developmental biology, physiology, immunology, ecology and evolution. Starting from the 2nd semester, compulsory electives such as seminars, advanced lectures, excursions and advanced practical courses are included into the curriculum. The students can choose their compulsory electives from a broad variety of advanced courses (Fig. 2; A). Therefore, they are able to set their own focuses within the studies and develop their particular skills and interests. Students finish their studies in the 6th semester with a Bachelor thesis project in the research field of a selected host group. In the summer and winter semesters 2013/14, 40 % of all Bachelor graduate students in the field of biosciences finished their Bachelor theses at COS Heidelberg.

The large diversity of basic and advanced courses within the Bachelor of Biosciences program covers the broad spectrum of topics within biosciences and is organized by ten different institutes of the bioscience faculty and associated centres including the Centre for Organismal Studies Heidelberg (COS), the Interdisciplinary Center for Neurosciences (IZN), the Center for Molecular Biology Heidelberg (ZMBH), the Biochemistry Center Heidelberg (BZH), the Interdisciplinary Center for Scientific Computing (IWR), the German Cancer Research Center (DKFZ), the European Molecular Biology Laboratory (EMBL), the Karlsruhe Institute of Technology (KIT), the Max-Planck-Institute for Medical Research (MPIMF) and the Julius Kuhn Institute (JKI).

The lecturers of COS Heidelberg participate in teaching of all basic courses (≥ 20 %; Fig. 1; A and B) and compulsory electives (≥ 40 %; Fig. 2; B). The contribution of COS lecturers in basic courses is in 5 of 9 cases more than 80 % (Fig. 1; A and B). Additionally, more than 80 % of compulsory electives including advanced lectures, 3 weeks advanced practical courses and excursions are coordinated and supervised by COS lecturers (Fig. 2; B). Thus, the main part of basic and advanced teaching within the Bachelor of Biosciences program is managed by lecturers of COS Heidelberg.

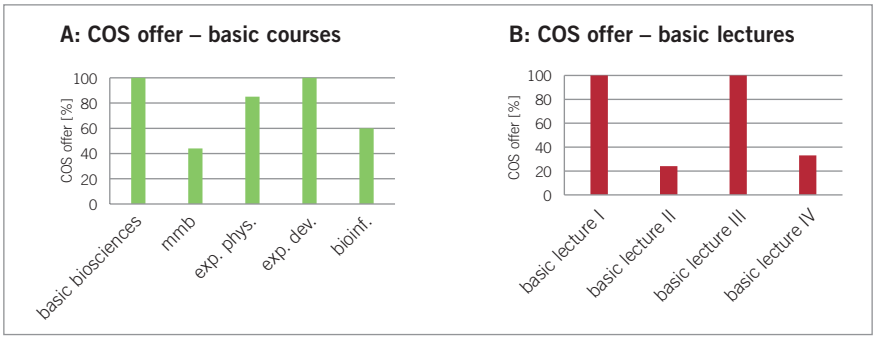


Figure 1
Basic practical courses and basic lectures offered by COS Heidelberg in the Bachelor of Bioscience program: (A) COS participation in teaching of basic practical courses. The numbers indicate the percentage of COS lecturers participating in the organization and supervision of particular practical courses. mmb: methods of molecular biosciences; exp. phys.: experimental physiology; bioinf.: bioinformatics; exp. dev.: experimental development (B) COS participation in teaching of basic lectures. The numbers indicate the percentage of semester week hours (swh) spend by COS lecturers. The estimate is based on the teaching offer in WS13/14 and SS14.

