

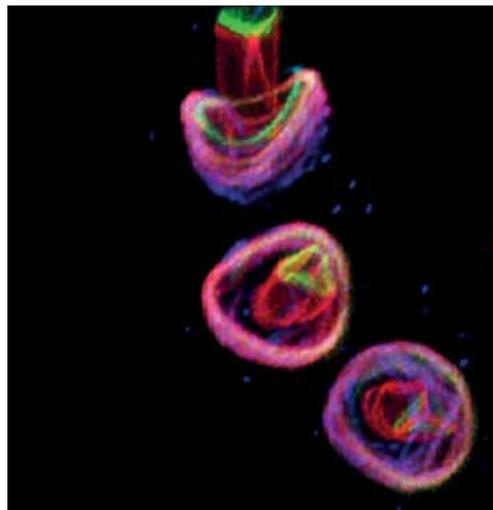
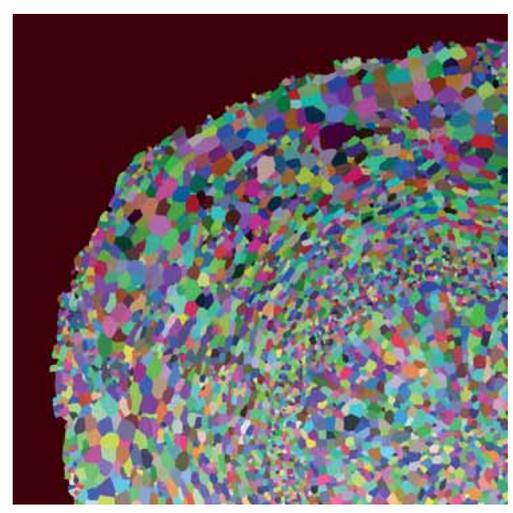


Centre for
Organismal
Studies
Heidelberg



UNIVERSITÄT
HEIDELBERG
ZUKUNFT
SEIT 1386

REPORT 2018-2021



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INTRO- DUCTION

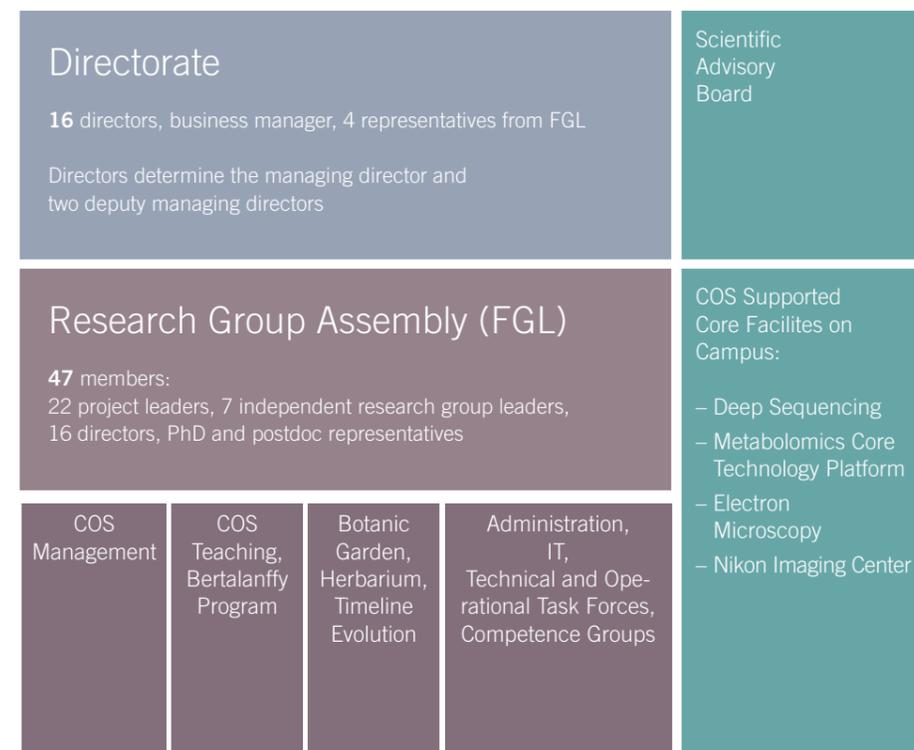
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1.1 PREFACE

The Centre for Organismal Studies (COS) was established in 2010 with the goal to bridge the gap between decoding complex molecular mechanisms and understanding of organismal development, physiology, and evolution. Consequently, research and teaching at COS embraces biological processes at all scales of complexity in plants, animals, and fungi, and in the context of their native environments. With key hires at the professorial level ongoing, our third report covering the years 2017-2021 represents a wrap up of the first decade of COS, in which we have established ourselves as a major player in the Heidelberg Life Sciences, as well as a sketch of directions for future developments.

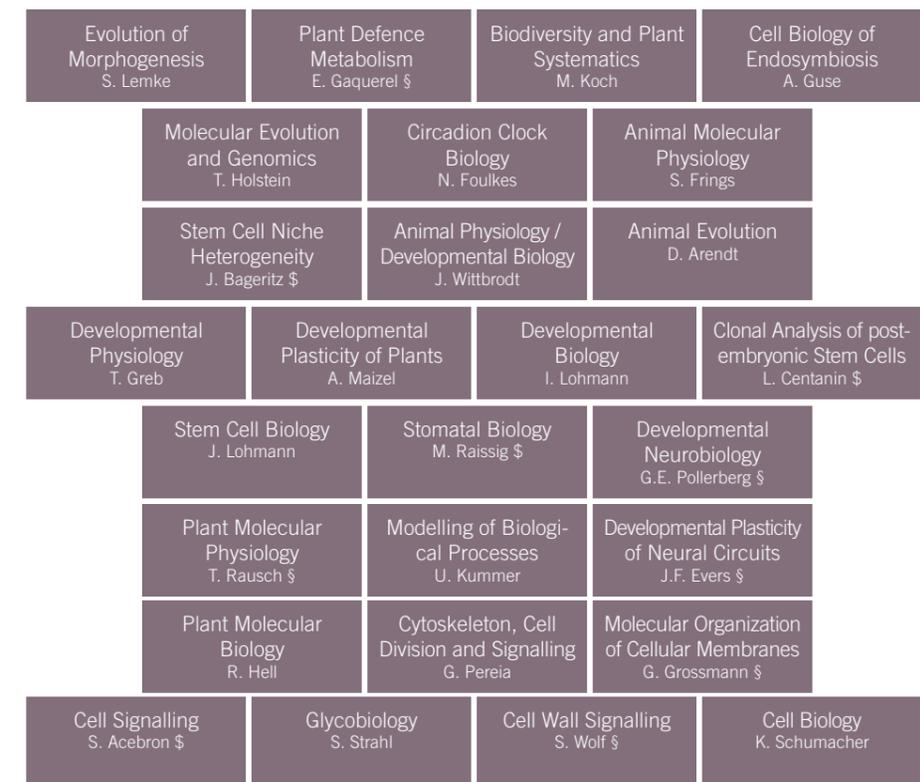
1.2 STRUCTURAL DEVELOPMENT OF COS

COS was founded in 2010 by merging the former Heidelberg Institutes for Plant Science (HIP) and Zoology (HIZ), and its scientific visibility has led to organic growth of our centre to a total of 24 independent research groups during the reporting period. The senior faculty of COS consists of 16 tenured professors with affiliations to the Heidelberg Faculty of Biosciences, the European Molecular Biology Laboratory, and the Karlsruhe Institute of Technology. Each professor serves as head of a department that may comprise several research groups. In addition to permanent faculty, independent junior groups are a key element of the COS strategy to create and maintain a dynamic and innovative research and teaching environment. Since 2010 COS has attracted 12 young group leaders with funding from the Emmy Noether program of the DFG (Annika Guse, Amal Johnston, Steffen Lemke, Sebastian Wolf, Michael Raissig), the Excellence Initiative (Emmanuel Gaquerel, Guido Grossmann), the SFB1324 (Sergio Acebrón, Josephine Bageritz), the SFB873 (Lazaro Centanin), the Chica and Heinz Schaller foundation (Alexis Maizel), and Heidelberg University (Jan-Felix Evers). Our junior faculty has significantly contributed to the development of COS, and in turn COS has made substantial efforts to support their scientific careers. In the reporting period, COS has further formalized the career support for junior faculty to offer reliable and transparent career options. In addition to the usual five- or six-year tenure of junior programs, COS committed to a two-year extension of the group leader position dependent on successful scientific review by the Scientific Advisory Board. Importantly, COS has introduced the option for a one-year bridging of the group leader salary to ensure the uninterrupted and smooth transition to a position outside of COS. This option was instrumental in supporting the transition of two of our junior group leaders into their next appointments and has now been adopted by the rectorate as standard for the entire University. During the reporting period, there was substantial turnover among our junior faculty with Emmanuel Gaquerel, Guido Grossmann, Jan-Felix Evers, and Sebastian Wolf moving to new institutions, Alexis Maizel being promoted to tenured full professor at COS, and Sergio Acebrón, Michael Raissig and Josephine Bageritz joining to start their groups.

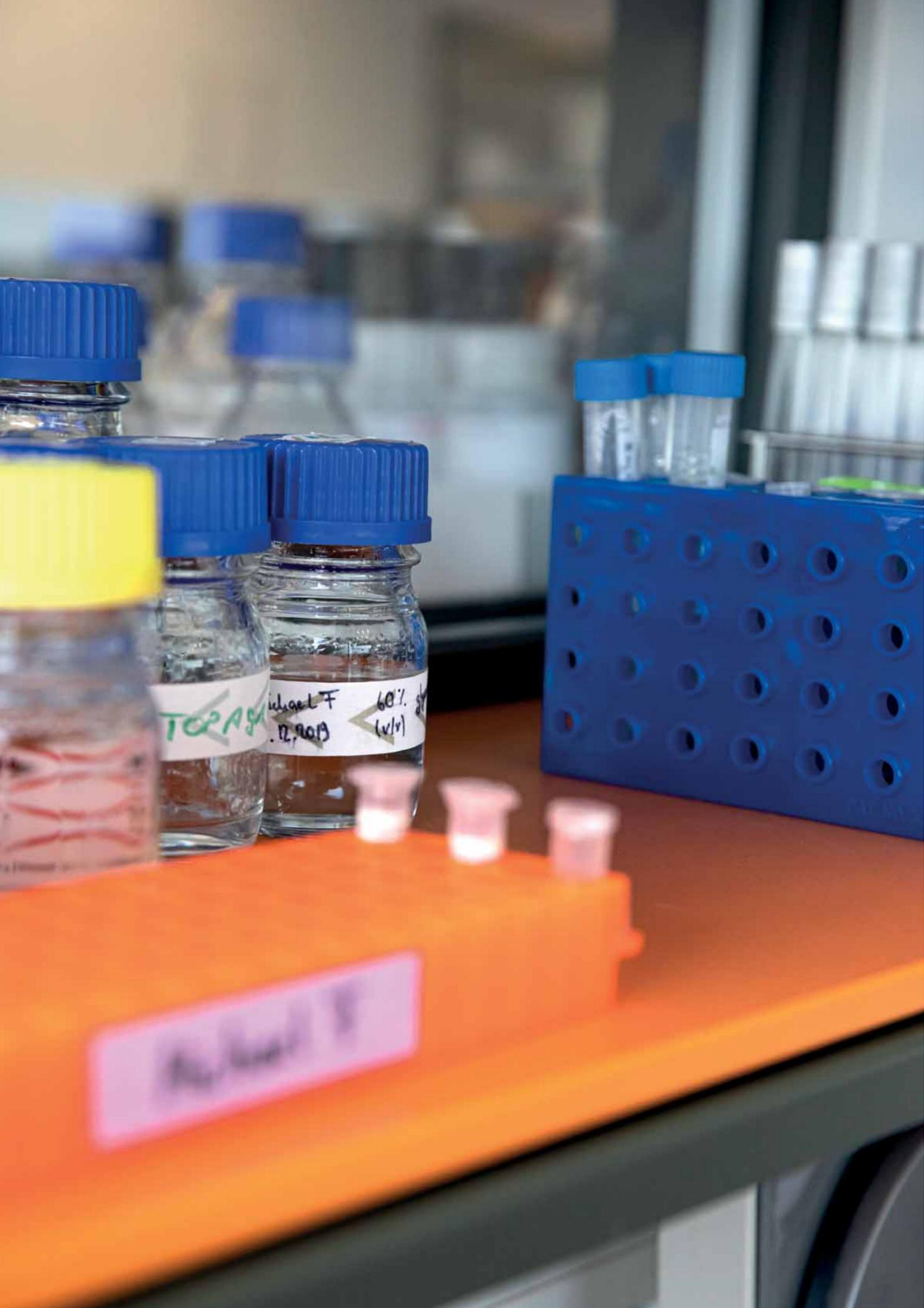


COS senior faculty was strengthened by three appointments: Thomas Greb took over the W3 position of Thomas Rausch to lead the department of Developmental Physiology. Gislene Perreira was appointed to lead the new department Cytoskeleton, Cell division and Signalling with a W3 professorship provided by the ZMBH and Heidelberg University to create a bridge between COS and ZMBH. Finally, Alexis Maizel was selected for the W3 professorship for Cell and Developmental Biology.

The development of COS faculty is a continuous process, and we are currently hiring three new colleagues at the tenure track or full professor level in the areas of Developmental Signalling and Physiology of animals, as well as Neurodevelopment. In addition, we have agreed to support the Emmy-Noether applications of two young scientists extending our portfolio in the plant and animal sciences. We expect that our new colleagues will enrich the scientific community at COS, advance our teaching programs, and contribute to the strategic development of our centre.



§ Left COS during reporting period
 \$ Joined COS during reporting period



1.3 RESEARCH AND COMMUNITY INTEGRATION

With its more than 50 PIs, COS covers a wide range of excellent and innovative research programs ranging from the molecular to the cellular and organismal level. Our success is visible at multiple levels, such as publications, awards, third party funding, participation in research consortia, or appointments. Since these aspects are described in detail in the contributions from individual PIs, we only give a brief overview over some of the key facts and highlights.

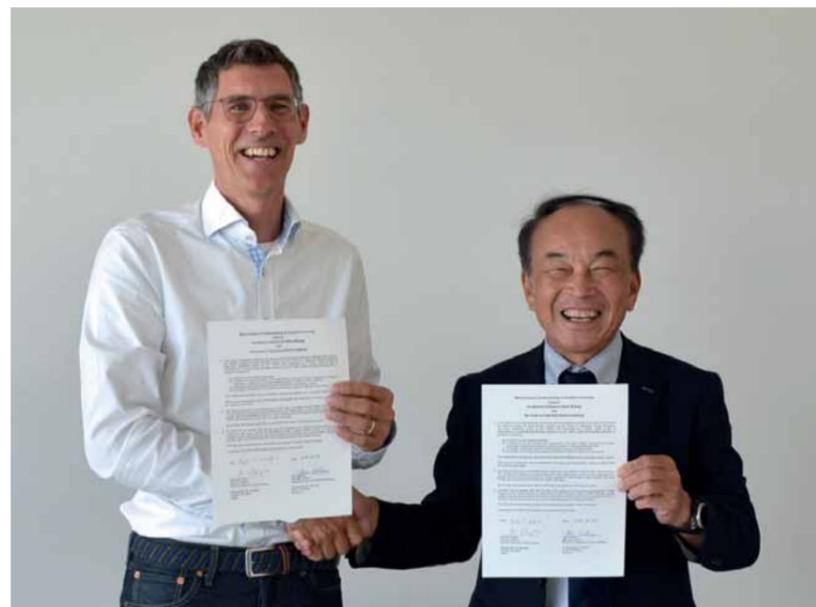
COS PIs have pledged to adhere to open access publication wherever possible, and we are proud that during the reporting period, 132 manuscripts with COS participation have been posted on BioRxiv. In total, PubMed reports more than 400 publications with COS affiliation published from 2017 to 2021 with many appearing in leading journals and most as open access.

Another important and quantifiable readout of scientific performance is third-party funding. We are therefore very pleased that during the reporting period, the funding portfolio of COS further diversified and increased. Compared to 2009, the last year before the merger of HIP and HIZ into COS, our third-party funds have more than doubled from 5.2 M€ to 11.8 M€ in 2020. Importantly, the positive trend continued in the past years with 9.3 M€ in 2017 and 2018, 11.4 M€ in 2019 and 11.8 M€ in 2020. There are three main drivers to this development, namely the success of COS junior PIs in securing Emmy-Noether-Program funds, the strong representation of COS PIs in research consortia, such as Collaborative Research Centers (CRC) or Research Units (RU), and the coordination of four of these prestigious consortia by COS PIs (CRC873 by Jan Lohmann, CRC1324 by Thomas Holstein, RU2509 by Sabine Strahl, and RU2581 by Alexis Maizel), and finally the streak of ERC grants to COS PIs: From 2010 to 2013 three ERC grants were secured by Detlev Arendt, Jan Lohmann, and Joachim Wittbrodt. From 2014 to 2016, two ERC Consolidator grants were won by Thomas Greb and Annika Guse. In the current reporting period from 2017-2021, two ERC Synergy grants were awarded to Jan Lohmann and Jochen Wittbrodt (as parts of small consortia with PIs from other institutions), and a second ERC advanced grant went to Detlev Arendt. While our third-party funding has doubled, core-funding allocated to COS by our University was only modestly increased from 3.8 M€ to 5.3 M€ in 2020. Hence, for every Euro invested in COS by the state, we currently secure more than two Euros of outside funding, underlining the commitment and competitiveness of our PIs. CRCs and RUs not only represent prominent funding lines, but more importantly, foster the collaboration and interaction of COS scientists in house, as well as with colleagues in the Heidelberg community and beyond. Therefore, we are proud that COS representation in DFG-funded collaborative networks has further increased in the reporting period with eight of our PIs being active members in the CRC873 (Stem Cells, co-ordinated by COS) and CRC1324 (Wnt Signalling, co-ordinated by COS), five in the CRC1101 (Specificity in Plants, co-ordinated by Tübingen University), four in the CRC1036 (Cellular Damage, co-ordinated by ZMBH, now expired), one in the CRC1211 (Evolution at the dry limit, co-ordinated by Cologne University) three in RU2581 (Plant Morphodynamics, co-ordinated by COS) and two in RU2509 (Glycosylation, co-ordinated by COS). In total, more than 80% of our Independent PIs are part in nationally or internationally funded consortia, highlighting the excellence of our research and the visibility of COS PIs.

Meetings, workshops, and seminars are additional important building blocks to create and maintain a vibrant research community at COS and to connect to colleagues worldwide. Therefore, we have developed a three-tier strategy with COS seminars for guest speakers on more specialized topics, the COS Keynote on topics of general interest delivered by highly visible speakers twice a year, and the bi-annual international COS Symposia Series, which features COS researchers and top-level scientists from around the world. COS symposia focus on forefront topics in organismal biology, such as “Building beauty – from genes to shape” (2013), “Darwin 2.0 – new tools to go through time” (2015), “Senses and sensitivity” (2017) and “Genetics 2019 – old questions and new frontiers” (2019). Unfortunately, the Symposium 2021 entitled “Building functionality - the relevance of form across biological scales” had to be postponed due to the COVID-19 pandemic and is

now scheduled for October 2022. COS symposia have established themselves not only as highly visible platforms for scientific exchange, but also for career development and brainstorming for new scientific or strategic activities at COS.

The pandemic also limited the opportunity for the COS community to engage in forward looking strategic discussions. Still, major initiatives emerged built on the cornerstones of our research portfolio, namely cell biology, developmental biology and physiology, and taking advantage of more recently emerging scientific directions, including organismal plasticity and adaptation, as well as genetics. In this context it is noteworthy that four draft proposals for excellence clusters have been put forward by COS PIs following Heidelberg University's internal call in early 2021. The initiatives cover scientific topics ranging from gene x environment interactions (led by Michael Boutros, DKFZ and Jan Lohmann), stem cells (led by Jan Lohmann and Ana Martin-Villalba, DKFZ), or morphogenesis in plants and animals (led by Alexis Maizel), to a proposal focusing on biological robustness in plants (led by Thomas Greb and Erik Kemen, ZMBP Tübingen). We expect these initiatives to further enhance the embedding of COS into the Heidelberg life science community and to act as motors for the development of our research and infrastructure portfolios. Notably, COS is the major contributor of excellence cluster draft proposals from the bioscience faculty, highlighting our development into a leading institution on campus. This is also well reflected at the level of appointments to leadership positions by our community, since COS PIs serve in manifold strategic roles for our University. Karin Schumacher is the Vice-Rector for Quality Development of Heidelberg University, Jochen Wittbrodt is the Dean of the Faculty of Biosciences, Alexis Maizel the Vice-Dean for research, and Thomas Greb acts as the Dean of Studies. Furthermore, Jan Lohmann has been appointed the spokesperson for the Life-Science Research Council, a steering committee bringing together the University faculties of Biosciences and Medicine with our partner institutions represented by the DKFZ, EMBL and MPI for medical research. Overall, we feel that COS is now well positioned in the local community and in a strong position for further strategic developments.



Signing of the Memorandum of Understanding between, Kiyokazu Agata, NIBB, and Jan Lohmann, COS

In the reporting period, COS has also been successful in extending international interactions. Building on our core mission in organismal biology and the emerging research direction in organismal plasticity and adaptation, COS PIs have built increasingly strong ties with members of the National Institute for Basic Biology in Okazaki, Japan. Triggered by national competitions for major funding lines, initial informal contacts matured into an institutionalized exchange and common strategic planning in 2017. These steps have culminated in a memorandum of understanding between COS and NIBB, which was signed in summer 2019 and lays the foundation for a long-term scientific collaboration between the two institutions. The program focuses on regular exchange of PIs and students, as well as joint funding lines. The NIBB has secured support for one COS PI to run a satellite group in Okazaki and issued an open call to young COS PIs. Annika Guse has been selected among four highly competitive applications and has started a joint project on light sensing in cnidarians in the NIBB open lab in 2020. Unfortunately, a first joint workshop of COS and NIBB PIs scheduled for spring 2020 in Okazaki had to be cancelled due to the pandemic. To maintain momentum in this important international collaboration, we have held two online meetings in 2021 with more being planned. Importantly, we will use the COS Symposium 2022 to host a satellite workshop with NIBB PIs with the aim to further deepen our ties.

Scientific infrastructure

An essential element for research at COS is easy and fair access to scientific infrastructure for all groups. COS has developed a three-tier system to not only provide up-to-date instrumentation, but also to ensure that our equipment is maintained and constantly renewed. The first tier is represented by core facilities open to the entire Heidelberg life science community. COS is heavily invested by providing personnel and/or equipment for the Nikon Imaging Centre (NIC), the Metabolomics Core Technology Platform (MCTP), the Electron Microscopy Core Facility (EMCF), and the CellNetworks Deep Sequencing facility (DeepSeq). Therefore, these facilities are represented in the COS report with dedicated chapters. Access to these facilities is regulated by user agreements and fees that conform to DFG rules and hence are eligible for inclusion in grant proposals. The second tier of COS scientific infrastructure is shared equipment. Scientists at the individual COS locations have agreed to share equipment to maximise user base, minimize redundancies and reduce costs. Instruments, such as large centrifuges, plate readers, qRT-PCR machines, or confocal microscopes, are maintained by individual departments, but made available to all groups at COS through an online booking system. While COS PIs have the full freedom to decide about equipment purchases, we have agreed to tailor them to maximize the COS instrument portfolio. This is particularly important for junior groups, who fully participate in sharing COS infrastructure, despite the fact that they are unable to contribute to the equipment pool due to lack of core funding. COS PIs have been highly successful in securing grants for equipment by the DFG or the ERC and thus our current setup is cutting edge. For example, COS currently operates 15 confocal, spinning disk or light-sheet microscopes, which cover a large range of applications including upright and inverted settings, two-photon excitation and fluorescence lifetime imaging. The third tier of instrumentation is highly specialized equipment, which is specifically tailored to the needs of individual groups, such as climate-controlled imaging stations, slide scanners, or photosynthesis analysis systems. Taken together, COS has successfully implemented a system that provides direct and fair access to most types of equipment in house, while maintaining a maximum of individual freedom and responsibility for our PIs. Our equipment base is complemented by campus core facilities supported by other institutions, covering important technologies such as proteomics, lipidomics, or FACS.