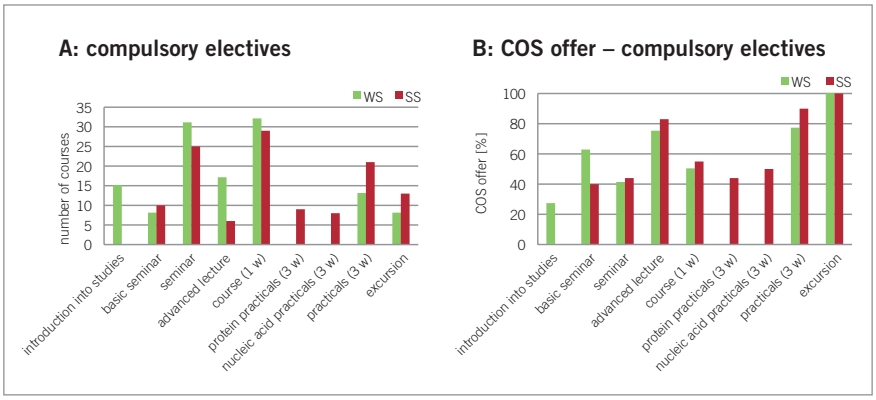


Figure 2
Compulsory electives of the Bachelor of Bioscience program. (A) Numbers of different compulsory electives offered during the winter semester (WS; green) and summer semester (SS; red). The estimate is based on the teaching offer in WS13/14 and SS14. (B) The percentage of compulsory electives offered by COS during WS13/14 and SS14. 1 w: one week practical course; 3 w: three weeks practical course.

The structure of the Teaching Degree program at the University of Heidelberg differs in many aspects from the structure of the Bachelor program. It is divided into basic and advanced study periods with defined course content and structure. The teaching of this program focuses predominantly on organismic biology and is completely organized by COS Heidelberg.

The extensive contribution to the teaching of Bachelor and Teaching Degree programs allows us to pursue our objectives and to implement our concept of organismal and research based teaching. The first step was the modularization of basic courses and the fusion of plant and animal bioscience teaching in lectures as well as in basic practical courses including basic biosciences, experimental physiology and experimental developmental biology. At present, our COS lecturers and our teaching team are working on the overall integration of cross-system teaching concepts into the Bachelor and Teaching Degree programs.

The international Master of Molecular Biosciences program of the University of Heidelberg offers eight different Majors. The teaching of every Major focuses on a specific bioscientific area, thereby allowing students to choose their Majors within the Master Molecular Bioscience program according to their specific interests.

The Master of Molecular Bioscience Program of University of Heidelberg lasts two years. From the 1st to the 3rd semester, students attend six different modules. Each module consists of a lecture with an accompanying tutorial and a practical course with an accompanying literature seminar. The teaching in the first semester provides a solid background in advanced biosciences for all students of the Master program, whereas the Major specific teaching starts in the second semester.

COS Heidelberg coordinates the Development and Stem Cell Biology, Evolution and Ecology- as well as Molecular Plant Sciences-Majors and is involved in teaching of the Neurosciences- and Systems Biology-Majors. According to our efforts to implement organismic biology beyond the borders of organizational levels into our studies and teaching, we try to break the boundaries between our Majors and encourage the students to gain experience in other related bioscientific areas.

Furthermore, we set a great value within our Majors on research based teaching, which reflects our large variety of courses and participating research groups, which offer the students possibilities to do their lab rotations and Master thesis projects. We encourage our young group leaders as early as possible to contribute to COS teaching to inspire young people for their research. Hence, between 12 and 21 lecturers from COS Heidelberg and other research centres and faculties are participating in our Majors (Development and Stem Cell Biology: 21 lecturers; Evolution and Ecology: 12 lecturers; Molecular Plant Sciences: 15 lecturers) and in summer and winter semesters 2013/14, 23 % of Master of Biosciences program students graduated at COS Heidelberg.

COS Heidelberg offers a three year PhD program with excellent training and research opportunities in stimulating and collaborative environment which links to DKFZ, EMBL, and the Max Planck Institutes on the Heidelberg Life Science Campus. It is embedded in the Hartmut Hoffmann-Berling International Graduate School (HBIGS) and emphasizes on independent research as well as a continuous and intense training program. The students receive experimental education in core courses, which offer practical training in biochemistry, imaging, genetics, molecular biology, FACS analysis etc. Additionally they improve their soft skills in courses on scientific writing, scientific presentation, and career opportunities.

In our PhD program we attach great importance not only to scientific exchange, which is ensured through seminar series, lecture series, and conference attendance, but also to scientific support and supervision. Therefore each PhD research project is supervised by a thesis advisory committee (TAC), which consists of supervisor of the thesis and at least two additional PIs and ensures progress and guidance in annual meetings.

A.3 COS EVENTS

The Centre for Organismal Study has created as well as is continuing several »events« for scientific exchange as well as communication of science to the interested public. Our aim is 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
In 2014, we are initiating the COS Lecture Series. On a monthly basis, a speaker is invited to talk about a research topic of general interest to the COS scientific community. This is the merger and formalization of two lecture series that have been running at the former Heidelberg Institute for Plant Sciences (HIP) and the Heidelberg Institute for Zoology (HIZ).

Seminars and Seminar Series at COS
Several seminar series are available at COS and in cooperation with other institutions on campus, which are dedicated to providing a forum for PhD students and postdocs for scientific exchange.

The »COS Plant Science Seminar« is a continuation of the »HIP Plant Science Seminar« and organized by the PhD students. The series »Systematic Approaches in Organismal Biology« has been created in 2009 and includes speakers from research groups at COS, DKFZ, ZMBH and the medical faculty Heidelberg. Depending on research interest, COS PhD students also join seminar series at other institutions and centres such as IZN, BZH and ZMBH.

Symposia at COS
The COS Inaugural Symposium in 2011 was followed by two international symposia:

»From Molecules to Living Systems« (COS Inaugural Symposium), May 13th, 2011

Herbert Jäckle, Göttingen	Towards epigenetics in development?
Jiří Friml, Gent, Belgium	Cell polarity and endocytic recycling in plant development
Elisa Izaurralde, Tübingen	Dissecting the molecular mechanisms of RNA-mediated gene silencing
Lothar Willmitzer, Potsdam	Response of *A. thaliana* to changing light and temperature: a systems analysis
Eddy de Robertis, Los Angeles, USA	From Spemann's organizer to the cellular mechanism of canonical Wnt signaling
Oliver Hobert, New York USA	How to make neurons: Lessons learned from the Worm
Pamela Soltis, Gainesville, USA	Angiosperm Phylogeny: The Role of Polyploidy in Diversification and Genome Evolution
Steve Kay, La Jolla, USA	Large Scale Biological Approaches to Understanding Circadian Networks
Philip Benfey, Durham, USA	Development rooted in interwoven networks

»At the Roots of Stemness«, July 6th, 2012

Elliot Meyerowitz, Cambridge, UK	Surprises from studies of the regeneration of stem cell niches in plants
Jan Lohmann, Heidelberg	It takes two to tango: Hormones and transcription factors in shoot meristem control
Volker Hartenstein, Los Angeles, USA	Stem cells and lineages of the intestine: a developmental and evolutionary perspective
Alexa Bely, College Park, USA	Evolution of annelid regeneration: pattern and process
Bruce Edgar, Heidelberg	Intestinal stem cell control on the healthy and hyperplastic *Drosophila* gut
Mitsuyasu Hasebe, Okazaki, Japan	Induction of a stem cell inducing factor STEMIN can change differentiating cells to pluripotent stem cells in the moss *Physcomitrella patens*
Thomas Holstein, Heidelberg	Cnidarian stem cells: On the evolution of metazoan stem cells
Noriko Funayama, Kyoto, Japan	What we can learn from sponges about the stem cell system
Jeremy Brockes, London, UK	Evidence for local evolution of mechanisms underlying limb regeneration and limb development in salamanders
Jochen Wittbrodt, Heidelberg	Lineage restriction and pluripotency in retinal stem cells