Overall, we are proud that COS has continued its positive development during the reporting period as shown by the quality and number of publications, the impressive amount of third-party funding, our prominent roles in campus strategy and, importantly, the success of our junior PIs on the job market. Our past success along with currently ongoing activities, such as excellence cluster initiatives and professorial hires, give us confidence that we will be able to take the next step along the developmental trajectory of COS in the coming years.



1.4 TEACHING AT COS

With an active teaching community encompassing 16 full professors and a large number of group leaders, postdocs and PhD students, COS remains the largest contributor to the teaching portfolio of the Heidelberg Faculty for Biosciences. The centre provides courses for:

- the bachelor program „Biosciences“ - spanning from introductory courses, lectures, and seminars to advanced lab work, research seminars, and thesis supervision
- the bachelor program „Biology 50%“ aimed at prospective teachers – covering the same range of biological topics as the biosciences bachelor and additional didactic teaching
- the master program “Molecular Biosciences”, where COS fully organizes two of the majors offered (Molecular and Applied Plant Sciences, Stem Cells and Development) and contributes to two more (Neurosciences and Systems Biology)
- the ‘Master of Education’ program for prospective teachers where COS provides biological lectures and courses
- the COS PhD-program, integrated into the HBIGS graduate school

In all these programs, COS teaching spans a broad range of topics from anatomy and biodiversity via developmental biology and physiology to molecular biology with strong emphasis on problem solving, on hands-on experience in modern laboratory techniques, and on current research questions.

In recent years, teaching at COS has faced some challenges and opportunities, which prompted us to flexibly adapt and to further develop our teaching program. One hurdle to cope with have been various construction projects limiting practical and seminar room space, a constraint which was further exacerbated by massive water damage in the ZMBH building, which required to find additional rooms on campus. The second, much bigger, hurdle to take was the SARS-CoV-2 pandemic reaching Germany in early 2020, and severely restricting the possibilities for on-site teaching for several semesters. Despite these challenges, COS has been able to provide the complete teaching program, including all practical courses which had to be reorganized to allow for the mandatory distancing, mask wearing and testing. To supplement the reduced on-site teaching in most practical courses and to offer lectures and seminars, COS lecturers have developed a variety of digital formats, ranging from live events via video-conferencing platforms to pre-recorded tutorials, and lab instructions, to innovative concepts to supplement the regular teaching offers.



One of these innovations has been the establishment of a media centre for recordings and live feeds, professionalizing the production of digital materials regarding video and audio quality. Another new concept is the establishment of field trips allowing students to individually explore local biodiversity by using supporting online material. Moreover, students from the Master of Education program have been integrated into the public outreach

initiative “Science goes School”, during which the students produce short videos as part of a seminar about biological topics of relevance to school curricula; these videos are offered via the Youtube channel of COS’ Bertalanffy outreach program.

Overall evaluations show that, in spite of students experiencing the coronavirus pandemic as a troubling time especially due to reduced social contact, the teaching courses offered were appreciated as valuable and the results in exams were comparable to previous years.

For the upcoming years, we are excited with the prospect to return to previous teaching modes where desirable, while retaining innovations having proven successful or even superior and to combine established and new formats into a modernized, appealing teaching offer. Together with the expected availability of the renovated teaching labs in early 2022 in the building INF 230 and the recruitment of three new professors in 2022, these developments provide exceptional opportunities for COS to renew and advance an already strong teaching program.



1.5 FUTURE PERSPECTIVES AND CHALLENGES

Having just passed its 10th anniversary, COS has come off age in the reporting period and now needs to develop a firm vision for its second decade. However, our future path will not only be shaped by our own goals and ambitions, but also by a substantial number of external factors, such as the progress on infrastructure renovation and construction, or further developments in Germany's excellence strategy.

Hiring faculty members

Arguably the most decisive influence over the developmental trajectory of COS will come from the ongoing hiring process leading up to the replacement of three out of five colleagues with zoological background. After careful discussion, the COS directorate has decided to look for candidates with research strength in developmental signalling for the succession of Thomas Holstein, neuro-development for the position so far filled by Elisabeth Pollerberg and physiology for the professorship held by Stephan Frings. These topics remain very close to the foci of the current departments, serving specific strategic needs for COS. The CRC1324 on Wnt Signalling was so far coordinated by Thomas Holstein and we now hope to be able to recruit a colleague who can bridge the strong biomedical interest in cell signalling on campus with more basic research focused questions rooted in developmental biology and evolution relevant for COS. With an appointment in neuro-development, we aim to re-connect COS with the Interdisciplinary Centre for Neuroscience (IZN), a stronghold of mechanistic organismal biology in Heidelberg. Finally, broadening the scope for the third opening from the current neurophysiology to physiology, we hope to attract talent that can either cater to the growing interest at the intersection of metabolism and development or strengthen the neurophysiology community. We have opted to advertise the three positions as a single package and to use a single committee for making the selection for all three professorships. To make this work, we have carefully assembled a fairly large group of colleagues from all relevant institutions in Heidelberg, including medical faculty, EMBL and DKFZ to cover all expertise and collaborative opportunities. While this strategy certainly has some risks, such as multiplying conflicts of interests in a large committee, or the massive workload for all members involved, we are convinced that we gain maximum flexibility in our decisions, since the committee oversees the entire process and will be able to adjust decisions across the spectrum of positions. As another novelty, we also have decided to advertise the positions for neuro-development and physiology as open rank at the W1 tenure-track and W3 full professor level. These positions only come with a fairly limited package and are therefore most suitable for up-and-coming candidates out of their postdocs (W1 tenure track) or right after a group leader phase (W3 full professor). Using this strategy, we hope to attract top talent and be able to offer young and promising scientists an ideal place to strive and develop.

Scientific directions

The choice of new colleagues at the professorial level will not only shape the research portfolio of COS for the coming 25 years, but will also have a major impact on how we will be able to position ourselves in the upcoming competition for excellence clusters in the framework of the federal excellence strategy. As laid out above, COS has put forward four proposals for excellence clusters following the internal call for ideas, and currently it appears that three will go forward in the development towards a full proposal. Two of these, namely the ones on Gene X Environment interactions and stem cells, will be affected by the new hires and we will emphasize this importance during the process. Of course, a strong participation of COS PIs and facilities in excellence clusters on topics central to our mission would substantially strengthen our portfolio and developmental options. However, with firm decisions in the excellence strategy still years ahead, it will take the combined efforts and visions of many of our PIs to succeed.

Building infrastructure

Whereas the decisions connected to new colleagues are in our hand, many other factors with substantial impact on our future trajectory are not. One important example is the continued struggle for space that COS has been facing for a decade. While the renovation of the main building complex INF230/231 will finally be finished in mid 2022 after roughly

ten years of gruelling construction work, COS will remain segregated into at least four locations and suffering a space deficit exceeding 3000 sqm. Hence, one of the major strategic goals for many years is the push for top-notch and flexible lab-space in close vicinity to INF230. With the whole campus undergoing continuous change due to renovations and construction of new buildings in the various disciplines, the road to a spatially unified COS is winding and long. Currently, the Universities developmental plan projects COS to gain a large amount of space in INF234, adjacent to our main building, not until the 2030s. Unfortunately, this option is dependent on a large number of variables, including financing for complete makeover of the building complex by the state government. Given the current situation with pandemic related costs and the climate crisis dictating the political agenda, it will need a strong push by the University to secure the necessary support to finally be able to provide adequate space for COS.

Strategies for junior groups and core facilities

Another essential topic for the COS agenda is the recruitment and the support of junior group leaders. While we have been extremely successful in attracting and promoting junior colleagues, using mostly external funding sources, this strategy has substantial shortcomings. Firstly, the number of suitable applications tends to vary over time, leading to peaks and troughs of incoming junior groups instead of evenly spread coming and going. This sometimes makes it difficult to plan for and support incoming groups and produces imbalance in opportunities and duties among the young PIs. Secondly, by relying on candidates bringing their own funding, COS is not able to set the agenda in terms of topics, which would be desirable for continuous scientific development, due to the much higher turnover rate of junior groups compared to tenured professors. We are therefore striving to secure internal funding for two junior groups to be able to better support junior PIs and to integrate them more tightly into the COS fabric.

A similar case can be made for our facilities, which so far have operated on a mix of COS core funding, University support and third-party funding. As with many organically grown organizational structures, this arrangement proved solid in the past but will need to be amended to secure future development. Hence, we aim to re-structure some of the budget related aspects, such as personnel costs, and consider to move them into the campus-wide CellNetworks Core Technology Platform, while at the same time keeping the scientists involved closely associated with COS departments. By doing so, we hope to create a sustainable and future-proof platform of core-facilities together with the University and the medical faculty.

Finally, once our new colleagues have settled in and the effects of the pandemic are overcome, COS will need to complement and expand its scientific mission, which is currently based on the white paper preceding the foundation of COS. Recent world-café style discussions between COS PIs have started to clarify and to partially re-define our goals and ambitions and emphasized the need that this is a top priority. Taken together, COS has profoundly matured as an institution and has been exceptionally successful in securing funding and providing an excellent platform for research and teaching. At the same time, essential issues, such as space, facilities, and internal funding for junior groups remain work in progress, along with an updated definition of what we aspire to achieve together as a team in research and teaching.

RESEARCH GROUPS

2.1 DR. SERGIO P. ACEBRÓN INDEPENDENT RESEARCH GROUP

DR. SERGIO P. ACEBRÓN

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Fields of Interest

Wnt signalling, Mitosis, Stem cells, Intestine homeostasis, Colorectal cancer, Genome stability, Cell lineage specification



Summary and outlook

Our group aims to understand how cells integrate extracellular signals to modulate their fate. We have a special focus on Wnt signalling, which is a master regulator of stem cell homeostasis and cell lineage specification in metazoans. Despite its relevance in health and disease, we still do not understand how Wnt activity is modulated during development and tissue renewal, as well as how it branches out to impact other functions beyond gene transcription. To address these questions, we use in vitro culture stem cells, as well as mouse models. Since joining COS in summer 2017, we have established culture and CRISPR-editing of mouse intestinal organoids. Together with detailed molecular studies, our work in organoids allowed us to identify and characterise a molecular brake that prevents Wnt-dependent unscheduled growth in adult stem cells and cancer cells. In collaboration with the Nikon imaging center, we also established a live cell imaging pipeline to investigate mitosis-specific phenotypes in somatic and stem cells. Using this approach, we have identified novel roles of Wnt ligands in directing chromosome alignment and segregation in dividing cells. These results raised the intriguing possibility that Wnts might have unexplored functions in genome maintenance during embryogenesis, tissue renewal and tumorigenesis. This question is central to our project in the recently renewed Wnt consortium SFB1324. To tackle it, we have established single cell genome and transcriptome sequencing (sc G&T-seq), and designed a new generation of reporters for cell signalling and chromosome instability.

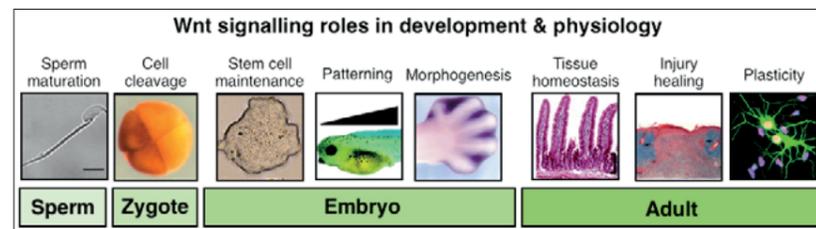


Figure 1
Wnt signalling plays critical roles in cell fate determination during the lifetime of all animals, and its misregulation is often associated with disease

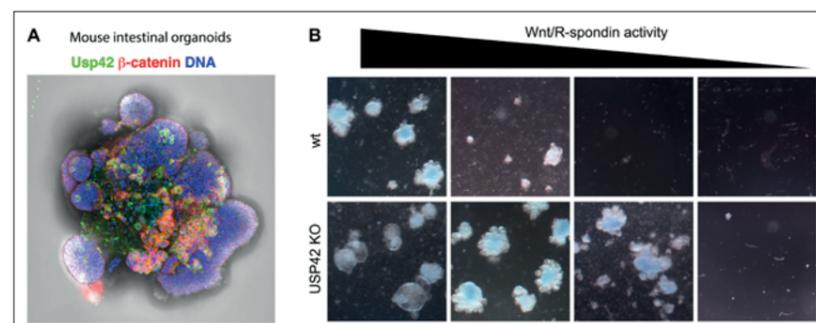
Research highlights since 2017

USP42 protects ZNRF3/RNF43 from R-spondin-dependent clearance and inhibits Wnt signalling (Giebel et al., EMBO Reports 2021)

Wnt signalling role in adult stem cell maintenance is overseen by the E3 ubiquitin ligases RNF43 and ZNRF3. As a clever evolutionary mechanism in mammals, RNF43/ZNRF3 function as a “dead man’s brake” by continuously destabilising the Wnt receptors in stem cells. The presence of stem cell factor R-spondin lifts the brake in the adult stem niches, allowing self-renewal. However, as soon as the stem cells leave the niche, the RNF43/ZNRF3 brake ensures that they differentiate and do not pose a risk to tissue homeostasis. The discovery of this mechanism has been at the core of the development of the organoid technology (notably in the intestine) and an important focus for therapeutical approaches in cancer (e.g. colorectal cancer). However, a key question remained open: What keeps the RNF43/ZNRF3 brake functioning in the first place? That question was not trivial, given that these proteins not only destabilise Wnt receptors, but also themselves.

We discovered the deubiquitinase USP42 as a novel regulator of Wnt signalling. In follow up molecular studies, we found that USP42 stabilises ZNRF3 and RNF43 at the plasma membrane to ensure that the *dead man’s brake* is operational. We found that USP42 is present in the crypts of mouse small intestine and, using mouse intestinal organoids, we demonstrated that genetic ablation of *Usp42* is sufficient to render intestinal stem cells hypersensitive to Wnt ligands, thereby phenocopying loss of RNF43/ZNRF3. We also identified that *USP42* levels are elevated in colorectal cancer cells, which our data predicted that will stall the R-spondin/ZNRF3 complex. Accordingly, we found that USP42 functions as roadblock for EMT and growth in colorectal cancer cells by blocking autocrine Wnt/R-spondin signalling. Our research suggested that USP42 is required to switch off the Wnt functional programmes as cells leave the stem cell niches, which we plan to validate using transgenic mouse models.

Figure 2
Organoids are exceptional tools to understand how tissue homeostasis is regulated. A, *Usp42* is a novel Wnt/R-spondin inhibitor expressed in intestinal cell lineages. B, Analysis of self-renewal and differentiation of intestinal stem cells in organoids upon withdrawal of Wnt and R-spondin. Using this approach, we identified that genetic ablation of *Usp42* renders intestinal stem cells hypersensitive to these signals



Wnt signalling recruits KIF2A to the spindle to ensure chromosome congression and alignment during mitosis (Bufe et al., PNAS 2021).

Wnt10b-GSK3 β -dependent Wnt/STOP signaling prevents aneuploidy in human somatic cells. (Lin et al., Life Science Alliance 2020)

Canonical Wnt signalling plays critical roles in development and tissue renewal by regulating β -catenin-dependent transcription of target genes. Our previous research demonstrated that

canonical Wnt also promotes a rich post-translational programme independent of β -catenin that peaks during cell division. However, the biological functions of this programme remain largely uncharacterised. Using comprehensive molecular and live cell imaging phenotypic analyses we identified that Wnt signalling is required for i) timely congression and alignment of chromosomes and ii) whole-chromosome segregation.

In phospho-proteomics analyses, we identified that Wnt signalling regulates the minus-end microtubule depolymerase KIF2A during mitosis. KIF2A is required in development for scaling of the spindle and neurogenesis. Mechanistically, KIF2A promotes microtubule depolymerisation at the spindle poles, which generate pulling forces on attached kinetochores, thereby ensuring the congression and alignment of chromosomes. We found that the Wnt receptor complexes, which are termed LRP6 signalosomes, recruit KIF2A during cell division. We identified that these mitotic signalling platforms ensure KIF2A activation by PLK1, which is critical for the localization and function of the depolymerase at the spindle poles. Accordingly, we demonstrated inhibition of basal Wnt signalling in pluripotent stem cells is sufficient to induce chromosome congression delay and misalignment in mitosis. In collaboration with colleagues at the SFB1324, we also identified that specific Wnts, such as Wnt10b, can restrict plus-end microtubule dynamics in the spindle through the signalling hub GSK3 (independently of KIF2A). Consequently, genetic ablation of Wnt10b or the Wnt secreting factor EVI is sufficient to induce whole-chromosome missegregation and aneuploidy, including in pluripotent stem cells. Taken together, we proposed that Wnt signalling monitors spindle dynamics during mitosis through at least two independent mechanisms to ensure timely alignment and faithful segregation of chromosomes, which could have important implications for genome maintenance, notably in stem cells.

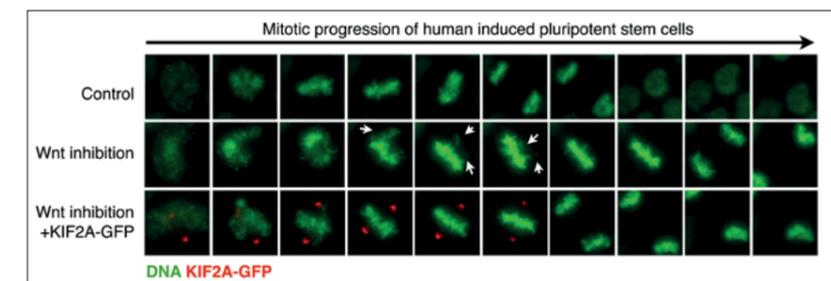


Figure 3
Wnt signalling promotes chromosome congression and alignment in pluripotent stem cells through the recruitment of KIF2A to the mitotic spindle poles.

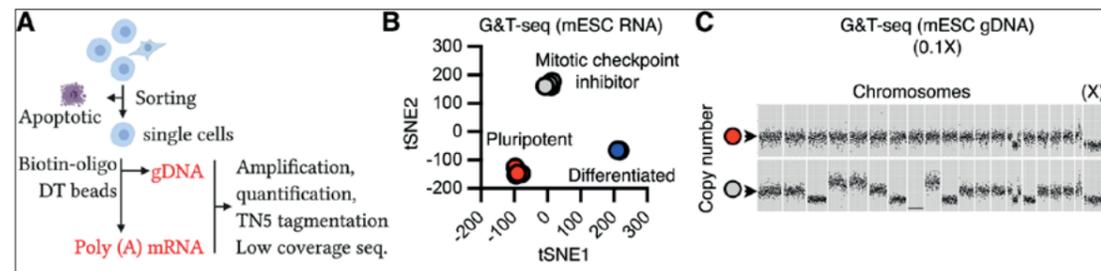
Future directions

Research in the next years will focus on i) characterising how the USP42/RNF43 molecular brake functions in vivo during tissue homeostasis, ii) unravelling how genome stability and cell fate specification are coordinated during development and tissue renewal, and iii) Developing novel reporting tools for signalling and aneuploidy to tackle the previous questions.

The USP42/RNF43 molecular brake: We plan to identify the signalling footprint associated with USP42 to determine how it coordinates adult stem cell renewal and differentiation in the intestine. To that end, we will use mouse transgenic models and organoids. Specifically, we will unravel the functional networks upstream and downstream of this molecular brake by combining proteomics (Split-BioID), single cell RNA sequencing and mass cytometry. We will also assess whether oncogenic mutations in the associated factors rewire USP42 functional network.

Coordination of genome stability and cell fate specification: Genome instability represent a major drawback in fertility and regenerative medicine, can disrupt tissue homeostasis, and is a hallmark of various diseases. In the context of the SFB1324, we will characterise the roles of Wnt signalling in monitoring faithful segregation of chromosomes during self-renewal using mouse blastocysts and organoid models. We will take advantage of single cell G&T-seq to characterise the bi-directional relationship between fate and genome maintenance. We will complement these studies with detail molecular analyses of the underlying cascades to characterise how specific Wnt ligands control different functions associated with chromosome segregation.

Novel reporters: The lack of scalable and non-invasive methods to monitor transient molecular processes in vivo represents a major drawback to address fundamental open questions in biology. Our project Synthetic DNA memories aims to develop novel molecular tools that orderly record cellular events into permanent and readable genetic memories. This collaborative and interdisciplinary project aims to establish high-throughput DNA recorders to unravel molecular dynamics in cell cycle (e.g. chromosome missegregation) and signalling across biological scales, including in mouse models. Furthermore, our goal is to implement these tools for a wide range of biological, industrial and therapeutical applications.



Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 4, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 3, Number of citations 2017-2021: 802

Bufe, A., García del Arco A., Hennecke, M.I., de Jaime-Soguero, A., Ostermaier, M., Lin, Y.C., Ciprianidis, A., Hattmer, J., Engel, U., Beli, P., Bastians, H., and **Acebrón, S.P.** ‡ (2021). Wnt signaling recruits KIF2A to the spindle to ensure chromosome congression and alignment during mitosis. *PNAS*. *In press* PNAS 118(34):e2108145118.

Giebel, N., de Jaime-Soguero, A., García del Arco, A., Landry, J.J.M., Tietje, M., Villacorta, L., Benes, V., Fernández-Saiz, V., and **Acebrón S.P.** ‡ (2021). USP42 protects ZNRF3/RNF43 from R-spondin-dependent clearance and inhibits Wnt signalling. *EMBO Rep.* 22(5):e51415.

Lin, YC., Haas, A., Bufe, A., Parbin, S., Hennecke, M., Voloshanenko, O., Gross, J., Boutros, M., **Acebrón, S.P.** and Bastians, H. (2020). Wnt10b-GSK3 β -dependent Wnt/STOP signaling prevents aneuploidy in human somatic cells. *Life Science Alliance.* 4(1):e202000855.

Berger, B.S.*, **Acebron, S.P.***,#, Herbst, J., Koch S., and Niehrs, C. (2017). Parkinson associated receptor GPR37 is an ER chaperone for LRP6. *EMBO Rep.* 18(5):712-725.

(‡ Last author; # Joint-corresponding author; * Equal contribution)

Figure 4

A, our newly established single cell genome and transcriptome (sc G&T-seq) pipeline will shed light on how Wnt signalling coordinates cell fate determination and genome stability in stem cells. B, C, failure to faithfully segregate chromosomes in stem cells impacts fate determination as shown by sc G&T-seq in collaboration with EMBL and Josephine Bageritz.





2.2 PROF. DR. DETLEV ARENDT ANIMAL EVOLUTION

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Fields of Interest

Animal evolution, cell type evolution,
planetary biology, evo-devo, marine biology,
nervous system evolution, eye evolution,
Platynereis dumerilii



Summary and outlook

The focus of our research is on the line of descent from the beginnings of multicellularity to human. What did these ancestors look like, how did they develop, function, feed and move in the changing planetary environment? What can we know about our last common ancestor with the nervous system-less sponges; with the nerve net-bearing cnidarians; with the annelids that have a centralized nervous system, or with the chordate amphioxus that has a neural tube with a very simple brain?

Our group has spearheaded the establishment of a new line of research to reconstruct these ancestors, which focuses on the comparison of cell types to understand their relatedness, origins and evolution. This approach builds on new technologies such as single-cell sequencing and volume electron microscopy, which allow us to build and compare multimodal cellular atlases for entire organisms. We can identify groups of related cell types, and then compare these data to that of other species, in order to establish a likely scenario of cell type diversification, i.e. an evolutionary cell type tree.

We have already built cellular atlases for the freshwater sponge *Spongilla lacustris*, the annelid *Platynereis dumerilii*, and the chordate amphioxus, outlined below. Ongoing and planned research expands this approach to the placozoan *Trichoplax adherens*, and basal vertebrates such as lamprey and shark. Building on these unique datasets, we will flesh out evolutionary scenarios on animal and nervous system origins and evolution in the form of comprehensive reviews and assays.

Research highlights since 2017

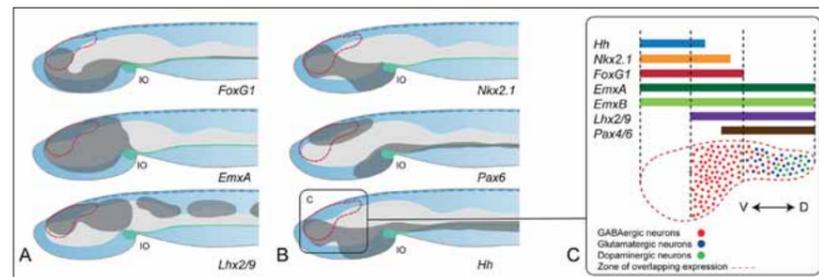
A telencephalon homolog in the chordate amphioxus (Benito-Gutierrez et al., 2021)

The evolutionary origin of the telencephalon, the most anterior part of the vertebrate brain, remains obscure. Since no obvious counterpart to the telencephalon has yet been identified in invertebrate chordates, it is difficult to trace telencephalic origins. One way to identify homologous brain parts between distantly related animal groups is to focus on the combinatorial expression of conserved regionalization genes that specify brain regions. We have investigated the combined expression of conserved transcription factors known to specify the telencephalon in the vertebrates in the chordate amphioxus. Focusing on adult specimens, we detect specific co-expression of these factors in the dorsal part of the anterior brain vesicle, which we refer to as Pars anterodorsalis (PAD). As in vertebrates, expression of the transcription factors FoxG1, Emx and Lhx2/9 overlaps that of Pax4/6 dorsally and of Nkx2.1 ventrally, where we also detect expression of the Hedgehog ligand. Importantly, this specific pattern of co-expression is not observed prior to metamorphosis, which explains why the PAD was not found in previous searches. Similar to the vertebrate

telencephalon, the amphioxus PAD is characterized by the presence of GABAergic neurons and dorsal accumulations of glutamatergic and dopaminergic neurons (Figure 1). Our findings suggest that the PAD in the adult amphioxus brain vesicle and the vertebrate telencephalon evolved from the same brain precursor region in ancestral chordates, which would imply homology of these structures. This ancestral brain may have already contained GABA-, glutamatergic and dopaminergic neurons, as is characteristic for the olfactory bulb of the vertebrate telencephalon. This makes the olfactory bulb a strong candidate for representing the most ancient part of the vertebrate telencephalon.

Figure 1

Combinatorial gene expression in the adult amphioxus brain. A, B Expression patterns projected on a lateral schematic brain representation. The dark grey shading represents the entire domain for each gene. The dashed red line indicates the region of expression overlap with a telencephalon-like profile. C Magnified view of the telencephalic-like region showing the dorsal-ventral distribution of gene expression and neurotransmitter identities. IO: infundibular organ. From Benito-Gutierrez et al., 2021

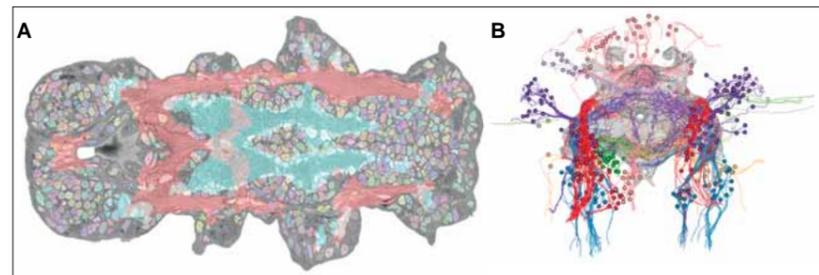


The Platynereis cell atlas integrates gene expression and single-cell morphology for an entire three-segmented young worm (Vergara et al., 2017 and Vergara et al., 2021)

In the 6-days-old three-segmented young worm of the nereid *Platynereis dumerilii*, the presence of a complex nervous system is paired with an overall remarkable anatomical stereotypy, which means that individuals resemble each other down to the cellular level. Furthermore, the worm starts feeding after only after this stage, which brings about another key advantage of the nereid model system: a pronounced synchrony of development. Together, stereotypy and synchrony have made it possible to register individual larval worms stained by whole-mount in situ hybridization for different genes of interest (Vergara et al., 2017), and, adding to this, to register such gene expression atlas to a single serial block-face scanning electron microscopy volume (Vergara et al., 2021) (Figure 2). For the first time, this key resource allows us to explore the link between cell-type-specific gene expression and subcellular morphology systematically and for an entire body. Having segmented all cellular somata and nuclei in the volume EM dataset, we have morphometrically characterized and compared all 11,400 cells. Unbiased analysis of cellular morphological parameters thus recognizes major cell classes and establishes a link between nuclear size, chromatin topography, and gene activation. Furthermore, clustering of segmented cells according to their assigned gene expression identifies spatially coherent groups of cells and indicates that combinations of regionally expressed transcription factors specify tissue identity. Genetically defined groups of neurons match anatomical boundaries and show similar axonal projections.

Figure 2

The multimodal cellular atlas. A. A snapshot from the PlatyBrowser, an online tool that allows exploring all data modalities that have been registered on the volume EM dataset. This picture shows segmentation of nuclei and chromatin for all body cells, and gene expression for a muscle and a neuropil marker (red and blue). Gene expression information taken from Vergara et al., 2017. Volume EM dataset and segmentation from Vergara et al., 2021. B. A set of ~500 randomly selected brain cells for which neurite projections have been traced. Similar colors demarcate cells that cluster together according to differential gene expression, which implies that genetically similar cells tend to show similar projections. Data from Vergara et al., 2021



The new resource has also allowed us to study the molecular anatomy of the nereid mushroom bodies, the presumed associative centers of the nereid brain. Surprisingly, we have found that the mushroom bodies contain sensory-neurosecretory cells and thus qualify as chemosensory organs themselves. These findings shed new light on the evolution of associative centers in Bilateria and of the telencephalon in the vertebrates.

A cell type atlas for the freshwater sponge Spongilla lacustris

To address the evolutionary origin of neurons and muscle cells, we have established single-cell transcriptomics and single-molecule fluorescent in situ hybridization (smFISH) for the freshwater sponge *Spongilla lacustris*, collected from Lake Constance. Sponges generally lack nervous system and musculature, and can thus be informative about cellular functions of conserved neuronal genes predating the evolution of the nervous system. Using smFISH directed against marker genes expressed in juvenile sponges, we have identified 18 distinct cell types (Figure 3A). These comprise four cell type families including nitric-oxide sensitive contractile cells, digestive cells active in macropinocytosis, and a novel family of amoeboid-neuroid cells involved in innate immunity and intercellular signaling. Specifically, we uncover 'presynaptic' genes in an amoeboid-neuroid cell type, and 'postsynaptic' genes in the digestive choanocytes, which is indicative of asymmetric and targeted communication. To explore the interaction between choanocytes and neuroid cells further, we have developed correlative X-ray electron microscopy (CXEM) to locate and image individual neuroid cells in choanocyte chambers (Figure 3B). Corroborating the molecular findings, we have observed long neurite-like extensions from neuroid cells that directly contact and enwrap choanocyte microvillar collars. Our data indicate a link between neuroid and immune functions in sponges, and suggest that a primordial neuro-immune system cleared intruders and controlled ciliary beating for feeding.

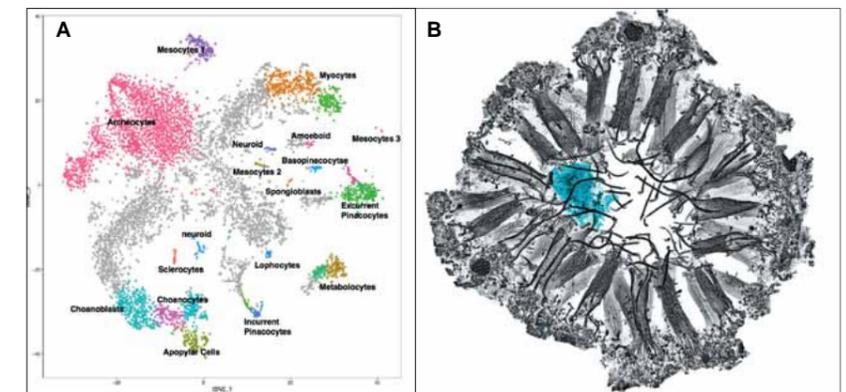


Figure 3

A cellular atlas for *Spongilla lacustris*. A. A 2-dimensional representation (UMAP) of all sequenced cells clustered according to expression similarity. Peripheral clusters represent cell types that have been identified via sm-FISH for marker genes on whole-mount juvenile sponges. B. A single neuroid cell populating a choanocyte chamber, captured by the newly-developed Correlative X-ray Electron Microscopy (CXEM) technique. The neuroid cell has multiple cellular projections that contact the cilia and the collar of choanocytes for cellular communication. Data from Musser et al., 2021

Future directions

The availability of cellular atlases for representatives of an ever-increasing number of animal phyla holds immense promise for the new field of cell type evolution. If we assume that the evolutionary lineage that led from the first metazoan to human has seen a never-ending series of cell type diversification event, with initial cell types giving rise to cell type families, we can infer the existence of an ever-growing cell type tree along this lineage. This tree can be reconstructed by the comparison of cell type trees between phyla, which should share amount of cell type diversification that was already present in their last common ancestor (Figure 4).

We have already started to cross-compare single-cell datasets generated in our laboratory (sponge, placozoan, annelid, polyplacophoran) or with collaborators (lamprey, sea urchin) with previously published datasets (vertebrates, insects, cnidarians). This requires new methodology for the construction of cell type trees for one species, and for the comparison and integration of cell type trees across species. Towards this aim we have initiated a collaboration with the Bo Wang lab at Stanford, with first results regarding the alignment of cell types and cell type families across phyla (Tarashansky et al., 2021). We are also in the process of creating a cross-comparative web-based database for the storage and comparison of single-cell datasets. To enable reasonable comparison, we are working with Jaime Huertas Cepas on the phylome resource, an automated pipeline for the establishment of orthology and paralogy relationships across entire genomes, via the systematic construction of gene family trees.

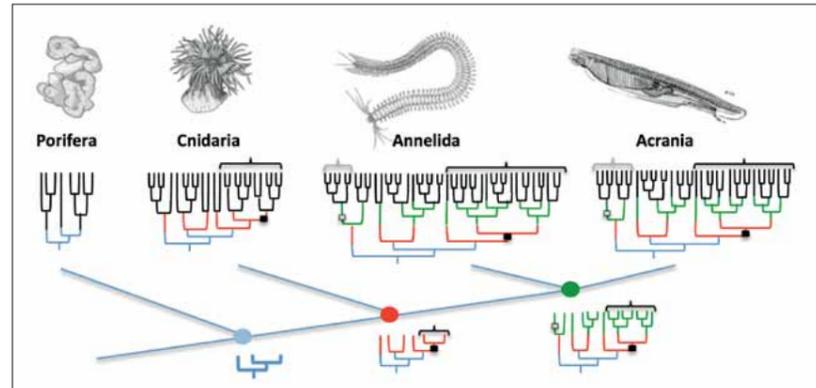


Figure 4

Hypothetical scheme illustrating cell type diversification in animal evolution. For each tree, the cell type branching events that had already occurred in a given ancestor are drawn in the color of that ancestor. The birth of the first neuron is indicated by a black box and the family of neurons demarcated by brackets, the (hypothetical) emergence of a second, neuron-like cell type family in stem bilaterians by grey box and brackets. From Arendt et al., 2019.

We are expecting to solve large part of the metazoan cell type tree in the next years. This has the potential to solve major issues in animal evolution, such as the single or multiple origin of neurons and nervous system, nervous system centralization, and brains, as well as other cell types, tissues, and organs such as musculature, nephridia, gut, or notochord. We are aiming at the integration of comparative cell biology, comparative development, and our knowledge of the fossil record of animals towards a comprehensive view of animal evolution.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 24, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 22, Number of citations 2017-2021: 4524 (Google Scholar)

Musser, J. M., Schippers, K. J., Nickel, M., Mizzon, G., Kohn, A. B., Pape, C., Hammel, J. U., Wolf, F., Liang, C., Hernandez-Plaza, A., Achim, K., Schieber, N., Francis, W. R., Sergio Vargas R., Kling, S., Renkert, M., Feuda, R., Gaspar, I., Burkhardt, P., Bork, P., Beck, M., Kreshuk, A., Wörheide, G., Huerta-Cepas, J., Schwab, Y., Moroz, L. L., and **Arendt, D.** (2021) Profiling cellular diversity in sponges informs animal cell type and nervous system evolution. *Science*, 374, 717-723.

Vergara, H. M., Pape, C., Meechan, K. I., Zinchenko, V., Genoud, C., Wanner, A. A., Titzte, B., Templin, R. M., Bertucci, P. Y., Simakov, O., Machado, P., Savage, E. L., Schwab, Y., Friedrich, R. W., Kreshuk, A., Tischer, C., and **Arendt, D.** (2020) Whole-body integration of gene expression and single-cell morphology. *Cell*, 184, 4819-4837

Arendt D. (2020). The Evolutionary Assembly of Neuronal Machinery. *Current Biology* 30(10), R603-R616.

Achim K, Eling N, Vergara HM, Bertucci PY, Musser J, Vopalensky P, Brunet T, Collier P, Benes V, Marioni JC, **Arendt D.** (2018) Whole-body single-cell sequencing reveals transcriptional domains in the annelid larval body. *Mol Biol Evol.* 35(5)

Vergara HM, Bertucci PY, Hantz P, Tosches MA, Achim K, Vopalensky P, **Arendt D.** (2017) Whole-organism cellular gene-expression atlas reveals conserved cell types in the ventral nerve cord of *Platyneris dumerilii*. *Proc Natl Acad Sci* 114(23):5878-5885





2.3 DR. JOSEPHINE BAGERITZ INDEPENDENT RESEARCH GROUP

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Fields of Interest

Muscle stem cell niche, cellular heterogeneity,
Wnt signaling, single-cell transcriptomics



Summary and outlook

We want to deconstruct the dynamic crosstalk between muscle stem cells and their niches during development, throughout adulthood and under experimental perturbations to understand how stem cell number and cellular identity are regulated. To address this, we apply modern approaches such as live cell imaging, cell type-specific CRISPR gene editing and single-cell RNA sequencing (scRNAseq) throughout development and adult life to dissect key principles and their underlying mechanism in a spatial context. We have previously generated a gene expression atlas of the *Drosophila* wing epithelium, which serves as a niche during development for the adjacent muscle stem cell populations (Figure 1). By selecting genes with expression patterns that correlate to known signaling pathway components, we identified clusters of genes with similar expression patterns and functionally linked the previously uncharacterized gene CG5151 to the Wnt signaling pathway (Bageritz et al., Nature Methods 2019). By focusing our studies on CG5151, we aim to mechanistically understand the role of novel Wnt pathway components for their role in muscle stem cell niche heterogeneity.

Furthermore, we want to understand how Wnt encoded specificity is achieved in different stem cell types (in collaboration with Ingrid Lohmann) and how this is linked to differences in muscle stem cell pool size. For this, a systematic analysis of all Wnt ligands, receptors and co-receptors in the epithelial-muscle stem cell niche will be conducted to unravel a specific 'Wnt code' important for muscle stem cell heterogeneity.

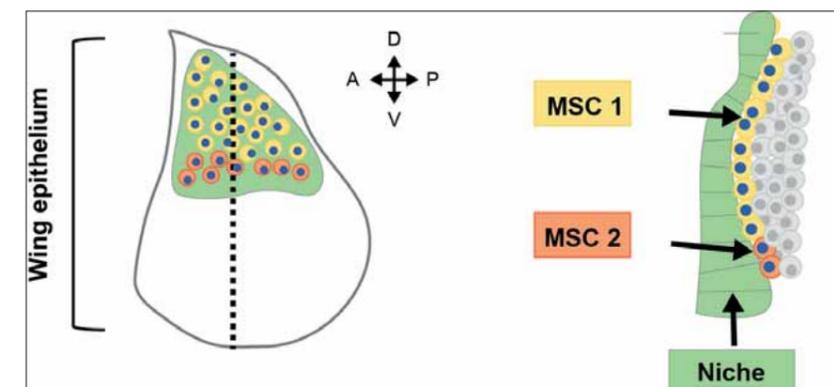


Figure 1
The *Drosophila* epithelial-muscle stem cell niche. Epithelial niche cells are colored in green, the two muscle stem cell (MSC) populations in yellow and orange, respectively.

Research highlights since 2017

Single cell transcriptome technologies rapidly developed in the last years allowing to profile thousands of individual cells at once. We have established Drop-Seq, a droplet-based single cell RNAseq (scRNAseq) technology and developed and benchmarked single-cell library protocols tailored for unfixed and glyoxal-fixed *Drosophila* cells (Bageritz and Raddi, 2010; Bageritz and Krausse, et al., 2020). Those methods allowed us to generate a gene expression atlas for the developing wing epithelium of *Drosophila* (Bageritz et al., 2019), a tissue composed of mainly undifferentiated stem-like cells. Importantly, we developed new bioinformatic approaches for computing expression maps of the wing epithelium that are based on gene expression correlations rather than cell mapping and we invented expression maps to identify spatially restricted expression patterns outperforming existing methods. Our methods will leverage the generation of atlases of undifferentiated tissues during development.

Using our established method, we identified clusters of genes with similar expression patterns and functional relevance and functionally linked the previously uncharacterized gene CG5151 to the Wnt signaling pathway. In a second approach, we performed scRNAseq on *Drosophila* intestines in homeostasis and bacteria-feeding conditions (Bageritz, Frauhammer, et al., manuscript in preparation). We performed in depth bioinformatic analysis, which enabled the separation of ISCs from their immediate progeny, two cell types found to have similar transcriptional profile. Consequently, our analysis now allows a better characterization of both cell types. Interestingly, we discovered that the progenitor population, contrary to the common assumption, directly responds to the pathogenic bacteria.

Future directions

With our current research we aim to decipher the role of Wnt signaling for muscle stem cell heterogeneity. Our developmental studies will elucidate Wnt pathway specificity in different muscle stem cell populations and its impact on muscle stem cell specification and stem cell pool size. We will extend this study to adulthood, where Wnt signaling is key for proper muscle fiber regeneration. Comparative analysis of developmental and adult processes will allow us to tackle some general overarching questions about stem cells and their niches: What is the contribution of the niche to stem cell cell-cycle exit and speed? How does the niche control stem cell pool size and in this way prevents tumorous outgrowth? How does the stem cell/niche interaction change during the lifetime of an organism? What are the molecular mechanisms underlying these complex processes? For this purpose, we will use live cell imaging, cell type-specific gene manipulations and scRNAseq together with our refined computational tool set.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 1, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 1, Number of citations 2017-2021: 32

Bageritz, J.,* #, Krauß, N.* #, Yousefian, S., Leible, S., Valentini, E., Boutros, M. # Glyoxal as alternative fixative for single cell RNA sequencing. *Contributed equally. # Corresponding authors. bioRxiv (2021); doi: <https://doi.org/10.1101/2021.06.06.447272>

Ernst, K.* #, Okonechnikov, K.* #, **Bageritz, J.**, Malm, JP., Maaß, K., Pfister, SM., Zuckermann, M., Jones, DTW. Establishment of a simplified preparation method for single-nucleus RNA-sequencing and its application to long-term frozen tumor tissues. *Contributed equally. bioRxiv (2020); doi: <https://doi.org/10.1101/2020.10.23.351809>

Bageritz, J., Willnow, P., Leible, S., Valentini, E., Boutros, M., Teleman, A. A gene expression atlas of the *Drosophila* wing imaginal disc at single-cell resolution. Nature Methods (2019) Jul;16:750–756.

Bageritz, J., Raddi, G. (2019) Single-Cell RNA Sequencing with Drop-Seq. In: Proserpio V. (eds) Single Cell Methods. Methods in Molecular Biology, vol 1979. Springer Nature published by Humana, New York, NY





2.4 JUNIOR PROF. DR. LÁZARO CENTANIN INDEPENDENT RESEARCH GROUP

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Fields of Interest

Post-Embryonic Stem Cells
Stem Cell Niche Formation
Coordination of Growth Within and
Among Vertebrate Organs,
Hierarchical Organisation of Lineages
Post-Embryonic Organogenesis
Phenotypic Variation Within a Genus



Summary and outlook

In animals with a fixed size, like mammals, adult stem cells (aSCs) maintain tissue homeostasis. Most vertebrates, however, keep growing during their post-embryonic life by the addition of new cells. Adult stem cells, therefore, need to fulfill two different functions: homeostatic replacement of lost cells, and the addition of new cells to increase organ size in a coordinated manner. We focus on the differences and similarities between homeostatic and growth stem cells.

Our animal model of choice is the teleost fish *Oryzias latipes*, medaka. Fish grow during their entire post-embryonic life, and permanent growth comes with major challenges like growth coordination. On the one hand, the extent of growth of each organ has to match that of the entire fish and the different organs within. On the other hand, and since most organs are composed of multiple lineages, the activities of the different lineage-restricted stem cells need to be coordinated in a given niche.

Our organs of choice are the sensory neuromasts of the lateral line system and the respiratory gills. Both organs are composed of cells from different lineages, but constitute opposite extremes in their complexity. Neuromasts contain a single differentiated cell type and only two lineages (Seleit, Kraemer et al, 2017)(Figure 1). Gills are composed of multiple differentiated cell types that we classified into 4 lineages (Stolper et al, 2019)(Figure 2). We tackle the hierarchical organization of the different lineages by generating genetic clones, wild-type/mutant mosaics, and inter-species chimeras.

Research highlights since 2017

We have implemented a non-biased, systematic induction of clones (Centanin et al, 2014) to tackle inter-lineage coordination. We have performed lineage analysis to characterize organ formation, regeneration, and growth in medaka, using a conventional system of organ growth and a non-conventional system of organ replication - both occurring during homeostatic growth along with post-embryonic life. Additionally, we have expanded our analysis of inter-lineage coordination by combining blastomeres of different species to form bona-fide chimeras. Our first attempts mixing medaka and zebrafish blastomeres allowed us to distinguish cell-autonomous programs from those influenced by the tissue environment.

b 1) The neuromasts — Lineage Induction and Organ Replication

Most organs consist of cells from different embryonic origins, so how do different cell types find each other during embryogenesis at the proper time and defined position? We exploited the simple composition of fish neuromasts to evidence two independent lineages (neural & niche) that are maintained fate-restricted life-long (Figure 1A). Following a 4D approach at critical developmental stages, we revealed that neural stem cells induce a morphological transformation of epithelial cells around, which eventually form a stem cell niche. The recruitment is mediated by transient cytoplasmic protrusions from the neural progenitors (Figure 1B), possibly mediators of the epithelial-to-niche transformation. We are following 4D imaging combined with transcriptome analysis of neural stem cell precursors to uncover the molecular determinants of niche induction.

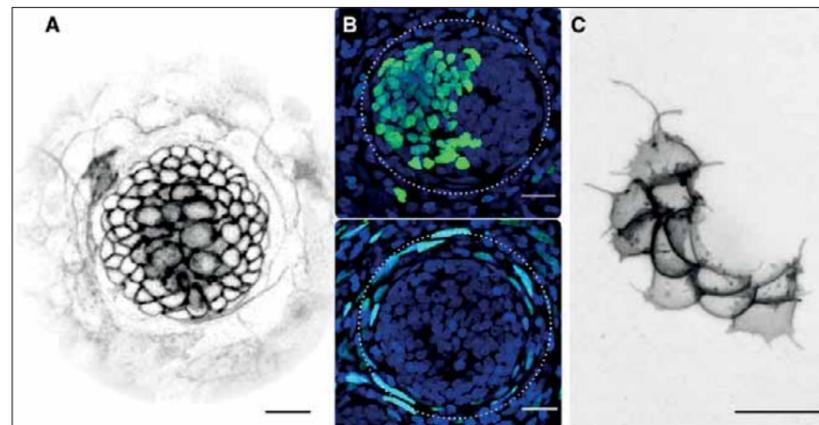


Figure 1
Neuromasts are small surface organs (A) composed of neural and niche lineages (B). During organogenesis, neural progenitors extend cellular protrusions (C) at the time that epithelial cells transform into niche stem cells. Scale bar: 10 μ m. Panels are adapted from Seleit, Krämer et al, 2017.