»Building Beauty – From Genes to Shape«, June 20th and 21st, 2013

Alain Prochiantz, Paris, France	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, Switzerland	Building a polarized fibroblast – From cytoskeletal dynamics to Shape
Mihaela Zigman, Heidelberg	From individual cell polarization to coherent tissue morphogenesis: building the zebrafish neural tube
Ulrich Schwarz, Heidelberg	On force and form: what cell and tissue shape can tell us on actomyosin force generation
Jan Traas, Lyon, France	From genes to shape: morphodynamics at the shoot apical meristem
Damian Brunner, Zürich, Switzerland	Cytoskeleton architecture, force generation and mechanistic principles of tissue closure
Ingrid Lohmann, Heidelberg	Hox Control of Drosophila Feeding Movements
Francois Graner, Paris, France	Fly thorax morphogenesis: from cell dynamics to tissue shape
Alexis Maizel, Heidelberg	Lateral root morphogenesis in Arabidopsis thaliana
Erez Raz, Münster	Motility and directed migration of primordial germ cells in zebrafish
Przemyslaw Prusinkiewicz, Calgary, Canada	Biology, Computation, and Art: Understanding the Form of Trees

In the future, symposia will be held on a yearly basis with an international symposium alternating with a COS internal event. The COS internal event, with oral as well as poster presentations, will serve as a forum for scientific exchange.

While the Inaugural Symposium was funded by a generous donation of the Klaus Tschira Foundation[1], the subsequent symposia were financed in part by the Klaus Tschira Foundation[2] and by other sponsors (Deutsch-Französische Hochschule[3], HBIGS[4], Nikon[5], the Company of Biologists[6], DFG[7]).

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 third year and with five events having taken place already, the lecture now attracts regularly about 150 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«[8] 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.

The lecture series is a project funded by the Klaus Tschira Foundation[9].

1 http://www.klaus-tschira-stiftung.de
2 http://www.klaus-tschira-stiftung.de
3 http://www.dfh-ufa.org
4 http://www.hbigs.uni-heidelberg.de
5 http://www.nikon.de
6 http://www.biologists.com
7 http://www.dfg.de
8 http://www.stuetzpunktschulen.de
9 http://www.klaus-tschira-stiftung.de


CENTRE FOR
ORGANISMAL STUDIES
UNIVERSITÄT
HEIDELBERG
ZUKUNFT
SEIT 1386
TALANFFY LECTURE AT COS
Prud'homme,
e Biologie du Développement de Marseille-Luminy
REGULATORY MECHANISM OF
EVOLUTION"
BERTALANFFY LECT
FÜR SCHÜLER
Benjamin Prud'homme,
„HOW DID THE FLY GE
Donnerstag, den 10. April 2014
14:00 Uhr Vortrag


Ludwig von Bertalanff
1901 – 1972
COS Heidelberg

Bertalanffy Lectures held so far:

October 4th and 5th, 2012

Alfonso Martinez-Arias, Cambridge, UK	**Genetic alchemy and the Making of animal embryos**
	Cell fate choice and decisions: An embryonic stem cell perspective

January 31st and February 1st, 2013

Enrico Coen, Norwich, UK	**Leaves, Loops and Leonardo: The generation of biological form**
	Hidden Signposts of Development: Tissue cell polarity and its role in development

May 2nd and 3rd, 2013

Russell Foster, Oxford, UK	**Do you take sleep and your body clock seriously?**
	Light and time – A New Look at the Eye

September, 26th and 27th, 2013

Hannah Monyer, Heidelberg	**Brain Plasticity, Learning and Memory**
	Can Studies in Mice help us understand how memory functions in humans?

April, 10th and 11th, 2014

Benjamin Prud`homme, Marseille-Luminy, France	**How did the fly get its spot**
	The regulatory mechanism of morphological pattern evolution

In 2014, the Bertalanffy Lecture Series will be complemented by a 3-week summer course for high school students, in which they will work on small research projects including experimental parts.