Fish grow massively during their post-embryonic and adult life, so organs and systems need to adapt to the growth pace of the fish. Some organs grow in size, where some systems expand by generating new post-embryonic organs. We are studying this process utilizing the caudal neuromast cluster that originates from the embryonic neuromasts PO. We have characterized the initial steps of post-embryonic organogenesis, which involve neural stem cells delaminating from the original organ, migrating short distances away, and organizing into a new functional neuromast. We are currently uncovering the molecular mediators triggering stem cells to abandon their organ of origin.

b 2) The Gills — Organ Growth.

Stem cells in fish drive constant organismal growth and maintain the homeostasis of functional organs. We are working on identifying the cellular and molecular features that differentiate growth from homeostatic stem cells. Following lineages in the medaka gills, we have functionally identified growth and homeostatic stem cells that locate to disparate regions and are subjected to differential forces (Stolper et al, eLife 2019). We follow the hypothesis that a strong physical niche is what we mammals use to prevent stem cell growth and restrict our stem cells to a homeostatic function. Additionally, lineage analysis has revealed that the medaka gill is built, maintained, and grown by 4 different fate-restricted types of stem cells (Figure 2A). These cells co-occur in every single of the ca. 800 filaments in an adult gill, and we focused on the hierarchical organization among fate-restricted stem cells. Are all equal in their role during organ growth? Or is there a dominant lineage imposing a growth rate to the others? Analyzing gill filaments in p53/wt chimeras (Figure 2B), we noticed that mosaic gills displayed a strong growth phenotype. Critically, filament's length is compromised when the wild-type clone in a p53 mutant gill contained pillar cells, indicating that individual lineages have indeed different roles during coordinated growth. Similar data were obtained in chimeras generated by mixing blastomeres of *Oryzias* species that differ in their growth dynamics. These expe-

riments constitute a proper experimental setup to address mechanistically the hierarchical organisation of stem cells in composite organs.

b 3) Chimeras — Self-established vs Coordinated Programs

Unlike intra-species blastomere transplantations, where the donor and host blastomeres intermingle during gastrulation, we noticed that inter-species transplantations between medaka and zebrafish resulted in donor and host cells that stayed apart from each other (Figure 3). The foreign group of cells would eventually develop into a retina (Fuhrmann et al, 2020), and we have taken advantage of the different spans of embryogenesis (3 and 9 days for zebrafish and medaka, respectively) to explore self-established vs coordinated programs. We show that the time taken for the retina to develop follows a genetic program:

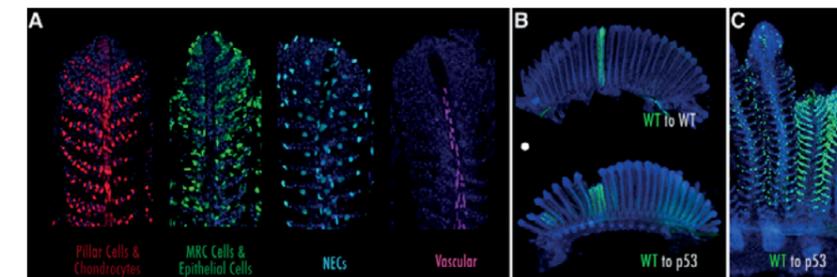


Figure 2
Stem cells are fate-restricted and each filament contains at least four stem cells of different fates (A). Transplanted wild-type cells generate coordinated filaments in wild-type hosts but shorter filaments in p53-mutant hosts (B). The phenotype occurs when pillar cells are labelled in the clone (C). Panels are adapted from Stolper et al, 2017.

an ectopic zebrafish retina in medaka develops with zebrafish dynamics. Despite this, the ectopic retina interacted with host tissues to complete axonal navigation and lens induction. Our results provide a new experimental system for addressing temporal decoupling along with embryonic development and highlight the presence of largely autonomous but interconnected developmental modules that orchestrate organogenesis.

Future directions

We study animals as models in which different cell types are in constant communication and respond to each other. The systems we use in the lab are crucial for the animal to read their environment, and at the same time can adapt to the changing conditions that fish live in. We are following three complementary approaches to investigate the impact of the environment on stem cell activity: i) responses to external stimuli, ii) responses to mutations in neighboring tissues, and iii) adaptation to alternative growth rates by inter-species transplantations.

i) *Oryzias latipes* display extreme resilience when it comes to changes in temperature, salinity, and water flow. Adaptation to saline water occurs by producing more ionocytes in the gill, and our knowledge on gill stem cell organization will allow us to determine whether the excess of MRC cells results from the enhanced activity of an already active MRC growth/homeostatic stem cell, the recruitment of a previously dormant MRC stem cell, or a change-of-fate of stem cells previously committed to another lineage. The functional data on stem cell behavior will be complemented with transcriptome analysis on fate-restricted clones at several time points during the adaptation to saline water.

ii) We will exploit the stereotypic formation and distribution of neuromasts in medaka to reveal the contribution of tissue environment to organogenesis and stem cell function. We have recently generated several *keratin* mutants that display an aberrant distribution of neuromasts, and the analysis on wt/mutant chimeras indicates that these phenotypes originate from a defective adhesion in the epithelium. This impacts as well in neural stem cell behavior within the neuromasts, and their visual accessibility allow us to characterize the impact of tissue architecture at the cellular level.

iii) We have recently incorporated into our experimental toolbox *Oryzias* species that show differential morphological traits, physiological responses, and growth rates. We pro-

duce hybrids between the compatible species to identify dominant and receive traits, but most interestingly, we generate inter-species chimeras, mixing cells that display disparate genetic content and physical properties. The generation of interspecies chimeras is an entry point into how the cellular environment impacts long-term stem cell activity, and an optimal paradigm to reveal the hierarchical organization of stem cells within a niche. Combining cells among different teleosts, and going as far as mixing medaka with zebrafish, species that have diverged 250My ago, we are revealing both fixed and adaptable programs during organ formation, maintenance, and growth.

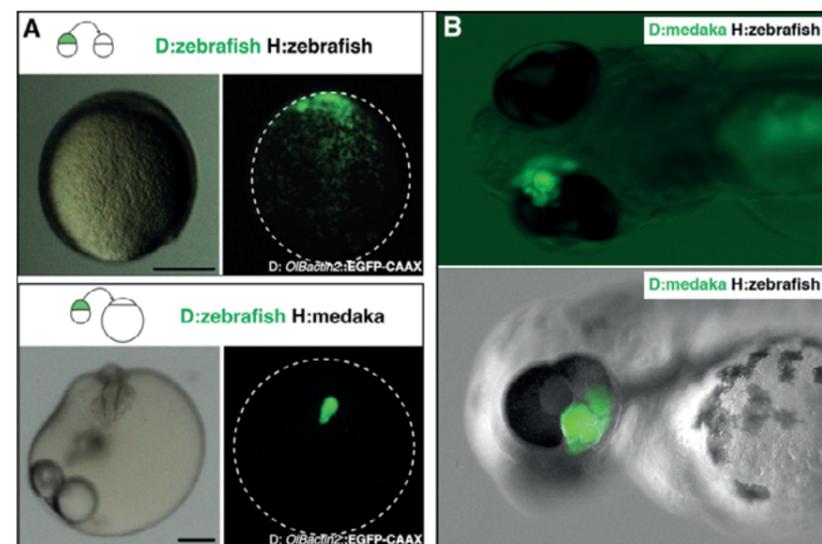


Figure 3
Inter-species transplantations between zebrafish and medaka result in proper chimeras where host and donor blastomeres do not intermingle (A). The donor blastomeres develop into a retina (B), which we use to tackle self-established versus coordinated developmental and growth programs.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 10, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 7, Number of citations 2017-2021: 5431

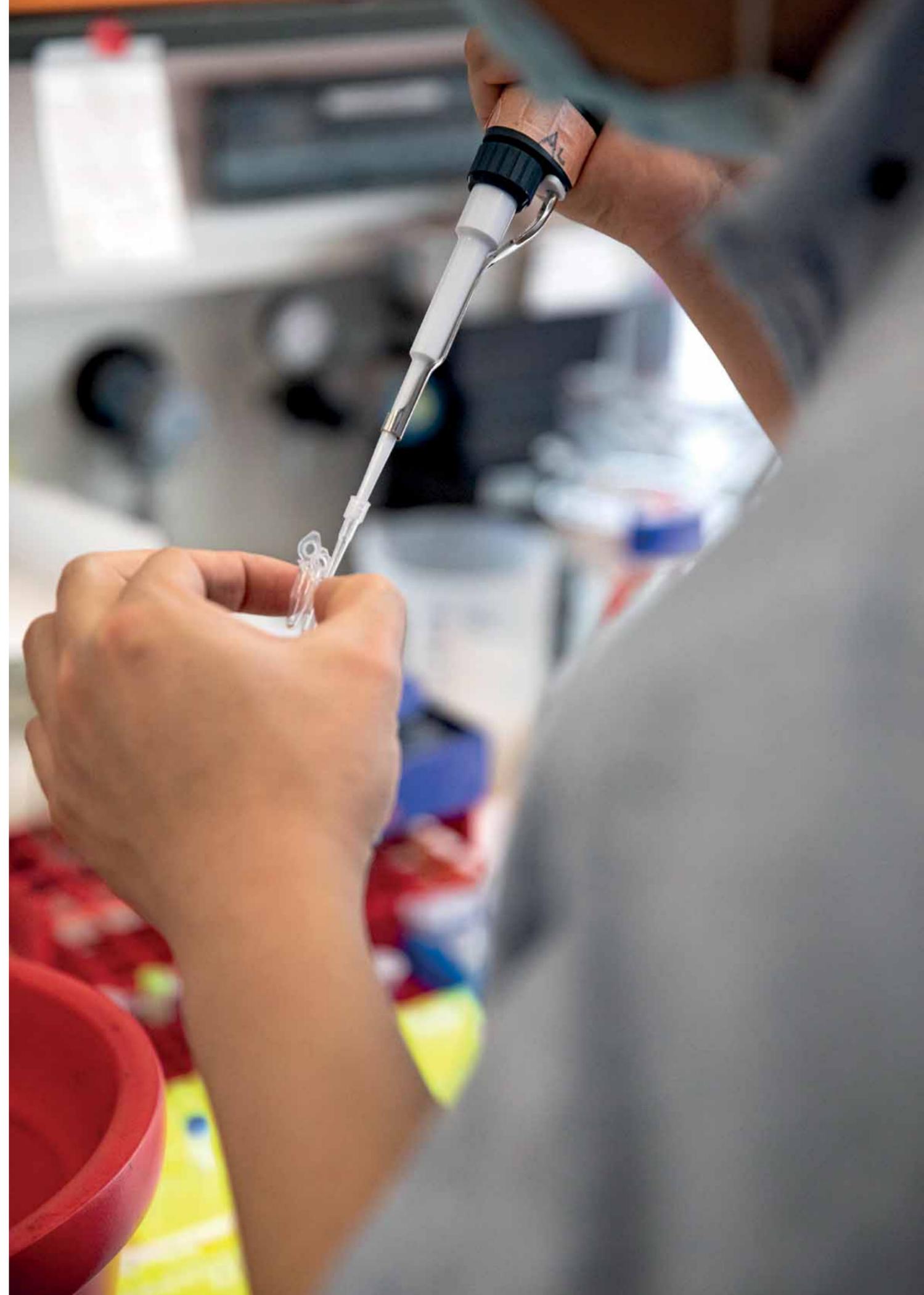
Fuhrmann, J.F., Buono, L., Adelman, L., Martinez Morales, J.R., **Centanin, L.** (2020). Genetic Developmental Timing Revealed by Inter-Species Transplantations in Fish. *Development* 147, dev192500. doi:10.1242/dev.192500

Seleit, A., Gross, K., Onistschenko, J., Woelk, M., Autorino, C., **Centanin, L.** (2020). Development and Regeneration Dynamics of the Medaka Notochord. *Dev Biol.* 463(1), 11-25

Stolper, J., Ambrosio, E.M., Danciu, D.P., Buono, L., Elliott, D.A., Naruse, K., Martínez-Morales, J.R., Marciniak-Czochra, A., **Centanin, L.** (2019). Stem cell topography splits growth and homeostatic functions in the fish gill. *eLife* 16, 8:e43747

Seleit, A.*, Krämer, I.*, Riebeseh, I.B., Ambrosio, E., Stolper, J., Lischik, C.Q., Dross, N., **Centanin, L.** (2017). Neural Stem Cells Induce the Formation of Their Physical Niche During Organogenesis. *eLife* 6, e29173.

Seleit, A., Krämer, I., Ambrosio, E., Dross, N., Engel, U., **Centanin, L.** (2017). Sequential organogenesis sets two parallel sensory lines in medaka. *Development* 144, 687-97.





2.5 DR. JAN FELIX EVERS INDEPENDENT RESEARCH GROUP

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Fields of Interest

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



Summary and outlook

Coordinated behaviour is the result of successful neuronal network assembly. During embryogenesis, excitability and connectivity of neurons must be carefully adjusted so that functional networks can arise. We investigate how individual neurons select from the vast number of potential partner neurons and form synapses with only a few in the inherently variable environment of the central nervous system.

Using the locomotor system of *Drosophila melanogaster* as a model system, we develop and apply a number of cutting edge techniques to uncover the molecular mechanisms that regulate developmental plasticity and functional homeostasis of neurons during network assembly:

1. live 3-dimensional imaging of dynamic growth and synaptogenesis of individual pairs of neurons;
2. 3-dimensional computational analysis of neuronal structure and interaction with synaptic partners;
3. optogenetically inducible labeling of endogenous synaptic proteins in individual cells with a conditional marker.

Research highlights since 2017

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

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

We show that neuronal growth in the embryo is geared to occupy synaptic territory; the emergent neuronal architecture then adapts to an increasing body size during post-embryonic animal growth, adding dendritic branches and synaptic connections while

maintaining the same synaptic territory that has been established in the embryo. During this time synapses not only increase in number, but also change their molecular composition with increasing developmental age: individual release sites accumulate more of the scaffold protein Bruchpilot (Brp), which likely subserves an increase in release probability and therefore gives rise to stronger and more reliable synaptic connectivity. We now have evidence that Brp is synthesized in the axon proximal to the synaptic release sites by local translation from long-lived mRNA.





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

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Fields of Interest

The regulation, function and evolution of the circadian timing system and light-dependent control of gene expression. Regulation and evolution of DNA damage repair systems.



Summary and outlook

The circadian clock is a key biological timing mechanism which temporally coordinates most aspects of plant and animal biology with respect to the environmental day-night cycle. Central to its function is its daily resetting by environmental signals, (so called “zeitgebers”), such as light and temperature which are indicators of the time of day. This ensures that endogenous clock time remains synchronised with the external day-night cycle. In turn, a wide range of systemic and cell autonomous mechanisms relay timing information from the clock to its regulatory targets. These include DNA repair mechanisms, many of which are also regulated directly in response to sunlight exposure. We use a complementary set of model species including zebrafish, medaka and blind cavefish, fish-derived cell lines as well as the anthozoan, *Aiptasia*, to tackle fundamental questions in circadian clock biology. Since 2017, we have continued to focus on understanding the signalling pathways whereby light, UV and ROS exposure regulates the clock and DNA repair systems via triggering changes in gene expression. We have also studied the evolution of sunlight sensing mechanisms under extreme environmental conditions such as constant dark cave environments as well as shallow, strongly sunlit marine environments. We investigate how light shapes the function of DNA repair mechanisms not only over the timescale of minutes and hours, but also over the course of evolution. Our future goal is to pinpoint the critical regulatory elements that have been modified during evolution and which shape long-term adaptations to sunlight exposure.

Research highlights since 2017

Our previous work has focused on exploring how direct exposure to sunlight induces the transcription of a subset of key clock genes as well as genes involved in the repair of sunlight-damaged DNA. Studying various fish models including zebrafish, medaka and blind cavefish, we have implicated a set of non-visual opsins in the detection of light. Bioinformatics analysis and functional testing of the promoters of batteries of light regulated genes have pinpointed the D-box enhancer element as the primary light responsive promoter enhancer element. We have also revealed that the MAPK signaling pathway plays an important regulatory role upstream of the bZip transcription factors which bind to D-boxes (Pagano et al., 2018).

During the reporting period, we have studied the evolution of light-dependent DNA repair systems in species of blind cavefish. We have examined the photoreactivation mechanism which repairs UV-induced DNA damage and relies upon visible light to drive the enzymatic repair process (Zhao et al., 2018). This highly conserved DNA repair system is based on the function of photolyases. These are highly conserved flavoproteins which are encoun-

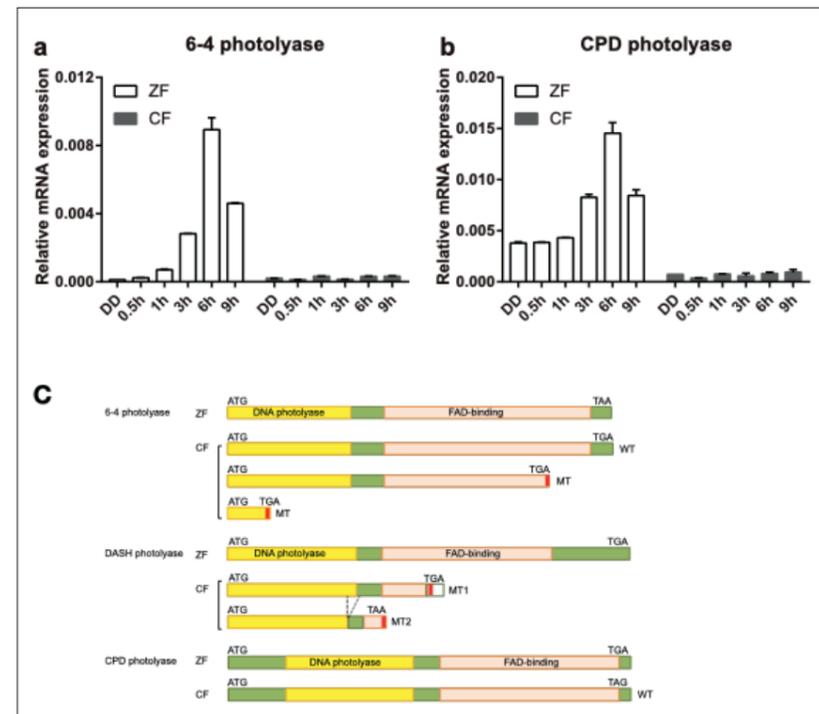
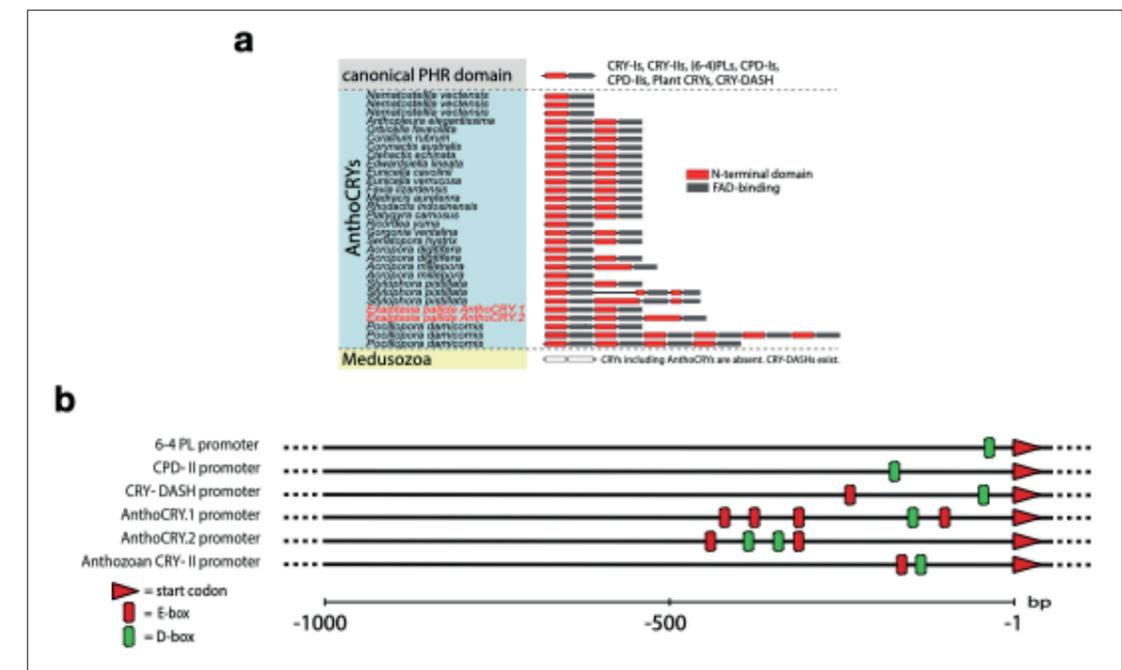


Figure 1
a,b Loss of light inducible expression of 6-4 and CPD photolyase genes in cavefish (CF) compared with zebrafish (ZF) cells, **c**, Truncation mutations affecting cavefish (CF) 6-4 and DASH photolyase in cavefish (CF).

tered in most prokaryotes and eukaryotes and absorb photon-derived energy via electron transfer between a specific chromophore and flavin. Photolyases are also closely related to cryptochromes which serve as key, negative regulatory elements of the core circadian clock machinery. Our work has revealed loss of photoreactivation in the Somalian blind cavefish, *Phreatichthys andruzzii*. This results from a general loss of D-box-mediated, light-induced photolyase gene expression as well as truncation mutations affecting two of the three main classes of photolyases. The C-terminally truncated proteins are restricted to the cytoplasm and so unable to contribute to DNA repair (See Figure 1). Loss of photoreactivation function is consistent with evolution in a perpetually dark cave environment, where *P.andruzzii* is neither exposed to UV nor visible light.

In contrast, other DNA repair systems such as Nucleotide Excision Repair (NER) are conserved during cavefish evolution. Interestingly, studying the regulation of the cavefish *ddb2* gene which encodes a key NER recognition factor, has revealed that D-box enhancers in its promoter cooperate with an adjacent E2F binding site and thereby retain light, UV and ROS inducibility (Zhao et al., 2021). Although the adaptive significance of retaining this D-box driven transcription in the cave environment is uncertain, it does potentially enable an upregulation of NER DNA repair in response to elevated levels of oxidative stress that might be encountered in cave water systems. Furthermore, these observations provide more insight into the regulation and function of the sunlight-responsive D-box enhancer. In collaboration with the group of Annika Guse (COS, Heidelberg) we have also started to investigate how evolution in shallow marine environments with sustained daytime levels of intense sunlight has affected clock and DNA repair evolution in the anthozoa (corals and sea anemones). Anthozoa are particularly interesting due to the many examples of endosymbiosis with photosynthetic dinoflagellates in these species. This symbiotic relationship places the host animals under additional photic stress, since they need to be exposed to sustained sunlight to maximize levels of photosynthesis. Furthermore, because of this photosynthetic activity, host cells also experience elevated levels of oxidative stress. Our first step has been to perform a detailed phylogenetic analysis of photoreceptors in the phylum cnidaria (Gornik et al., 2021). This has included documenting the opsin as well

as the cryptochrome and photolyase gene families in a range of anthozoa and medusozoa (the jellyfish). These results have revealed a surprising diversity of opsins in the sessile anthozoa compared with their more motile medusozoan relatives. Furthermore, we have identified a novel anthozoan cryptochrome, which we have termed AnthoCry, that shows similarity with the 6-4pp photolyase proteins as well as multiple tandem repeats of the conserved functional domains (Figure 2). Such a tandem repetition has not been encountered previously in any other prokaryotic or eukaryotic species and we speculate this may represent an anthozoan-specific adaptation which enhances DNA repair in these challenging marine environments. Consistent with this prediction, AnthoCry gene expression in the model species *Aiptasia*, is robustly induced upon both visible light and UV exposure. Furthermore, D-box enhancer elements are enriched in the promoter regions of the AnthoCry genes (Figure 2), suggesting that these enhancers play an ancestral role in the transcriptional response to sunlight exposure.



Future directions

In relation to the D-box mediated transcriptional response to light, UV and ROS exposure, we are currently comparing the sunlight-regulated transcriptome in zebrafish cells with that of blind cavefish cells (which lack D-box-induced transcription). By this approach we aim to obtain a global picture of the relative contribution of D-box-regulated transcription to the cellular response to sunlight. This should also provide more general insight into the involvement of other transcription control mechanisms in these responses. In parallel, in collaboration with the Han Wang group at Soochow University, China in a DFG-funded project (LIGHT BOX) we are comparing the transcriptome, clock and DNA repair phenotypes of a panel of zebrafish lines where each of the 13 transcription factors that bind to and regulate the D-box enhancer has been inactivated by CRISPR-Cas9 mediated mutagenesis. This will enable us to decode the differential functional contributions of this family of D-box regulator transcription factors.

Figure 2
a, Multimerized cryptochrome structure in AnthoCry proteins.
b, D- und E-box enhancer elements in the *Aiptasia* AnthoCry gene promoters.

In relation to studying sunlight responses in anthozoa, in our continued collaboration with the Guse lab, we plan to perform a detailed analysis of the functionality of the AnthoCry protein in *Aiptasia*. This will involve biochemical analysis aiming to test for DNA repair activity in vitro as well as gain and loss of function studies in vivo, including ectopic expression in cultured cells and the establishment of transgenic lines in the other anthozoan model, *Nematostella*.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 14, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 6, Number of citations 2017-2021: 2263

Gornik SG, Bergheim BG, Morel B, Stamatakis A, **Foulkes NS**, Guse A. (2021) Photoreceptor Diversification Accompanies the Evolution of Anthozoa. *Mol. Biol. Evol.* 38: 1744-1760.

Zhao H, Li H, Du J, Di Mauro G, Lungu-Mitea S, Geyer N, Vallone D, Bertolucci C, **Foulkes NS**. (2021) Regulation of ddb2 expression in blind cavefish and zebrafish reveal plasticity in the control of sunlight-induced DNA damage repair. *PLoS Genet.* 17: e1009356.

Ceinos RM, Frigato E, Pagano C, Fröhlich N, Negrini P, Cavallari N, Vallone D, Fuselli S, Bertolucci C, **Foulkes NS**. (2018) Mutations in blind cavefish target the light-regulated circadian clock gene, period 2. *Sci Rep.* 8: 8754.

Pagano C, Siauciunaite R, Idda ML, Ruggiero G, Ceinos RM, Pagano M, Frigato E, Bertolucci C, **Foulkes NS**, Vallone D. (2018) Evolution shapes the responsiveness of the D-box enhancer element to light and reactive oxygen species in vertebrates. *Sci Rep.* 8: 13180.

Zhao H, Di Mauro G, Lungu-Mitea S, Negrini P, Guarino AM, Frigato E, Braunbeck T, Ma H, Lamparter T, Vallone D, Bertolucci C, **Foulkes NS**. (2018) Modulation of DNA repair systems in blind cavefish during evolution in constant darkness. *Curr Biol.* 28: 3229.





2.7 PROF. DR. STEPHAN FRINGS ANIMAL MOLECULAR PHYSIOLOGY

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Fields of Interest

We are interested in the molecular basis of sensory function. Our focus lies on olfaction, pain and motor coordination. Within this context, we look into the functions of ion channels in the regulation of excitability of sensory neurons.



Summary and outlook

In recent years, we have identified a species of calcium-dependent chloride channels - anoctamin channels - as modulators of olfactory sensory neurons and of cerebellar Purkinje neurons. In both types of neurons, anoctamin channels couple cellular calcium signals to electrical phenomena. In olfactory neurons excitability is boosted, while anoctamin limits excitability in Purkinje neurons. Thus, anoctamin channels can subserve quite different modulatory functions, depending on local chloride gradients across the plasma membrane. Moreover, we have characterized a modulatory pathway that inhibits the processing of pain signals in the brainstem. This pathway is triggered by the olfactory system, and the site of interaction between the olfactory and pain pathways is the spinal trigeminal nucleus caudalis where pain-induced network activity is downregulated by olfactory co-stimulation. The outlook for the coming year is to further analyze this cross-modal interaction.

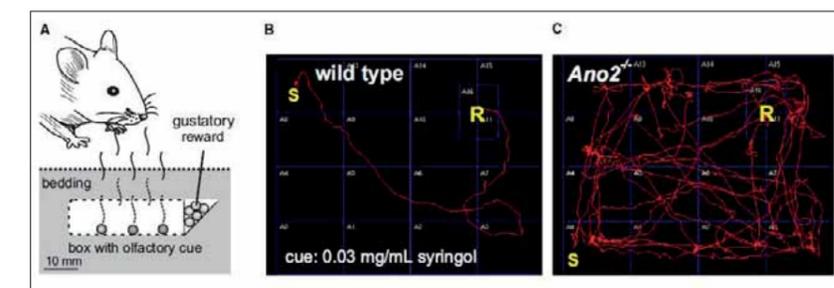


Figure 1

Anoctamin 2 and high-performance odor tracking. Wildtype animals are able to track the weak scent of an unknown odor quite effectively. This can be seen in the middle panel where the running path of a test animal was filmed from start (S) to reward (R). The panel on the right shows that a mouse lacking anoctamin 2 (ANO2) requires extensive explorative running to find the reward by chance.

Research highlights since 2017

Since 2017, we had four research highlights:

1. We found that anoctamin chloride channels are required in olfactory sensory neurons for detection of unfamiliar odors at low concentrations. Neuromodulation is thus necessary to achieve high-performance sensory function under challenging conditions.
2. We found that anoctamine chloride channels in cerebellar Purkinje neurons provide a modulatory role that is necessary for motor coordination and motor learning. Thus, proper network performance in the cerebellar cortex requires inhibitory modulation of neuronal excitability.

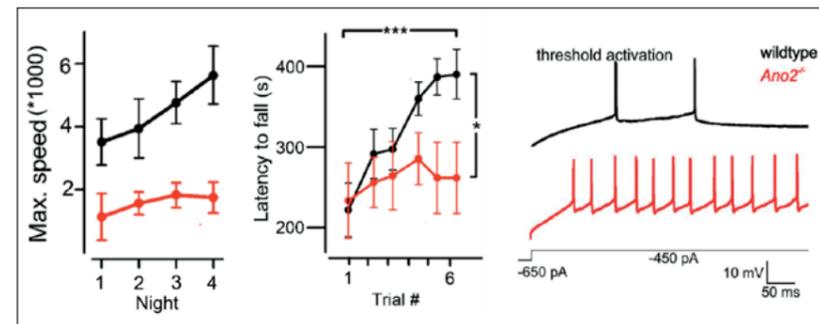


Figure 2
Identifying the role of anoctamin 2 in motor coordination and learning. Mice lacking anoctamin 2 (red) reach lower speed in the running wheel (left) and show poor coordination and learning on the rotarod (center). Whole-cell recordings from cerebellar Purkinje neurons (right) demonstrate that anoctamin 2 channels reduce the excitability of Purkinje neurons.

3. We found that anoctamin channels fulfil distinct functions in maintaining the mucociliary layer covering the airway epithelia on the evels of nasal, tracheal and bronchial epithelia. This project provided essential information for a collaboration with the Heidelberg Center of Cystic Fibrosis Research.
4. We found that the olfactory system has access to the central trigeminal system at the input stage in the medullary brainstem. Olfactory stimuli trigger an analgesic mechanism of descending inhibition that effectively reduces pain signaling.

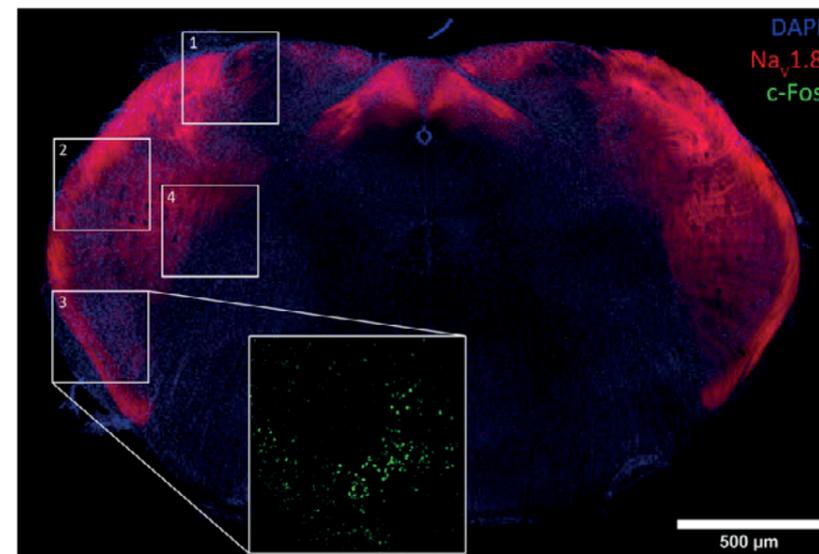


Figure 3
Coronal section of the medullary brainstem with the spinal trigeminal nucleus caudalis visualized by tdTomato (red) staining Nav1.8-expressing sensory neurons. Exposure to the painful stimulus mustard oil induces neural activity, which is visualized by c-Fos expression (green). c-Fos expression was quantified to examine pain intensity and pain modulation through olfactory co-stimulation.

Future directions

Our working hypothesis for the odor-induced inhibition of pain signaling addresses the signal flow from the olfactory bulb through amygdala and hypothalamus to the spinal trigeminal nucleus caudalis, where the inhibitory effect is now well documented by our work. The next step to examine this hypothesis is to look for a functional connection between hypothalamus and the spinal trigeminal nucleus. Good candidates so far are the hypothalamic neuropeptides orexin and oxytocin, which both are known to impinge on pain processing. Furthermore, dopaminergic and serotonergic fibers reach the spinal trigeminal nucleus from various hypothalamic centers. Eventually, these modulatory inputs to the trigeminal brainstem may activate GABAergic interneurons, which then inhibit other neurons of the neural network. The way to look into this is to repeat our experiments with mouse models for neuromodulation and to examine whether any mouse line deficient of receptors for hypothalamic peptides or neurotransmitters fails to produce the odor-induced analgesia. Such models are available in the Heidelberg pain-research community so that this important next step can be carried out.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 8, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 3, Number of citations 2017-2021: 37

Auer, F., Franco-Taveras, E., Klein, U., Kesenheimer, C., Fleischhauer, D., Möhrlein, F., and Frings, S. (2021) Anoctamin 2-chloride channels reduce simple spike activity and mediate inhibition at elevated calcium concentrations in cerebellar Purkinje cells. *PLoS ONE* 16, e0247801.

Hahn, A., Salomon, J.J., Leitz, D., Feigenbutz, D., Korsch, L., Lisewski, I., Schrimpf, K., Millar-Büchner, P., Mall, M. A., Frings, S., Möhrlein, F. (2018) Expression and function of anoctamin 1/TMEM16A calcium-activated chloride channels in airways of in vivo mouse models for cystic fibrosis research. *Pflügers Archiv - Eur J Physiol* 470, 1335-1348.

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

Neureither, F., Stowasser, N., Frings, S., and Möhrlein, F. (2017) Tracking of unfamiliar odors is facilitated by signal amplification through anoctamin 2 chloride-channels in mouse olfactory receptor neurons. *Physiol. Reports* 5, e13373

Neureither, F., Ziegler, K., Pitzer, C., Frings, S., and Möhrlein, F. (2017). Impaired motor coordination and learning in mice lacking anoctamin 2 calcium-gated chloride channels. *Cerebellum* 16, 929-937.

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Fields of Interest

Sensory physiology and biochemistry,
signal transduction in sensory systems,
ion channels



Summary and outlook

We are working on several aspects of sensory transduction. We are particularly interested in ion channels of the anoctamin family (ANO) of calcium-gated chloride channels. We studied their expression in rodent airway epithelia and were able to gain an understanding of the functional role of ANO1 in cystic fibrosis. Moreover, we identified anoctamins in the olfactory system and in the cerebellum. We could report that the detection of weak odors requires signal amplification through ANO2 channels in olfactory receptor neurons and ANO2 in the cerebellar cortex play important roles in calcium-dependent short term ionic plasticity. Currently, our main interest is a modulatory pathway triggered by olfactory stimulation that inhibits pain signals in the spinal trigeminal nucleus of the brainstem.

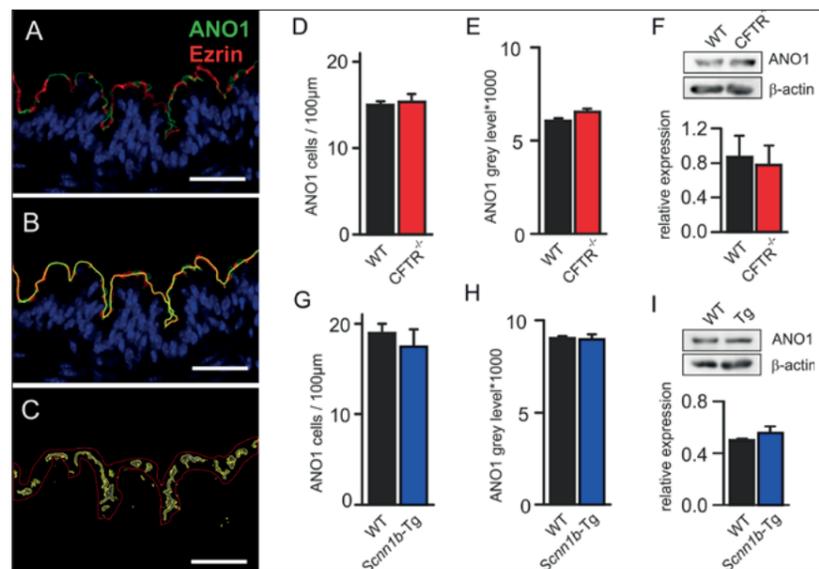


Figure 1
Quantification of ANO1 protein expression in murine tracheal epithelia (Hahn et al. 2018)

Research highlights since 2017

We identified the subcellular localization of the anoctamin channel proteins in epithelia in the nasal cavity, as well as in tracheal and bronchial epithelia. Our study revealed that ANO1 Ca²⁺-activated Cl⁻ channels are co-expressed with CFTR and ENaC channels in the apical membranes of non-ciliated cells of mouse airways. Moreover, we found that ANO1 is not upregulated in cystic fibrosis disease models, indicating that airway epithelial cells

cannot spontaneously recruit ANO1 channels to rescue the CFTR-mediated Cl⁻ secretion in cystic fibrosis patients. Moreover, we found that ANO2 channels expressed in the olfactory epithelium of the nasal cavity boost the excitability of ciliated chemosensory neurons. In this study we demonstrated that the ANO2-mediated amplification mechanism enables mice to track weak, unfamiliar olfactory cues. In contrast, ANO2 channel expressed in Purkinje neurons in the cerebellar cortex modulate the inhibitory input to cerebellar Purkinje cells and limit their excitability. Thus, in the cerebellar neurons ANO2 is involved in a calcium-dependent mode of ionic plasticity that reduces the efficacy of GABAergic synapses. In behavioral studies we found that ANO2^{-/-} mice display deficiency in motor coordination and motor learning. This study illustrated the behavioral significance of calcium-dependent modulation of inhibitory-network activity through short-term ionic plasticity.

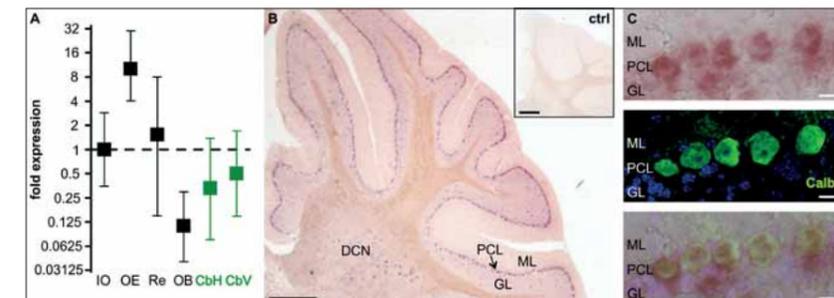


Figure 2
Ano2 is expressed in cerebellar Purkinje cells (Auer et al. 2021)

Future directions

Our future interest lies in the role that ion channels play in the processing of olfactory and nociceptive signals. Previous studies have shown that within the nasal epithelia, the excitability of trigeminal fibers was found to be unaffected by activity in the olfactory system. To advance into the cross-talk between the olfactory and trigeminal systems, we therefore study the brainstem subnucleus SpVc as initial relay center for nociceptive signals. As an initial step we analyze changes of neural activity in the brainstem by monitoring activity-induced expression of the proto-oncogene c-fos in response to nociceptive and combined nociceptive/olfactory stimulation. Our working hypothesis is that the olfactory system has access to the central trigeminal system at the input stage in the brainstem and, thus, olfactory stimuli can trigger an analgesic mechanism that effectively reduces trigeminal pain signaling, e.g. for headaches.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 7, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 4, Number of citations 2017-2021: 37

Auer F, Franco Taveras E, Klein U, Kesenheimer C, Möhrlén F, Frings F (2021) Anoctamin 2-chloride channels reduce simple spike activity and mediate inhibition at elevated calcium concentration in cerebellar Purkinje cells. PLoS ONE 16(3): e0247801

Hahn, A., Salomon, J.J., Leitz, D., Feigenbutz, D., Korsch, L., Lisewski, I., Schrimpf, K., Millar-Büchner, P., Mall, M.A., Frings, S., Möhrlén F. (2018) Expression and function of Anoctamin 1/TMEM16A calcium-activated chloride channels in airways of in vivo mouse models for cystic fibrosis research. Pflügers Archiv - European Journal of Physiology 470(9):1335-1348

Neureither, F., Stowasser, N., Frings, S., Möhrlén F. (2017) Tracking of unfamiliar odors is facilitated by signal amplification through anoctamin 2 chloride channels in mouse olfactory receptor neurons. Physiological Reports 5: e13373

Neureither, F., Ziegler, K., Pitzer, C., Frings, S., Möhrlén, F. (2017) Impaired motor coordination and learning in mice lacking anoctamin 2 calcium-gated chloride channels. Cerebellum 16(5): 929-937

Hahn, A., Faulhaber, J., Srisawang, L., Stortz, A., Salomon, J.J., Mall, M.A., Frings, S., Möhrlén F. (2017) Cellular distribution and function of ion channels involved in transport processes in rat tracheal epithelium. Physiological Reports 5: e13290



2.8 PROF. DR. EMMANUEL GAQUEREL INDEPENDENT RESEARCH GROUP

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Since 2018: University of Strasbourg
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Fields of Interest

Plant specialized metabolism, Metabolomics,
Chemical ecology



Summary and outlook

Plants adapt to their environments by diversifying their phenotypes in various ways. This diversification is intimately reflected in plants' fascinating capacity to evolve novel specialized metabolites. Shedding light on the evolutionary history of plant specialized metabolism can illuminate a key question in biology: how complex phenotypic traits evolve? Eventually, the same approach can also provide molecular insights for engineering programs on plant metabolites with high economical and societal values. From 2013 to 2018, the "Plant Defense Metabolism" junior group was funded by the University's Excellence Initiative, supported by the department of Prof. Dr. R. Hell and has pursued three inter-connected research lines. First, a central focus has been dedicated to the analysis of metabolic innovations in the genus *Nicotiana* and of their defensive roles against specialized insect herbivores. The latter research line led to several gene function discoveries in specialized metabolism. Critical to this research has been the development of computational approaches to support mass spectrometry-based metabolomics data mining. In a third research area, the group collaborated its metabolomics expertise to other COS groups' project centered on plant metabolic plasticity in connection with developmental processes. Thanks to the support by the COS of junior group leaders' research programs, E. Gaquerel could secure, at the end of the junior group's funding period, a Professor of Plant Physiology at the University of Strasbourg and has transitioned there on October 2018.

Research highlights since 2017

Advances in the resolution and sensitivity of MS instrumentations allow the measurement of an exponentially growing volume of metabolites. But, mining the resulting data remains a cornerstone challenge. Thanks to the technical support and scientific expertise at the Metabolomics Core Technology Platform (MCTP) headed by Prof. Dr. R. Hell, an increasing interest of the junior group has been in the development of novel approaches to navigate large-scale MS data. Prior to 2017, research of the group led, along with that of other groups, to the implementation of a "BLAST-like" tool for metabolomics data in which metabolite mass spectra are aligned and their degree of similarity is scored for metabolic tree construction. After 2017, we further refined this approach as part of the Master thesis in Bioinformatics of Thomas Naake. This work led to the publication of an open-access tool (R package MetCirc, <https://bioconductor.org/packages/release/bioc/html/MetCirc.html>, Figure 1), which includes an interactive graphical user interface, and a companion publication (Naake and Gaquerel, Bioinformatics, 2017, see "Selected publications"). An additional research article that stems from this approach is currently in preparation. In a collaboration with Dr. Dapeng Li (MPI-CE Jena; Center for Excellence in Molecular Plant Science, Shanghai), we combined the above metabolomics informatics approach with information theory statistics to quantify the robustness of metabolic responses elicited by

insect herbivory in 7 wild tobacco species. This study (Li et al., Science Advances, 2020, see “Selected publications”) allowed to revisit two seminal ecological theories on the defensive role of plant specialized metabolism diversity and provided an additional example on how computational metabolomics can be used to test ecological hypotheses.

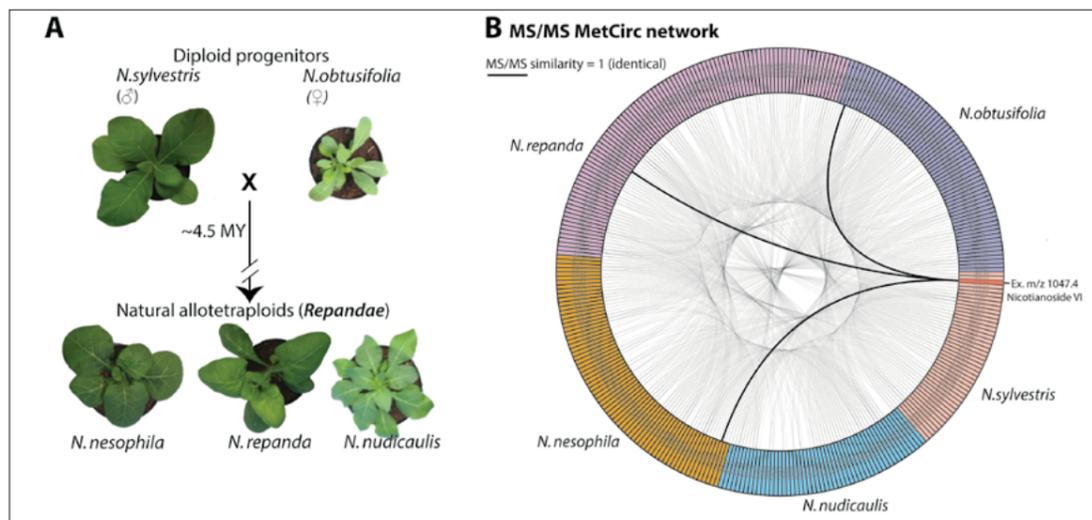


Figure 1
Implementing comparative metabolomics to explore metabolic innovations. A) *Nicotiana* allotetraploids species of section Repandae. B) The group implemented the MetCirc R package (Naake and Gaquerel, Bioinformatics 2017) to facilitate the exploration of cross-species comparison of high-resolution MS/MS spectra acquired by metabolomics analysis. In the Circos-based visualization of MS/MS similarity implemented in MetCirc, colored sectors represented species (here diploid progenitors and Repandae allotetraploids) and smaller segments, MS/MS spectra. The Shiny interactive interface allows for visualizing edges that denote for MS/MS similarity scores from 0.7 (light grey) to 1 (identical MS/MS, example of Nicotianoside VI).