Santander International Summer School
Research group leaders from COS organized the first Santander International Summer School for Doctoral Students[10] in November 2013 in Santiago de Chile, Chile, titled »Getting in Shape – Visualization and Manipulation of Organismal Morphogenesis«.[11] The summer school was concluded by a two-day symposium.[12] This summer school program is funded by Santander Universidades in the framework of the cooperation agreement[13] between Heidelberg University and Banco Santander. Within Heidelberg University, funding for summer schools is available on a competitive basis.

Sunday Matinée
The Sunday Matinée is a highly successful lecture series, which is running every winter semester since 1980. The aim is to present current topics of life science research and related disciplines to the interested public.

10 http://www.uni-heidelberg.de/research/international/santander_summer_schools/
11 http://www.uni-heidelberg.de/research/international/santander_summer_schools/2013_getting_in_shape.html
12 http://www.uni-heidelberg.de/research/international/santander_summer_schools/2013_symposium_getting_in_shape.html
13 http://www.uni-heidelberg.de/international/profile/santander_cooperation_agreement.html

Topics in recent years have been:

WS 2008/09	Kooperation – Von der Zelle bis zur Gesellschaft
WS 2009/10	Brennpunkte der Biologie – Teil 1
WS 2010/11	Brennpunkte der Biologie – Teil 2
WS 2011/12	Orientierung – Von der Zelle bis zur Gesellschaft
WS 2012/13	Im Fokus: Der Mensch
WS 2013/14	Der Lebensbogen – Werden, Sein, Vergehen

This series is very well received in Heidelberg and it surrounding communities with several hundreds of attendees at each lecture. Since 2001, the series is organized by Prof. Dr. Dr. h.c. Volker Storch, »Seniorprofessor« at the Heidelberg University since 2010 and formerly professor at the Heidelberg Institute for Zoology. The matinée is financed through the »Verein der Freunde und Förderer des Zoologischen Museums der Universität Heidelberg e. V.«.

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«[14] and the »International Summer Science School Heidelberg«[15].

14 http://www.klaus-tschira-stiftung.de/aktivitaeten.php?we_objectID=1096
15 http://www.ish-heidelberg.de/