During the previous evaluation period, the group established allopolyploids of the *Nicotiana* Repandae section (Figure 1) to explore how subtle innovations in alkaloid metabolism evolved. This research initially involved the participation of the research group to evolutionary analyses of recently sequenced *Nicotiana* genomes (Xu et al., PNAS, 2017, see “Selected publications”) and the establishment of a transcriptomic screen to identify BAHD acyltransferases responsible for the production of unique and superior acylated alkaloid toxins (termed NANNs) in the Repandae species. During the period 2017-2018 and prior to E. Gaquerel’s transition to the University Strasbourg, research has been focused on the characterization of one of the BAHD candidates, hereafter referred to as NAT1. Functional studies indicated that NAT1, which originates from a gene fusion event post-allopolyploidization, is responsible for the NANN defensive innovation. This work was further supported by post-ingestive metabolomics analyses to investigate the mode of action of NANN as superior toxins. This research is currently continued at the University of Strasbourg and a manuscript will be in preparation at the end of 2021.

Finally, the group brought in its metabolomics data mining knowledge to collaboration projects. The group contributed, together with the group of Prof. Dr. A. Maizel and in the context of the CellNetworks EcTop6, to the investigation of central carbon metabolic reprogramming associated with lateral root formation (project is continued in A. Maizel’s group). As part of a research project led by the group of Prof. Dr. M. Koch, the junior group contributed metabolomics measurements of the cold-responsiveness of the primary metabolome of species of the genus *Cochlearia* to explore the enigmatic evolutionary history of cold adaptation of this genus (manuscript submitted).

Future directions

Thanks to the mentorship of Prof. Dr. R. Hell and support by the COS of junior group leaders’ research, E. Gaquerel could secure, at the end of the junior group’s funding period, a Professor of Plant Physiology at the University of Strasbourg and started there on October 2018. E. Gaquerel is additionally head of a research group on plant specialized metabolism at the Institut de Biologie Moléculaire (IBMP) du CNRS. The junior group “Plant Defense Metabolism” hence stopped progressively its research activity at the COS in the fall of 2018. The COS research environment and support of the MCTP facility were key for the junior group in shaping a niche research line in plant metabolomics applied to organisms’ adaptation to their environment. Research at IBMP notably expands on the use of multi-species comparative metabolomics approaches to investigate specialized metabolism functions in the context of plant defenses to herbivory and colonization of lands by plants.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 17 (8 with COS affiliation), Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 7 (5 with COS affiliation), Number of citations 2017-2021: 1079

Li, D., Halitschke, R., Baldwin, I. T., and Gaquerel, E*. (2020). Information theory tests critical predictions of plant defense theory for specialized metabolism. *Science advances*, 6(24), eaaz0381. (*co-corresponding authors)

Naake, T., Gaquerel, E., and Fernie, A. R. (2020). Annotation of Specialized Metabolites from High-Throughput and High-Resolution Mass Spectrometry Metabolomics. In *Computational Methods and Data Analysis for Metabolomics* (pp. 209-225). Humana, New York, NY.

Naake, T., and Gaquerel, E. (2017). MetCirc: navigating mass spectral similarity in high-resolution MS/MS metabolomics data. *Bioinformatics*, 33(15), 2419-2420. (*co-corresponding authors)

Xu, S*, Brockmüller, T., Navarro-Quezada, A., Kuhl, H., Gase, K., Ling, Z., Zhou, W., Kreitzer C., Stanke, M., Tang, H., Lyons, E., Pandey, P., Pandey, S. P., Timmermann, B., Gaquerel, E*, and Baldwin, I. T*. (2017). Wild tobacco genomes reveal the evolution of nicotine biosynthesis. *Proceedings of the National Academy of Sciences*, 114(23), 6133-6138. (*co-corresponding authors)

Gaquerel, E*. and Stitz, M. (2017). Insect resistance: An emerging molecular framework linking plant age and JA signaling. *Molecular plant*, 10(4), 537-539. (*corresponding authors)

2.9 PROF. DR. THOMAS GREB DEVELOPMENTAL PHYSIOLOGY

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Fields of Interest

Radial growth of plant shoots and roots is essential for wood formation and, thus, for the creation of terrestrial biomass. Our lab uses this process as an example to reveal principles of growth and cell fate regulation in multicellular organisms.



Summary and outlook

Multi-cellularity is a fundamental concept of life on our planet. Whereas single-cell organisms comprise an essential part of the living world, only multicellular organisms are able to build the more complex repertoire of forms and functions observed in nature. Plants represent ideal objects for deciphering concepts of multi-cellularity. Plant cells do not move relative to neighboring cells and display a high degree of developmental plasticity. Thus, cell fates are constantly readjusted by signals specific for the respective cellular and environmental context. Our group uses cambium-driven radial plant growth and the related formation of xylem and phloem tissues as a paradigm for revealing fundamental concepts of multicellularity and organismal growth (Figure 1). The last years were highly productive in this regard as we could make major contributions to the field: We functionally mapped central cambium domains, identified central cell fate regulators, explored the role of auxin in controlling wood formation and established single cell transcriptomics in the context of the cambium. In addition, computational modelling of cellular dynamics targeting regulatory networks and mechanical aspects of radial plant growth is currently developing into a central part of our work. Building on these achievements, we are in the process of establishing integrated and comprehensive views on one of the most productive terrestrial growth processes with regard to biomass production and long-term sequestration of CO₂.

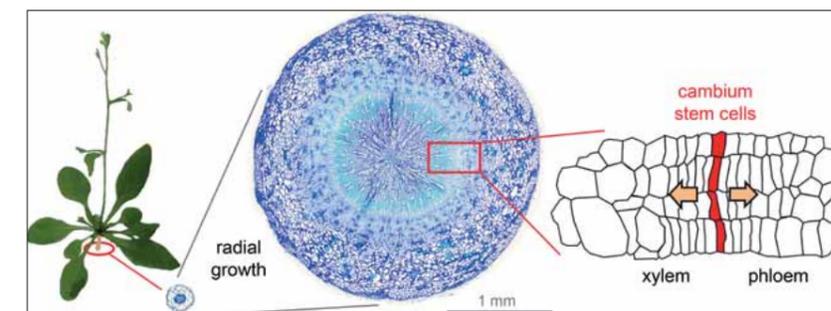
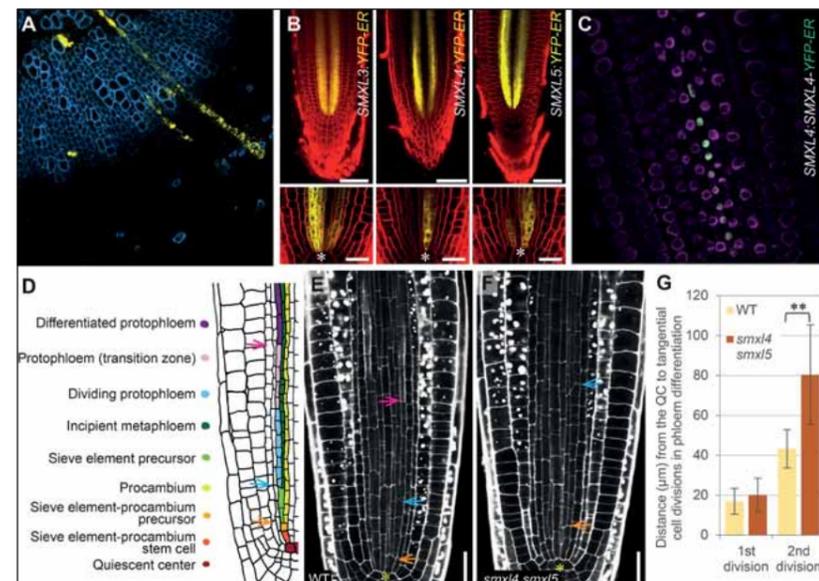


Figure 1

Arabidopsis organs grow radially driven by cambium stem cells. Shown is a comparison between hypocotyl cross sections collected from two and six weeks-old plants. The hypocotyl connects the root and the shoot system and the production of the vascular tissues xylem (wood) and phloem (bast) by cambium stem cells is essential for fulfilling this function.

Research highlights since 2017

As an ultimate characterization of cambium organization and representing the foundation of a large body of future work, we discovered in the last reporting period that, in a given cell file, a single bifacial stem cell generates both xylem and phloem cell lineages (Figure 2). These findings settled a long-standing debate about the nature of the stem cell population driving radial plant growth. By using pulse labeling and genetically encoded lineage tracing we found that bifacial stem cells are characterized by a specific combination of PXY, SMXL5 and WOX4 gene activity and a high division rate in comparison with tissue-specific progenitors. We further revealed that a proximal domain characterized by PXY and WOX4 expression, represents a site of xylem formation and a distal cambium domain, characterized by SMXL5 expression, contains cells that are determined for phloem development. Thus, we clearly mapped functional domains of the cambium and developed genetic tools for visualizing and targeting these domains in a very specific manner. Using these tools, we provided gene expression profiles of the mature inflorescence stem of *Arabidopsis thaliana* covering a comprehensive set of distinct tissues including different cambium domains. By combining fluorescence-activated nucleus sorting and Laser-capture microdissection with next generation RNA sequencing, we characterized the transcriptomes of xylem vessels, fibers, the proximal and distal cambium, phloem, phloem cap, pith, starch sheath, and epidermis cells. Our analyses classified more than 15,000 genes as being differentially expressed among different stem tissues and revealed known and novel tissue-specific cellular signatures. Our datasets predict the expression profiles of an exceptional number of genes and allow hypotheses to be generated about the spatial organization of physiological processes. Moreover, we demonstrated that information about gene expression in a broad range of mature plant tissues can be established at high spatial resolution by nuclear mRNA profiling.



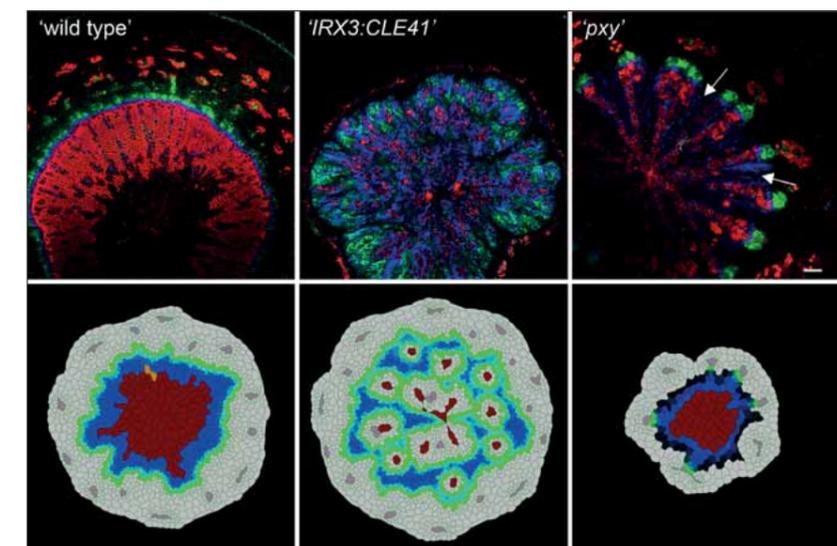
In addition to these achievements, we identified functional domains of auxin signalling in the *Arabidopsis* cambium by local short-term modulation of auxin biosynthesis and signalling. We revealed that, while cambial stem cells do not appear to be a site of elevated auxin signalling, auxin signalling in these cells is required for cambium activity. By analyzing transcriptional reporters and mutants of vasculature-associated AUXIN RESPONSIVE FACTORS (ARFs), we identified ARF3, ARF4 and ARF5/MP as cambium regulators with different tissue-specificities as well as distinct roles in cambium regulation. In particular, we identified ARF5/MP to cell-autonomously restrict the number of stem cells by directly

attenuating the activity of the stem cell-promoting WOX4 gene. Our results revealed an influence of auxin signalling on distinct cambium features by specific signalling components and allow the conceptual integration of plant stem cell systems with distinct anatomies. In fact we concluded that auxin signalling in the cambium shares features with both the situation in the root apical meristem where auxin regulates cell divisions and the shoot apical meristem where auxin, and particular ARF5/MP, is strongly correlated with cell differentiation. Thereby, we enlightened a long-observed role of auxin signalling in radial plant growth and revealed that its function is partly specific in different stem cell niches.

As a central discovery in the context of the question toward mechanisms of cambium-derived tissue formation, we identified and characterized an essential role of SUPPRESSOR OF MAX2 1-LIKE (SMXL) proteins. We demonstrated that, within the SMXL gene family, specifically SMXL3, SMXL4, and SMXL5-deficiency results in strong defects in phloem formation (Figure 2). At the beginning of our work, the three genes formed an uncharacterized subclade of the SMXL gene family which mediates hormonal strigolactone and karrikin signalling. We found that SMXL3/4/5 proteins function differently to canonical strigolactone and karrikin signalling mediators, although being functionally interchangeable with those under low strigolactone/karrikin signalling conditions. We also found that the SMXL5 protein interacts physically with the PhD finger protein OBERON3 (OBE3) forming a functional unit during phloem formation. We provided evidence that SMXL5 and OBE3 proteins interact in planta and elucidated a functional interaction between OBE3 and SMXL3/4/5 genes during phloem development using genetic means. Therefore, just like SMXL3/4/5, OBE3 is an important component during phloem initiation and differentiation. By characterizing the SMXL3/4/5-OBE3 interaction we provided also insights into the molecular network of phloem formation in plants and propose that the SMXL3/4/5-OBE3-dependent establishment of a distinct chromatin profile is an essential step during phloem specification.

Future directions

Considering the importance of the cambium and its derived tissues for land plant evolution on the one hand and biomass accumulation on the other hand, information on associated gene activities is certainly vital. The methods for extracting tissue-specific nuclei established in the context of our work are not only the basis for analyzing different cambium domains with a finer spatial resolution or single cell sequencing, but also for revealing



genome-wide patterns of chromatin conformation in distinct tissues and genetic backgrounds by applying the Assay for Transposase Accessible Chromatin with high-throughput sequencing (ATAC-seq). This will be a central direction for characterizing the role of SMXL/OBE protein complexes in vascular tissue formation in addition to the biochemical investigation of protein function.

Another essential future direction is based on cell-based computational modelling visualizing cambium activity and integrating the function of central cambium regulators (Figure 3). We envision that modelling allows recapitulating qualitative and quantitative variation in radial plant growth found in different mutants and when comparing different dicotyledonous species. Moreover, the modelling will help to predict targets of environmental stimuli inducing changes of cambium activity like seasonal changes or mechanical perturbation, allowing the generation of testable hypotheses. Thus, computational modelling will be a useful tool for investigating a process not possible to observe in real time and partly develops over exceptionally long periods. We expect that integration of quantitative information on cell size and number will result in a more realistic representation of radial plant growth allowing, for example, a better integration of mechanical properties of the system.

With respect to tissue mechanics, it is important to highlight that cambium-derived tissues are exceptionally rigid and, thus, cambium stem cells are embedded in a demanding physical environment. In an exciting research line leveraging the unique anatomical features of cambium-based organ growth, we probe the role of mechanical forces applied by surrounding tissues in instructing stem cell behavior. Based on preliminary data obtained by computational modelling and genetic approaches altering cell wall rigidity we hypothesize that mechanical stress generated by differentiating xylem cells, influence the division orientation of cambium stem cells. We expect that investigating the integration of mechanical cues on the subcellular level will be very instructive with regard to the role of inter-tissue forces in developmental processes like tissue patterning and stem cell activity.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 17, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 11, Number of citations 2017-2021: 2019

Shi, D., Jouannet, V., Agustí, J., Kaul, V., Levitsky, V., Sanchez, P., Mironova, V.V., **Greb, T.** (2021). Tissue-specific transcriptome profiling of the Arabidopsis inflorescence stem reveals local cellular signatures. *Plant Cell* 33, 200–223.

Shi, D., Lebovka, I., López-Salmerón, V., Sanchez, P., **Greb, T.** (2019). Bifacial cambium stem cells generate xylem and phloem during radial plant growth. *Development* 146, dev171355.

Miyashima, S., Roszak, P., Sevilem, I., Toyokura, K., Blob, B., Heo, J., Mellor, N., Help-Rinta-Rahko, H., Otero, S., Smet, W., Boekschooten, M., Hooiveld, G., Hashimoto, K., Smetana, O., Siligato, R., Wallner, E.S., Mähönen, A.P., Kondo, Y., Melnyk, C.W., **Greb, T.**, Nakajima, K., Sozzani, R., Bishopp, A., De Rybel, B., Helariutta, Y. (2019). Mobile PEAR transcription factors integrate positional cues to prime cambial growth. *Nature* 565, 490–494.

Brackmann, K., Qi, J., Gebert, M., Jouannet, V., Schlamp, T., Grünwald, K., Wallner, E.S., Novikova, D.D., Levitsky, V.G., Agustí, J., Sanchez, P., Lohmann, J.U., **Greb, T.** (2018). Spatial specificity of auxin responses coordinates wood formation. *Nat Commun* 9, 875.

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

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Fields of Interest

Fructan and sucrose metabolism,
greenhouse gas production in plants,
cell wall polysaccharides



Summary and outlook

Many higher plants accumulate fructans as storage carbohydrates. These fulfill diverse physiological functions in plants but are also a valuable resource for humans with a wide range of applications. In collaboration with Südzucker AG, we are investigating the molecular physiology of sucrose and fructan metabolism (inulin type) in chicory, which serves as a model but also can be used industrially. The aim of this project is to understand the regulation of inulin metabolism as comprehensively as possible and thus provide a basis for targeted breeding of chicory to improve inulin content and composition. Another line of research is the contribution of plants to the global budget of greenhouse gases. Nitrous oxide and methane play a crucial role here, for while they occur in much lower concentrations than carbon dioxide, they are far more potent.

Research highlights since 2017

CiMYB17, a stress-induced R2R3-MYB transcription factor in chicory, activates promoters of genes involved in fructan synthesis and degradation (Wei et al., 2017).

At the regulatory network level, the role of R2R3-MYB transcription factors in the regulation of fructan-active enzymes (FAZY) was investigated by bioinformatically identifying R2R3-MYBs using a RNAseq database.

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

The joint induction of CiMYB17 with 1-SST and 1-FFT (and, less consistently, with 1-FEH1/2) in nitrogen-depleted or abscisic acid (ABA)-treated chicory seedlings and in salt-stressed chicory hairy roots suggests a role in stress-induced fructan metabolism.

Nitrous oxide emitted by plants is a potentially important source to the atmosphere (Lenhart et al., 2019).

The global budget for nitrous oxide (N₂O), an important greenhouse gas and presumably the most important ozone-depleting substance emitted in the 21st century, is far from being fully understood. The cycling of N₂O in terrestrial ecosystems has traditionally focused exclusively on gas exchange between the soil surface (nitrification-denitrification processes) and the atmosphere. Terrestrial vegetation has for a long time been neglected in estimating the global budget although plants are known to release N₂O.

We were recently able to measure the N₂O emission rates of 32 plant species from 22 different families under controlled laboratory conditions. We also determined the first isotopic values (δ¹⁵N, δ¹⁸O, and δ¹⁵N_{sp}) of N₂O emitted by plants.

A robust relationship between N₂O emission and CO₂ respiration rate, which did not

change significantly over a wide range of changing environmental conditions, was used to quantify plant emissions at the ecosystem level. Stable isotope measurements of plant-emitted N_2O clearly show that the dual isotope fingerprint of plant-generated N_2O differs from that of currently known microbial or chemical processes. These studies suggest that vegetation is a natural source of N_2O in the environment, with a large fraction released by a previously unrecognized metabolic process.



Figure 1
Establishment of hydroponic chicory cultures to study fructan metabolism under environmentally controlled conditions.

Future directions

Meanwhile, through bioinformatic analysis, we identified other transcription factors (TFs) besides R2R3-MYBs that play crucial roles in stress-mediated regulatory aspects of fructan metabolism. We will investigate TF networks with chicory plants grown under field conditions and comparisons of different chicory genotypes with varying fructan accumulation. Results from this research will provide fundamentally new insight into fructan metabolism and may open new routes for biotechnology. In this context, we also establish CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) / Cas Technology with *in vitro* cultured chicory plants for *in planta* verification of the results.

In the future, we will also extend our climate research regarding nitrous oxide in the environment from the laboratory to field conditions, in cooperation with the geosciences, geography and environmental physics. To this end, we have recently been granted a laser spectrometer for real-time concentration and isotope determinations and will be able to better analyze the sources and quantification of fluxes.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 8, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 0, Number of citations 2017-2021: 986 (according to Google Scholar)

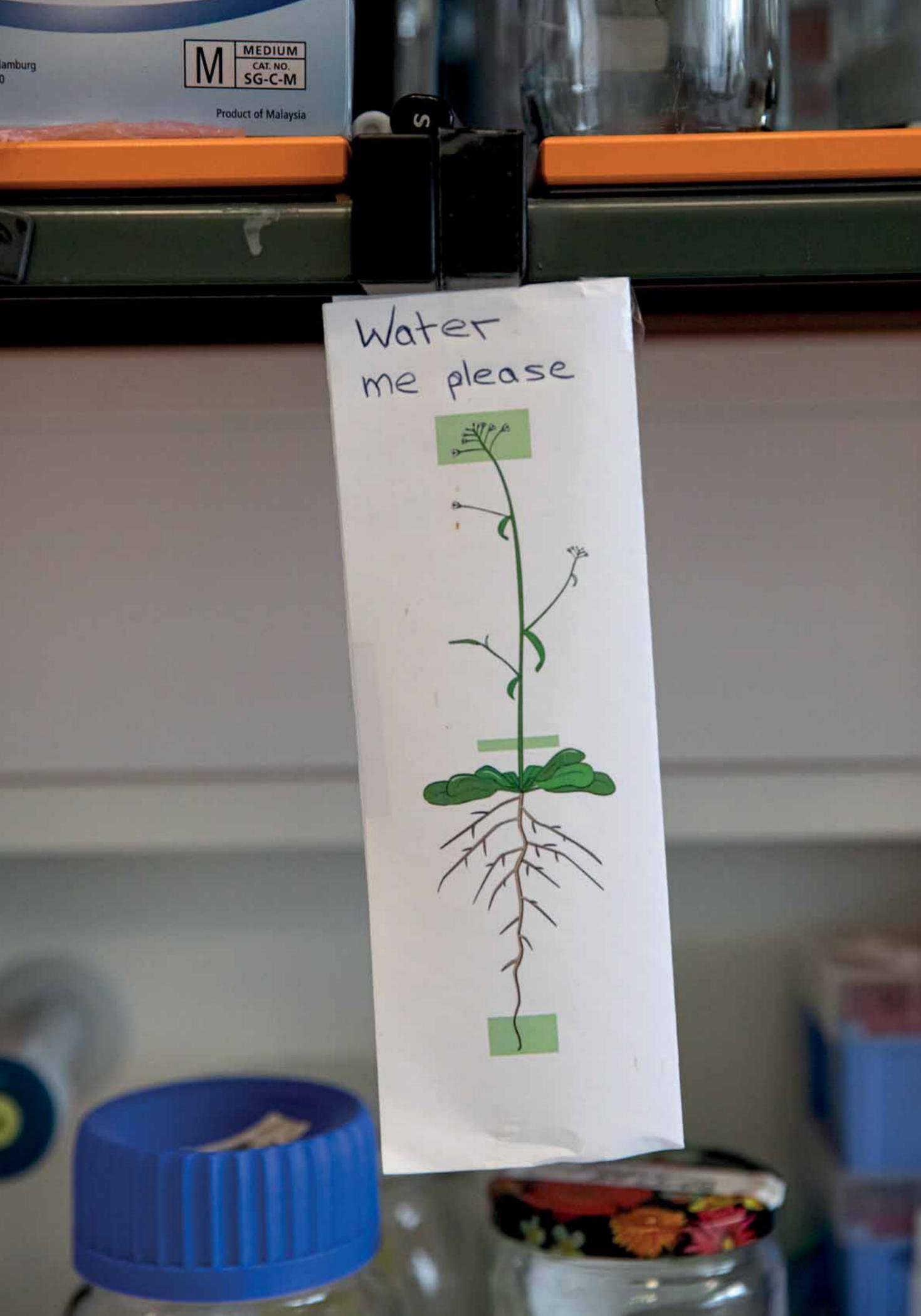
Wu, S., Greiner, S., Ma, C., Zhong, J., Huang, X., Rausch, T., and Zhao, H. (2021) A Fructan Exohydrolase from Maize Degrades Both Inulin and Levan and Co-Exists with 1-Kestotriose in Maize. *Int. J. Mol. Sci.* 22(10), 5149

Huang, X., Luo, W., Wu, S., Long, Y., Li, R., Zheng, F., Greiner, S., Rausch, T. and Zhao, H. (2020) Apoplastic maize fructan exohydrolase Zm-6-FEH displays substrate specificity for levan and is induced by exposure to levan-producing bacteria. *International Journal of Biological Macromolecules* 163, 630-639, <https://doi.org/10.1016/j.ijbiomac.2020.06.254>.

Lenhart, K., Behrendt, T., Greiner, S., Steinkamp, J., Well, R., Giesemann, A. and Keppler, F. (2019) Nitrous oxide effluxes from plants as a potentially important source to the atmosphere. *New Phytol.* 221: 1398-1408. <https://doi.org/10.1111/nph.15455>

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

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



2.10 PROF. DR. GUIDO GROSSMANN INDEPENDENT RESEARCH GROUP

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Fields of Interest

How are shapes of plant cells determined?
How do environmental conditions influence cell shape and growth? How are stimuli communicated between plant cells?
Using quantitative microscopy, we want to answer these fascinating questions and help make future crops more resilient.



Summary and outlook

The integration of environmental conditions into growth regulation is of fundamental importance for the sessile lifestyle of plants. Roots prosper in favourable environments, but cannot retract when conditions become precarious. This poses a challenge to plant development because natural environments are spatially heterogeneous: water availability, nutrient distribution or soil structure and composition can be highly variable. Biological activity by pathogenic or beneficial microbes, herbivores or competing plants lead to local and dynamic changes.

As an independent junior group leader at COS, I have established a research program targeting mechanisms of plant cell morphogenesis and growth regulation. Specifically, my group aims to understand how plasma membrane constituents become compartmentalized, how this dynamic membrane organization is influenced by environmental signals or conditions and how such membrane subcompartments are involved in growth regulation, signalling and cell-to-cell communication.

In summary, our main achievements include (I) the establishment of a temporal network of plant cell polarization using high-resolution live cell imaging and the identification of a mechanism for polar ROP GTPase recruitment during the emergence of root hairs; (II) the identification of stimulus-specific signalling and signal propagation in roots by employing quantitative imaging and genetically-encoded sensors; (III) the first demonstration of cell-autonomous regulation of growth in *Arabidopsis thaliana* under asymmetric growth conditions using novel custom-designed microfluidic imaging platforms.

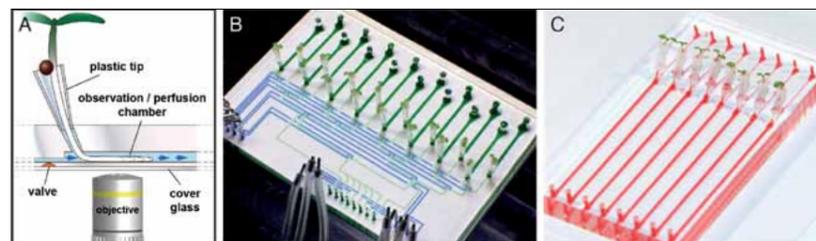
In 2020, I joined the Heinrich-Heine-University Düsseldorf as Heisenberg Professor (W3) for Cell and Interaction Biology, where my group will be able to build on and expand the research directions we started in Heidelberg.

Research highlights since 2017

Research in my group focusses on the regulation and spatiotemporal dynamics of the plasma membrane-associated growth machinery and its response to environmental conditions to uncover principles of cell morphogenesis and growth in roots. Signaling responses to changing environmental conditions are investigated in roots of the reference plant *Arabi-*

Figure 1

The RootChip microfluidic perfusion device. (A) Schematic of the device (modif. from Grossmann et al. 2011). (B) The RootChip16 with 16 observation chambers (green) and an integrated valve system (blue) (from Jones et al. 2014 eLife). (C) An 8-chamber version without valves (Denninger, Reichelt et al. 2019 Curr Biol).



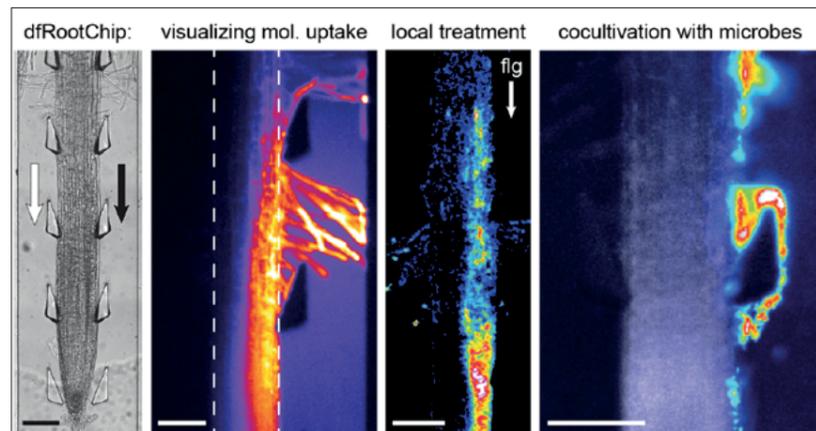
dopsis thaliana. As model system for our cell biological studies, we use root hair formation in *Arabidopsis*.

To investigate the cellular growth machinery and its regulation under changing environmental conditions, to measure protein localization and quantify signalling dynamics we need direct microscopic access with subcellular resolution and precise environmental control. To this end we have, over the past years, continuously advanced our RootChip technology, employing microfluidic platforms for root imaging (Fig. 1) (Grossmann et al., 2011 *Plant Cell*). In combination with the application of genetically encoded sensors for small molecules, the RootChip technology has enabled us to quantify Ca^{2+} signalling in a spatiotemporal manner that allowed us to track the spreading of signals across tissues. Ca^{2+} elevations are often among the fastest responses that can be measured using genetically encoded fluorescent sensors and can serve as a readout for intercellular communication. We have been collaborating with Karin Schumacher (COS) to decipher elicitor-specific three-dimensional (XYt) Ca^{2+} signatures (Keinath et al. 2015 *Mol Plant*) and contributed to a study targeting receptor type-specific differences in immune signalling (Wan et al. 2019 *New Phytol*) led by Thorsten Nürnberger (U. Tübingen). In collaboration with Jürgen Pahle (BioQuant Heidelberg), we are developing approaches to trace calcium elevations quantitatively as they propagate within the root and combine genetic and pharmacological approaches with multivariate statistical analyses to identify stimulus-specific mechanisms of signal propagation (Župunski, Brugman et al., in preparation).

To investigate how roots perceive and respond to local stimuli and develop in asymmetric environments, we designed, in collaboration with Claire Stanley (IC London), a novel experimental setup, where the same root can be exposed to different conditions on opposite sides. The dual-flow-RootChip (dfRootChip) features a micropillar array for guided root growth and uses laminar flow to treat both sides of a root separately (Fig. 2; Stanley, Shrivastava et al. 2018 *New Phytol*). We used the dfRootChip to monitor physiology and development under asymmetric conditions, to track molecular uptake and for local inoculation with microbes. Upon asymmetric perfusion of one side of the root with biotic

Figure 2

Asymmetric growth conditions in the dfRootChip. Root growth is guided by an array of micropillars. Asymmetric perfusion on either side enables the selective treatment of opposite sides of the root, e.g. with fluorescent stains or elicitors of Ca^{2+} responses (flg = the bacterial peptide flagellin). Right: the triangular design of the pillars serves as trap for fluorescent microbes (GFP-expressing *P. fluorescens*). Individual panels are taken from figures in Stanley et al. 2018.



(bacterial peptide flg22) or abiotic (100 mM NaCl) stress elicitors, we found that Ca^{2+} responses to NaCl propagated rapidly across tissues, while Ca^{2+} release upon flg22 remained confined to treated cells (Fig. 2). These different behaviours indicate the involvement of stimulus-specific intercellular signalling mechanisms.

Using the dfRootChip and its ability to stimulate the root locally, we were further able to investigate whether root hair growth is regulated in a cell-autonomous or a systemic manner. Under asymmetric availability of phosphate, a nutrient that is often unevenly distributed in soil, we observed that hair length was positively correlated with the local phosphate concentration (Fig. 3) and that growth rates could be tripled within minutes. Our study not only demonstrated the ability of root hairs to cell-autonomously regulate growth, but also provided evidence for a non-genomic, direct regulation of the growth machinery at the hair apex. Such plasticity on the single-cell level may be key to acclimate under the heterogeneous conditions in soil.

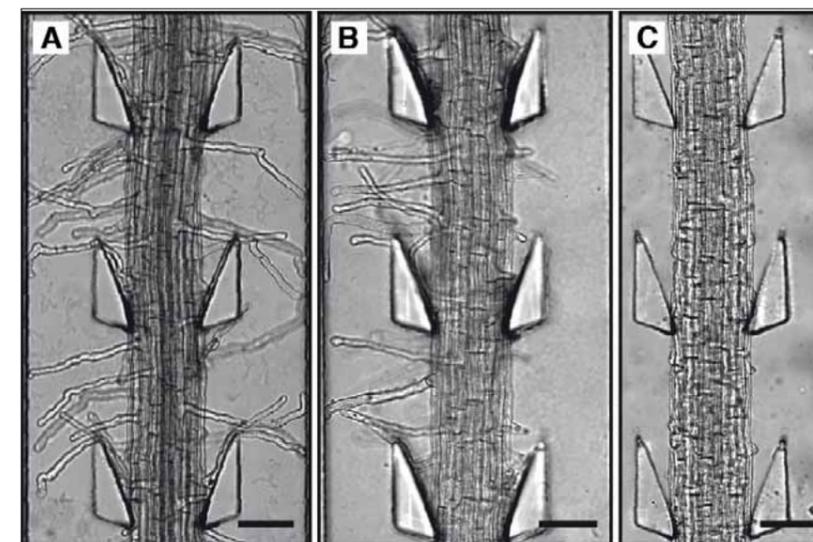


Figure 3

Root hair growth responds to asymmetric phosphate availability. Root hair response to perfusion with (A) symmetric 2.5 mM Pi, (B) asymmetric 2.5 mM left, 0.01 mM right Pi, (C) symmetric 0.01 mM Pi. Figure taken from Stanley et al. 2018.

In several collaborations on root hair development we could obtain deeper insights into molecular mechanisms of tip growth. In a study led by Christopher Grefen (U. Bochum), we helped identify a requirement of the novel GET (guided entry of tail-anchored proteins) pathway for root hair growth (Xing et al. 2019 PNAS). In a collaboration with Petra Dietrich (U. Erlangen), we contributed to the identification of CNGC-family Ca^{2+} channels that are responsible for cell integrity at the growing tip through regular calcium oscillations (Brost et al. 2019 *Plant J*).

Using root hairs as a model to uncover mechanisms of cell morphogenesis, we resolved the stepwise assembly of the tip growth machinery and followed the temporal association of over 30 proteins involved in root hair initiation (Denninger, Reichelt et al. 2019 *Curr Biol*). In collaboration with Christopher Grefen (U. Bochum) and Jan-Felix Evers (COS), we found that cell polarization and outgrowth of root hairs are temporally distinct processes, with different RopGEFs playing roles in each phase (Fig. 4). An early polarized RopGEF3 serves as a landmark to target Rho-like GTPases of plants (ROPs) to the future site of hair formation. Once the cell begins to bulge, RopGEF4 is recruited to positively regulate tip growth. Our results suggest a mechanism involving recruitment factors that mark the root hair initiation domain and are necessary and sufficient for the targeted deployment of the growth machinery to the plasma membrane – a key step during the initiation of polar growth.

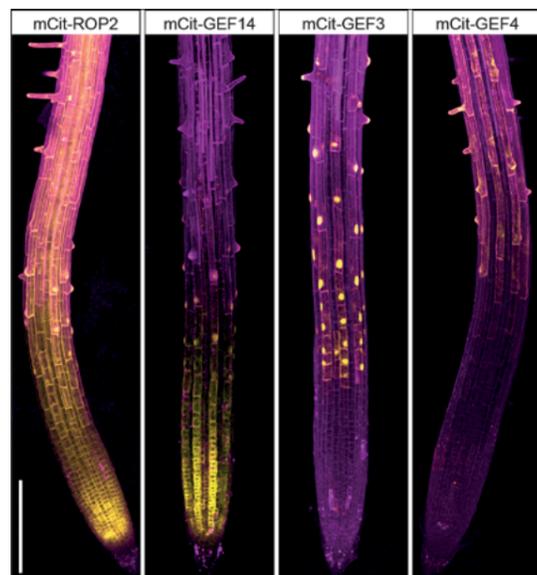


Figure 4
Distinct Distinct RopGEFs successively drive polarization and outgrowth of root hairs. mCitrine-fused ROP2 (left) and root hair-specific RopGEFs are shown sorted by the timing of their expression during root development. Note the highly polar localization of mCit-GEF3. Scale, 200 μ m. Figure taken from Denninger, Reichelt et al. 2019.

Future directions

Our group has recently moved to Heinrich-Heine-University Düsseldorf, Institute of Cell and Interaction Biology, where we have joined the Cluster of Excellence on Plant Sciences (CEPLAS) and recently became members of the Collaborative Research Center SFB1208 „Identity and Dynamics of Membrane Systems“. Here, we will continue our research on cell morphogenesis, growth regulation and root-environment interactions, as well as our efforts to develop technologies that enable quantitative cell biology at the root-soil interface.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 14, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 6, Number of citations of papers published 2017-2021: 384 (Google Scholar)

Guichard, M, Bertran Garcia de Olalla, E, Stanley, CE and **Grossmann G.** (2020) Chapter: Microfluidic Systems for Plant Root Imaging. *Methods in Cell Biology: Plant Cell Biology*, eds. Dixit, R, Haswell, E, Anderson, C; Vol. 160; Academic Press; doi: 10.1016/bs.mcb.2020.03.012.

Denninger, P*, Reichelt, A*, Schmidt, VAF, Mehlhorn, DG, Asseck, LY, Stanley, CE, Keinath, NF, Evers, J-F, Grefen, C, and **Grossmann G.** (2019) Distinct ROPGEFs successively drive polarization and outgrowth of root hairs. *Curr Biol*. doi: 10.1016/j.cub.2019.04.059

Brost, C, Studtrucker, T, Reimann, R, Denninger, P, Czekalla, J, Krebs, M, Fabry, B, Schumacher, K, **Grossmann, G**, and Dietrich P. (2019) Multiple cyclic nucleotide-gated channels coordinate calcium oscillations and polar growth of root hairs. *Plant J*. doi: 10.1111/tj.14371

Stanley, CE*, Shrivastava, J*, Brugman, R, van Swaay, D, and **Grossmann, G.** (2018) Dual-flow-RootChip reveals local adaptations of roots towards environmental asymmetry at the physiological and genetic levels. *New Phytol*. 217(3):1357-69;

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





2.11 PROF. DR. ANNIKA GUSE CELL BIOLOGY OF ENDOSYMBIOSIS

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Fields of Interest

Cell Biology of Endosymbiosis,
Adaptation to the Environment,
Phagocytosis, Innate Immunity,
Genome Organization



Summary and outlook

Symbiotic interactions between organisms occur in all domains of life. A prime example is the symbiosis between corals and eukaryotic, photosynthetic dinoflagellates. Each generation, initially symbiont-free coral larvae take up dinoflagellates from the environment and a new, stable symbiotic interaction is established. Symbionts provide essential nutrients such as sugars, amino acids and lipids to their host powering the productivity of reefs ecosystems. This 'photo-symbiosis' is evolutionary ancient and considered a key adaptation to shallow, sunlit tropical oceans where food is scarce, and likely the main driver for coral diversification as well as onset of reef formation in the Triassic. Despite of its importance, key aspects about coral symbiosis establishment, maintenance, its evolution and ecosystem functions are still largely unknown, mainly because of the poor accessibility of corals as experimental models. The long-term goal of my research is to dissect the mechanisms of coral symbiosis and to uncover fundamental principles of organismal interactions across scales and through time by using the emerging model species *Aiptasia*.

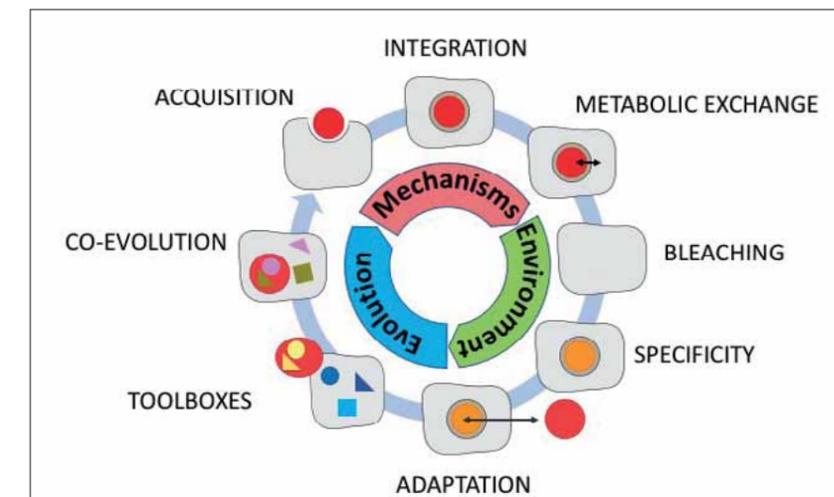


Figure 1
The cell biology of endosymbiosis. We aim to uncover the molecular mechanisms of how symbionts are acquired, integrated into host cell function and exchange nutrients. In the context of the environment, we plan to understand the mechanism of coral bleaching and symbiont-host specificity. Evolutionary, we investigate what was the original toolbox to promote the symbiosis and partner co-evolution.

Symbiotic associations are a powerful way to gain metabolic novelties, a prerequisite for adaptation to new environments and lifestyles, and a major driver for evolutionary diversification. A prime example is the acquisition of mitochondria, a key step for the evolution of cellular and organismal diversity. Coral symbiosis powers the entire food web of coral-reef ecosystems and presents a unique opportunity to elucidate how molecular interactions between distinct species lead to stable and complex eco-systems, which have co-evolved over millions of years.

Research highlights since 2017

Establishing intracellular symbiosis between animal cells and photosynthetic symbionts is a complex cell biological process involving phagocytosis, modulation of host cell immunity, vesicular transport and fusion, metabolism and nutrient exchange. Untangling this web has been hindered by the notorious difficulty of corals as experimental subjects, which we have now overcome by establishing a new model system: the sea anemone *Exaiptasia diaphana* (commonly Aiptasia) a close relative of reef-building corals that hosts the same dinoflagellate symbionts as corals. In contrast to corals, Aiptasia can be easily maintained in the laboratory, cleared from symbionts, maintained in this bleached state and re-infected with various symbionts. We had a lead role in establishing essential resources for Aiptasia including defined host and symbiont lines, sequenced genomes and transcriptomes. Importantly, we can induce Aiptasia spawning in the lab, larvae are naturally non-symbiotic and acquire symbionts anew each generation with similar specificities as coral larvae. Due to the larvae's small size and transparency they are amenable to high-resolution microscopy, which we routinely combine with protein biochemistry, live imaging, metabolomic, transcriptomic and phylogenetic analyses for Aiptasia, and we are developing functional approaches. What we learn in Aiptasia then informs targeted comparative fieldwork with corals. Thus, Aiptasia offers unparalleled power for dissecting the mechanistic core of endosymbiosis and coral bleaching.

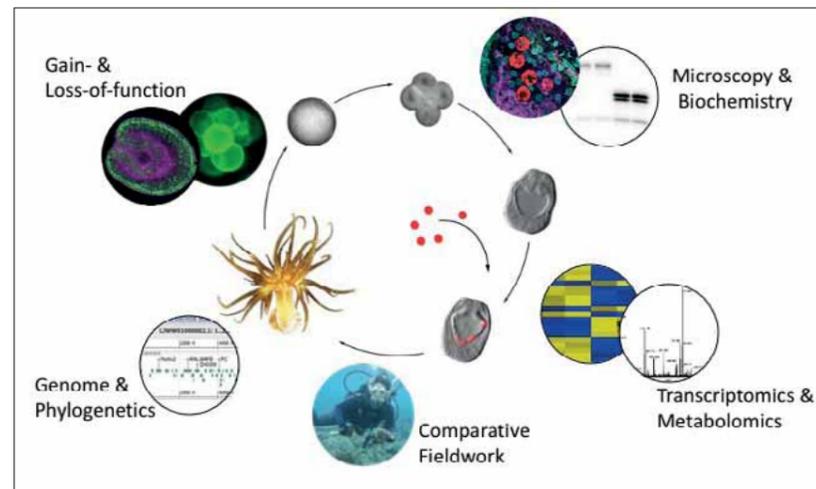


Figure 2

The marine sea anemone *Aiptasia*. We have established a comprehensive tool kit for using *Aiptasia* as an experimental system to mechanistically dissect the full complexity of coral endosymbiosis as the basis for understanding its evolution and adaptability for future change.

We are dissecting the basic mechanisms underlying key steps of symbiosis establishment. Acquisition & avoiding detection | The mechanism of symbiont uptake is not well understood. Using a comparative approach with non-symbiotic algae, we find that an unspecific trial-and-error mechanism is used to phagocytose particles. Surprisingly, we found that non-symbiotic algae are removed by expulsion (and not by digestion) which is reminiscent to 'vomocytosis', a process observed in amoeba and macrophages to remove certain fungal pathogens. Symbionts induce host cell immune suppression, likely by targeting the adapter MyD88 to suppress NF- κ B, TLR and TNF signaling, and form a lysosomal-associated membrane protein 1-positive niche. We propose that symbiosis establishment relies on local innate immune suppression, to avoid expulsion and promote niche formation.

Metabolic integration | All animals have to eat to survive. Endosymbiosis is a unique way to acquire nutrients from within the cells. Using *Aiptasia* larvae, we find that the symbiosis establishment induces host cell proliferation and lipid accumulation. Symbiosis activates, similar to food, mTORC1 signaling in a light-dependent manner. Small molecule inhibition of this signaling pathway impairs LAMP1-niche formation and symbiosis stability. This suggests that the endosymbiotic partnership between corals and dinoflagellates repurposes pre-existing machinery to integrate symbiont-derived nutrients into host cell physiology.

Metabolic exchange | The nutrient transfer from symbionts to host is key for ecosystem function. One example are sterols, essential building blocks for membranes in all animal cells. We found that sterol-auxotroph anemones and corals receive their bulk sterols from their symbionts. Host sterol composition is flexible and varies with the symbiont type housed. During evolution, symbiotic hosts have expanded their suite of sterol-binding NPC2 proteins which may be specifically adapted to the low pH within the 'symbiosome'.

Future directions

To uncover concepts of how coral-algal symbiosis originated, functions today and continues to adapt in the future, we will expand our model systems' approach at the bench and work in the field by adding new experimental and comparative approaches.

Mechanisms of endosymbiosis | A key feature of endosymbiotic interactions is that two distinct cells coordinate their functions, yet our understanding of the cell biology of endosymbiosis remains extremely limited. We will identify the core endosymbiotic community in corals and ask: How do the partners recognize each other? How do they integrate their cell functions and exchange nutrients? How are the cellular effects impacting the physiology of the organisms involved? Knowing the key mechanisms of cells required for endosymbiosis, we can explore why only some animals exploit the power of photo-symbiosis and attempt to genetically engineer animal and algal cells to form an endosymbiotic association that naturally does not occur.