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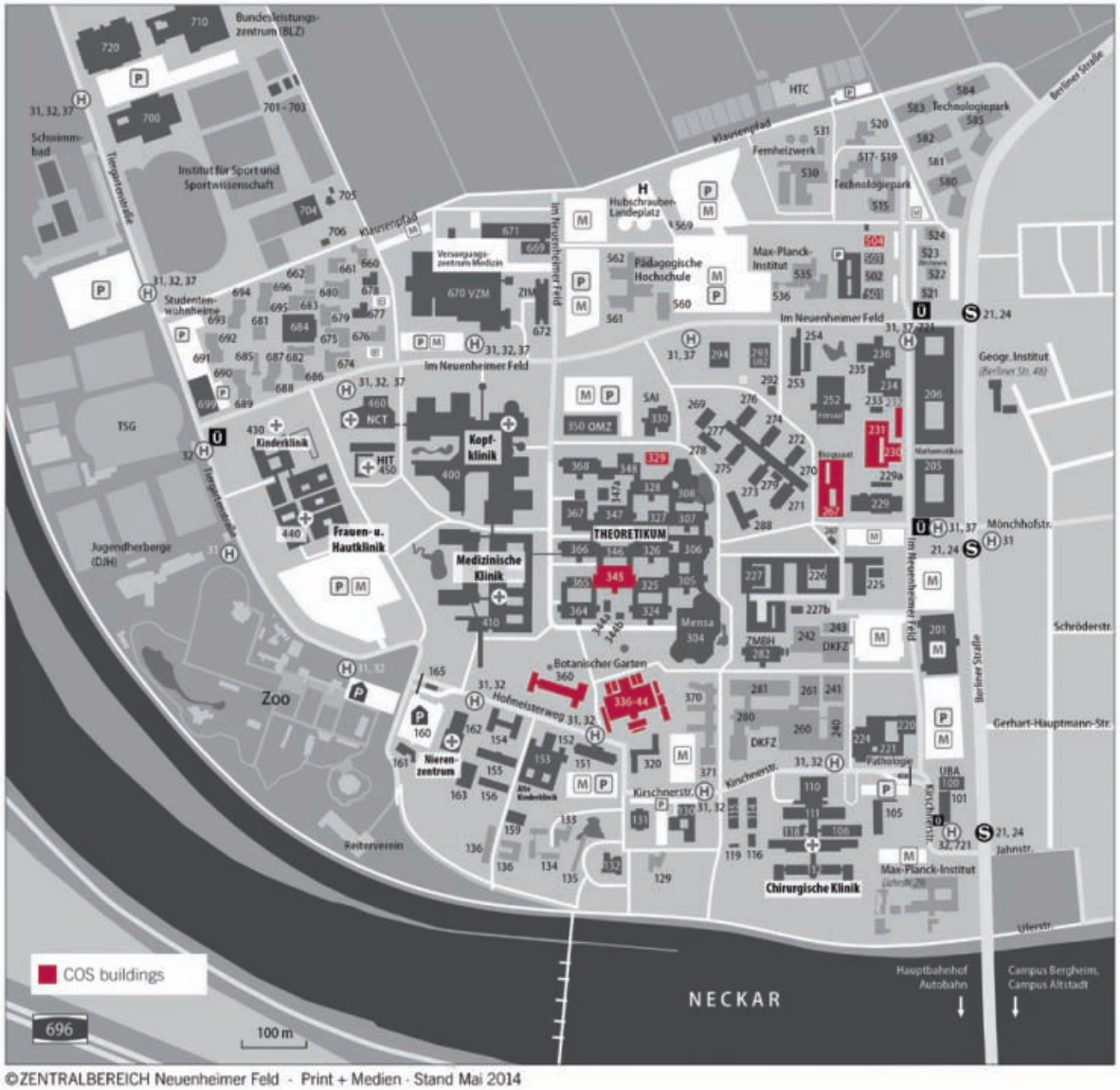

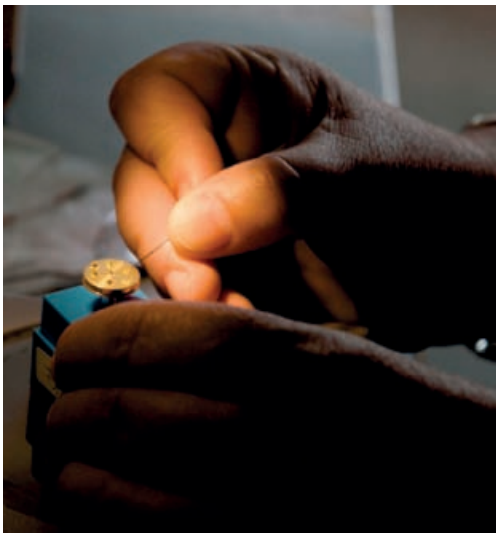


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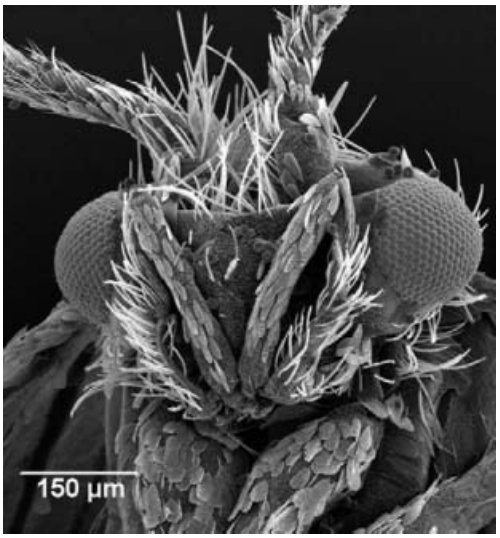
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