The endosymbiotic lifestyle requires the symbiont to quickly switch between living in the open water and inside the animal host cell, two immensely distinct environments. How do dinoflagellates accomplish that? Dinoflagellates have extraordinarily large genomes packaged into permanently condensed, liquid-crystalline chromosomes. We hypothesize that dinoflagellates exploit liquid-liquid phase separation for spatiotemporal compartmentalization into transcriptionally active (histone-based) and silent (non-histone-based) chromatin to transition between environments. Higher-order genome organization is considered unique to Bilateria, yet phase separated genomes have been observed in all domains of life and we plan to explore the underlying mechanism.

Environmental impacts on endosymbiosis | The loss of symbionts ('coral bleaching') due to environmental stress leads to coral starvation and death. Nutritional input by symbionts can be substituted by increased heterotrophic feeding, yet 90% of reefs globally are already affected by bleaching. What are the molecular mechanisms and nutritional consequences of bleaching? What capacities do corals have to recover and what is the bottleneck for recovery? Can we genetically engineer corals for example with enhanced uptake capacity to increase recovery rates and thus resilience?

Evolution of endosymbiosis | Dinoflagellate symbionts are closely related to the apicomplexan lineage comprising many potent intracellular pathogens such as *Plasmodium*. Both, dinoflagellates and Apicomplexa evolved from autotrophic, free-living ancestors, 'invade' coral cells and replicate, spread and co-exist as endosymbionts within coral tissue. Do dinoflagellates and apicomplexans hijack similar phagocytosis mechanisms to enter? How do they hijack host innate immunity? Or more broadly, what is the evolutionary relation of endosymbiosis to parasitism?

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 4, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 4, Number of citations 2017-2021: 823

Voss, P.A., Gornik S.*, Jacobovitz, M.R.*, Rupp, S., Dörr, M., **Guse, A.** Nutrient-dependent mTORC1 signaling in coral-algal symbiosis. *bioRxiv*, 10.1101/723312

Jacobovitz, M.R.*, Rupp, S.*, Voss, P.A., Gornik S., **Guse, A.** (2021) Coral symbionts escape 'vomocytosis' by host cell immune suppression. *Nature Microbiology* 6, 769–782

Gornik S.*, Bergheim, B.G.*, Morel B., Stamatakis, A., Foulkes, N.S.#, **Guse, A#** (2020). Photoreceptor diversification accompanies the evolution of Anthozoa. *Molecular Biology and Evolution*, msaa304

Hambleton, E.A., Jones, V.A.S., Maegele, I., Kvaskoff, D., Sachsenheimer, T., **Guse, A.** (2018). Sterol transfer by atypical cholesterol-binding NPC2 proteins in coral-algal symbiosis. *eLife*, 43923

Jones, V.A.S.*, Bucher, M.*, Hambleton, E.A., **Guse, A.** (2018). Microinjection to deliver protein, mRNA, and DNA into zygotes of the cnidarian endosymbiosis model *Aiptasia* sp. *Scientific Reports* 8, 16437





2.12 PROF. DR. RÜDIGER HELL PLANT MOLECULAR BIOLOGY

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Fields of Interest

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



Summary and outlook

The Department of Molecular Biology of Plants investigates the role of metabolism in plants for driving growth in relation to developmental programs and changing environments. To this end the metabolism of sulfur proved to be highly suitable not only to study plant nutrient sensing, but growth control in general and stress responses at cellular and organismal levels. These aspects are linked to a second major theme represented by Dr. Markus Wirtz, consisting in cellular adaptation reactions to stress by protein turnover and damage control via co-translational acetylation of protein N-termini.

Projects related to sulfur metabolism during the report period were redox regulation via sulfhydryl groups (DFG Priority Program 1710), distribution control in primary sulfur assimilation, sulfur nutrient sensing by target of rapamycin and drought acclimation via cysteine-dependent ABA synthesis (all DFG funded). Together with the Wirtz group protein stability control (DFG CRC 1036) and the role of N-terminal acetylation in chloroplasts (ERA-CAPS) were addressed with respect to proteome surveillance during environmental stress. The department further hosts the Metabolomics Core Technology Platform that provides scientific services for the entire Heidelberg Life Science Campus. The third party funded cooperation with medical cell biology groups extended our interest to metabolic diseases (CRC 1118, on late diabetic disorders), metabolic processes in tumor cells (German Cancer Research Center, DKFZ Heidelberg) and metabolomics of recurrent cancer (BMBF SMART-CARE). Until 2019 the department supported the independent junior group of Dr. Emmanuel Gaquerel on plant secondary metabolism and insect defense who accepted a Professorship at Strasburg University.

Research highlights since 2017

The regulation of protein activities by redox control beyond photosynthesis is a rapidly developing field based on greatly improved protein thiol biochemistry, sulfur metabolite analytics and molecular genetics. The department has contributed with its expertise in these fields to several projects at COS and within DFG-SPP1710 during the report period: integration of cysteine synthesis and oxylipin signaling during high light stress (Müller et al., 2017), redox activation of plastid glutathione synthesis (cooperation with T. Rausch; Yang et al., 2019), redox interplay between chloroplasts and mitochondria (Marty et al., 2019), redox activation of mitochondria during germination (Nietzel et al., 2020), and the function of the only mitochondrial glutaredoxin (Moseler et al., 2021).

The prerequisite of thiol-based regulatory redox functions and control of reactive oxygen species for stress defense is the partitioning on reduced sulfur between glutathione-mediated processes and protein translation, or in brief: the decision between growth and survival. The key component in this context is cysteine, the first stable organic sulfur compound in the assimilation pathway. Labeling experiments under steady state conditions showed

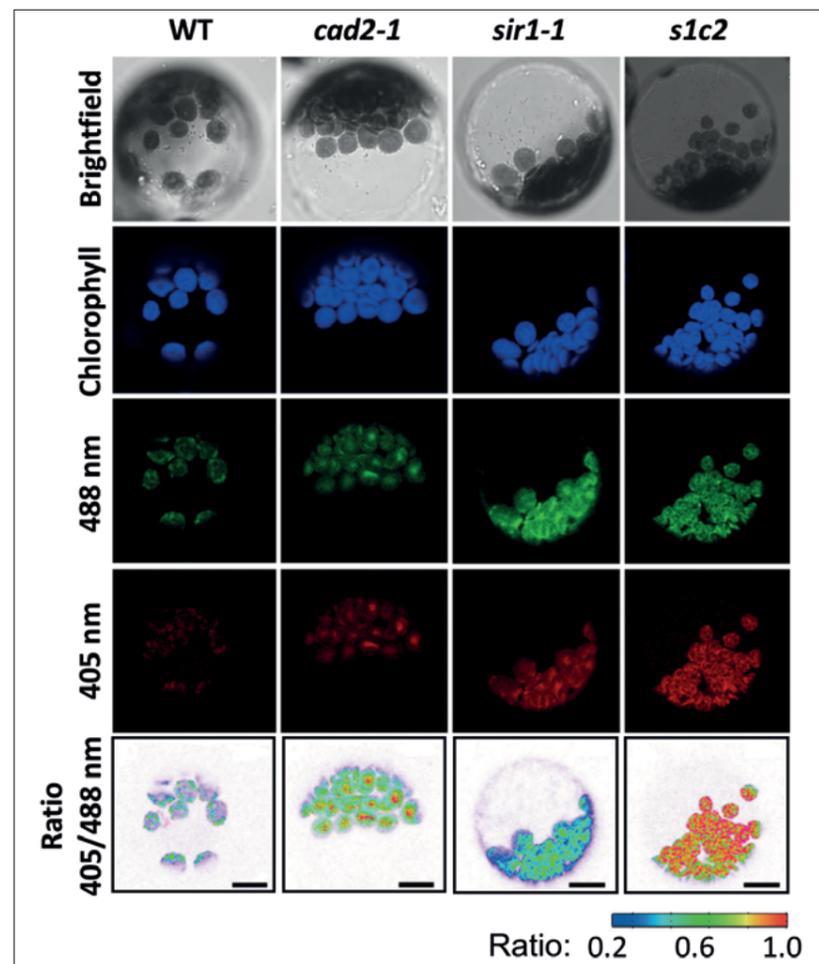


Figure 1

The plastid GSH redox pool is more oxidized in plants with decreased sulfur flux into glutathione. The *s1c2* double mutant is a genetic hybrid of *sir1-1*, which displays lowered sulfur assimilation, and *cad2-1*, suffering from low GSH biosynthesis capacity. The redox-potential is indicated by the signal ratio of the plastid targeted Grx1-roGFP2 sensor (blue = reduced, red = oxidized).

that substantial amounts of cysteine can be partitioned between glutathione and proteins. Using knock-down mutants of Arabidopsis impaired in either glutathione synthesis (*cad2-1*) or sulfite reduction (*sir1-1*) or crosses thereof, the complementation of reduced growth phenotypes demonstrated the principal channeling of these two major routes and their consequences for chloroplast and cytosolic redox state as shown by live cell imaging with compartment-specific redoxGFP2 probes (Speiser et al., 2018). In cooperation with U. Kummer (COS) data from steady state and labeling studies of Arabidopsis plants were used for a computational approach with a kinetic model including all biochemical reactions of primary sulfur metabolism. Remarkably, the model confirmed a shared role of the sulfite reductase and APR catalyzed reduction steps that had first been experimentally determined by us in Kahn et al. (2010).

Partitioning of flux can take place at several branch points in sulfur metabolism and depends on the environmental challenge, e.g. nutrient limitation, high light, insect defense etc. We discovered that Arabidopsis senses sulfur availability by the Target of Rapamycin (TOR) receptor kinase, while carbon/nitrogen supply for cysteine synthesis is transmitted by General Carbon Nitrogen limitation (GCN2) kinase. These fundamental eukaryotic sensor kinases are encoded by single copy genes in Arabidopsis and allowed a deep transcriptomic and metabolomics analyses that revealed that surprisingly sulfur availability is not transmitted directly but by the so-called glucose-TOR axis (Dong et al., 2017). Further research showed that TOR is also involved in cold acclimation (Dong et al., 2019).

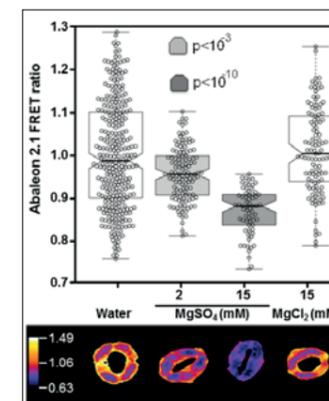


Figure 2

Detection of the cytosolic ABA concentration in guard cells after external application of increasing sulfate concentration (MgSO_4). MgCl_2 and water were applied as controls. Physiologically relevant concentrations (2-15 mM) of the xylem-mobile sulfate induced internal accumulation of ABA in guard cells as shown by the decrease of Abaleon 2.1 FRET ratio (Shown in false color).

Molecular mechanisms of drought resistance have become the major target of environmental stress research in the department (see report by M. Wirtz). Earlier reports had suggested sulfate as a signal during early onset of water deficit, even preceding ABA in the vasculature. In cooperation with the team of H. Rennenberg (Freiburg) it was shown that Arabidopsis and poplar indeed can close stomata by sulfate during onset of drought (Malcheska et al., 2017). This was followed up by mechanistic investigations focusing on an Arabidopsis set of mutants in sulfur metabolism and transport as well as ABA synthesis and signaling. These studies surprisingly revealed that not sulfate, but cysteine from chloroplasts in guard cells is prerequisite for de-novo synthesis of ABA for stomatal closure. Using live cell imaging with the Abaleon ABA sensor we could show that indeed ABA concentrations increase specifically after sulfate feeding and that cell-autonomous synthesis is sufficient for closure. Mutants lacking plastidial cysteine synthesis are consequently drought resistant (Batool et al., 2018; Rajab et al., 2019)

The discovery of de novo synthesis of cysteine in chloroplasts as prerequisite for ABA synthesis in guard cells complements our long-standing interest in the subcellular and biochemical organisation of cysteine synthase complexes (CSCs). Our earlier studies had revealed that the mitochondrial CSC is the driver for the formation of O-acetylserine, the precursor of cysteine, whereas the cytoplasmic site is responsible for the bulk of cysteine synthesis and the chloroplast is particularly important during stress (Müller et al., 2017; Rajab et al., 2020). In this respect the cooperation with Prof. F. Zhao, Nanjing Agricultural University, led to the functional characterization of a rice mutant carrying a point mutation in a subunit of the chloroplast CSC (Sun et al., 2021). This leads to constitutive association of the CSC with concomitant enhanced synthesis of cysteine and subsequently enhanced formation of phytochelatin and arsenic resistance. These findings received broad attention and are now continued within the framework of a Humboldt postdoctoral fellowship.

Future directions

Starting from pathway elucidation and dissection of subcellular compartmentation of primary sulfur metabolism and N-terminal acetylation in the previous report period new avenues will lead to fundamental questions of growth control under environmental stress conditions. This general theme is pursued in the department with emphasis on biomass formation and the accompanying developmental acclimations using mainly water limitation as stress factor.

Future work will address the differential function of TOR in shoot (Dong et al., 2017) as compared to root, aiming to explain the important trait of shoot growth retardation and root acceleration under limiting water and nutrient supply. Ongoing experiments suggest that photosynthesis, autophagy and sugar export at lowered TOR activity in the shoot are crucial for the activated state of TOR in the root, going along with inactive autophagy, en-

hanced translation and root growth with the aim to tap on water or nutrient resources in the soil. These investigations will be extended to the other major sensor kinase Snrk1 that is known to interact with TOR regarding changes of energy status under stress.

The successful unraveling of sulfur-dependent signaling during early water deficit (Batool et al., 2018) indicated novel links to OPDA signaling and the plastid cysteine synthase complex. In a newly funded DFG project the signaling leading to OPDA synthesis under water deficit and the mechanism of interaction of OPDA with an interactor of the plastid cysteine synthase complex be addressed.

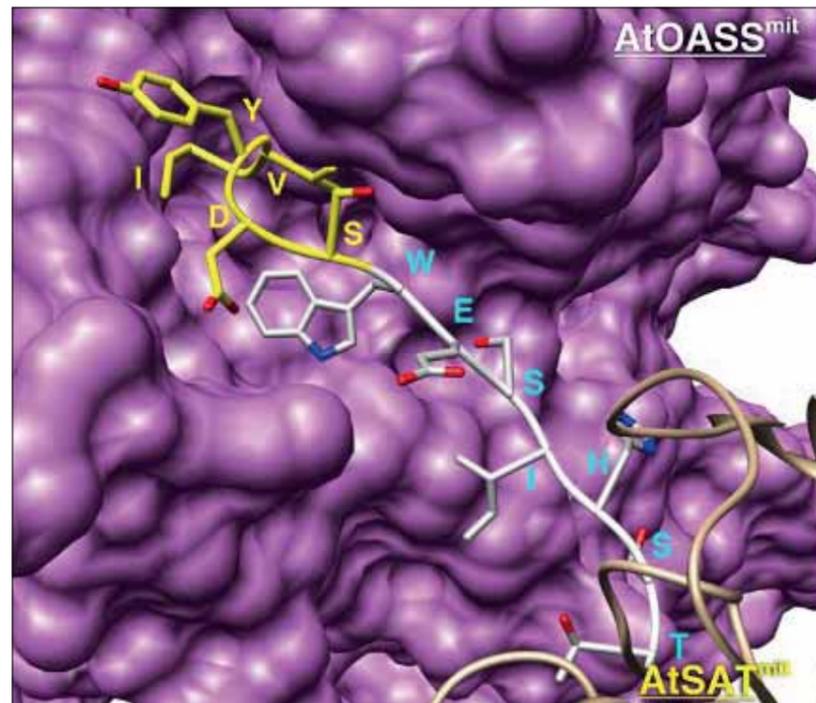


Figure 3
Top view on the modelled sensor site of the cysteine synthase complex consisting of serine acetyltransferase (AtSATmit) and O-acetylserine(thiol)yase (AtOASSmit). The C-terminal tail of AtSATmit engages the active site of AtOASSmit. Consequently, the OASS substrates, sulfide and OAS, dynamically regulate this transient interaction. Modified according to Feldman-Salit et al. (2009).

The discovery of constitutively activated cysteine synthesis in rice plastids (Sun et al., 2021) is currently being engineered into Arabidopsis mutant backgrounds to make this tool available for structural and regulatory experiments. Interaction sites of cysteine synthase complex subunits and binding partners will be resolved by crystallization or cryoEM to unravel the mechanism of constitutive activation with the final aim of a full structure that is still missing for this unusual protein complex. The subcellular compartments will be independently equipped with activated CSCs to dissect their individual functions. In addition to basic questions this engineering is supposed to identify the most effective steps in setting up arsenic resistance that may later be transferred back to rice.

The joint projects with M. Wirtz are based on shared methodologies (proteomics, life cell imaging, metabolite analyses) and overlap in the understanding of stress responses, i.e. mainly drought stress. Following both major lines proved to be complementary and very successful (Linster et al., 2020, Huber et al., 2020, Armbruster et al., 2020).

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 41, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 7, Number of citations 2017-2021: 4011

Sun, S.-K., Xu, X., Tang, Z., Tang, Z., Huang, X.-Y., Wirtz, M., **Hell, R.**, and Zhao, F.-J. (2021) A molecular switch in sulfur metabolism to reduce arsenic and enrich selenium in rice grain. *Nature Commun.* 12: 1392.

Armbruster, L., Linster, E., Boyer, J.-B., Brünje, A., Eirich, J., Stephan, I., Bienvenut, W. V., Weidenhausen, J., Meinnel, T., **Hell, R.**, Sinning, I., Finkemeier, I., Giglione, C., and Wirtz, M. (2020) NAA50 is an enzymatically active N α -acetyltransferase that is crucial for the development and regulation of stress responses. *Plant Physiol.* 183: 1502–1516

Speiser, A., Silbermann, M., Dong, Y., Haberland, S., Uslu, V., Wang, S., Bangash, S., Reichelt, M., Meyer, A., Wirtz, M., and **Hell, R.** (2018) Partitioning of sulfur between glutathione and protein synthesis determines growth of Arabidopsis. *Plant Physiol.* 177: 927-937.

Batool, S., Uslu, V.V., Rajab, H., Ahmad, N., Waadt, R., Geiger, D., Malagoli, M., Xiang, C.B., Hedrich, R., Rennenberg, H., Herschbach, C., **Hell, R.**, and Wirtz, M. (2018). Sulfate is incorporated into cysteine to trigger ABA production and stomatal closure. *Plant Cell* 30, 2973-2987.

Dong, Y., Silbermann, M., Speiser, A., Forieri, I., Linster, E., Poschet, G., Allboje Samami, A., Wanatabe, M., Sticht, C., Teleman, A.A., Deragon, J.-M., Saito, K., **Hell, R.**, and Wirtz, M. (2017). Sulfur availability regulates plant growth via glucose-TOR signaling. *Nature Commun.* 8, 1174.

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Fields of Interest

N-terminal acetylation, cotranslational modifications, N-degron pathways, ubiquitin-proteasome system, autophagy, proteostasis, translation control, amino acid biosynthesis, Target of Rapamycin, stress-induced developmental plasticity, sulfur metabolism, abscisic acid biosynthesis, stomatal aperture regulation, root-to-shoot signaling



Summary and outlook

Plants have to face diverse environmental challenges onsite. The key to adaption towards environmental stimuli is the reorganization of resources to optimize stress responses and growth. We apply sulfur or water supply shortage as proxies to understand these intricate adaptation mechanisms and identify novel stress-related signals. In this report term, we addressed how sulfur depletion impinged on the sensor-kinase “Target of Rapamycin” to control transcriptional networks, protein translation, and autophagy in the leaves when sulfur supply via the root is limited. We unraveled the function of xylem-mobile sulfate as a root-to-shoot long-distance signal upon soil drying. Substantial advances have been achieved regarding the role of ribosome-associated N^o-acetyltransferases for stress-induced proteostasis. Remarkably, we identified a novel N-degron pathway controlled by N-terminal protein acetylation.

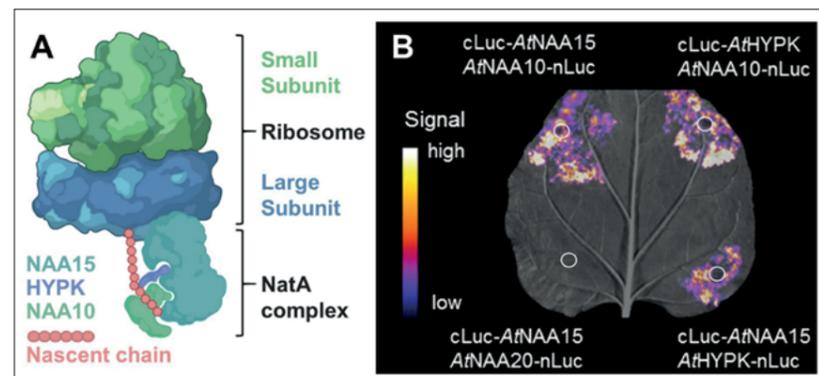


Figure 1

The plant HYPK protein is a novel subunit of the NatA complex
A) Schematic overview of the NatA complex consisting of the ribosome anchoring subunit NAA15, the catalytically active subunit NAA10 and the novel regulatory subunit HYPK. B) Split-luciferase complementation assays demonstrate close proximity of NAA15, NAA10 and HYPK. Luciferase signal intensity is shown in false colors.

Research highlights since 2017

The Target of Rapamycin (TOR) is a highly conserved sensor kinase in phototropic and chemotropic eukaryotes. TOR stimulates cell division and translation and inhibits nutrient remobilization by autophagy (self-eating) under favorable growth conditions. We found that TOR is a critical regulator of these fundamental processes in the leaves of the reference plant *Arabidopsis* when the macronutrient sulfur becomes limiting (Dong et al., 2017).

In the last report term, we identified xylem-borne sulfate as an early root-to-shoot signal upon soil drying. We now could establish sulfate as a physiologically relevant early long-distance drought-stress signal by demonstrating that sulfate triggers ABA production in the guard cells (Batool et al., 2018).

N-terminal protein acetylation (NTA) is a prevalent protein modification widely conserved across eukaryotic kingdoms and is essential for viability in animals and plants. The dominant executor of NTA is the N^o-acetyltransferase A (NatA) complex, which is tethered to the ribosome and accounts for cotranslational acetylation of 40% of the proteome. Drought stress and ABA application deplete NatA abundance, which causes substantial tolerance to soil drying.

Motivated by this previous finding, we identified the remaining cytosolic Nat machinery and contributed to the functional characterization of the plastid-localized NTA machinery (see full publication list). Identifying the plastid localized Nats uncovered a novel and unexpected layer of complexity in the protein acetylation machinery of plants. Surprisingly, eight out of ten plastidic Nats possess dual acetylation activity on N-termini and internal lysine residues. The diversification of the plant Nat machinery during the evolution of eukaryotes is further corroborated by discovering that the plant NatF is plasma membrane resident. NatF post-translationally acetylates plasma membrane proteins and is critical for protein-harming stress (Linster et al., 2020). We also found that loss of the ribosome-associated NatB and NatE complexes impair responses to individual stresses and affect plant development (Huber et al., 2020 and full publication list).

Furthermore, we have identified the plant homolog of the Huntingtin Yeast interacting Partner K (HYPK) as a central regulator of NatA activity in eukaryotes. Like in humans, HYPK interacts with the ribosome-associated core NatA complex in plants (Figure 1). In contrast to animal HYPK, the plant HYPK facilitates NTA of NatA substrates, stabilizing a significant subfraction of cytosolic NatA substrates (Miklankova et al., in preparation).

Future directions

A hallmark of the sulfur deficiency response is the increase of the root-to-shoot ratio by stimulating the root apical meristem. Based on the identification of TOR as the dominant sensor of sulfur supply in leaves, we will focus on the role of TOR for the sulfur-deficiency-induced nutrient remobilization in leaves to support root growth. The second focus of our research team will be on the control of proteostasis in response to environmental stimuli. To this end, we will establish ribosome profiling to determine the stress-induced loading of transcripts to ribosomes. One application will be the quantification of the translome in roots and shoots to sulfur limitation. In combination, with the establishment of a novel non-invasive sensor to quantify protein-half lifetimes in plants (Zhang et al., 2019), the expected result will uncover how dynamic adaptations of the proteome in response to nutrients are achieved. Furthermore, both techniques will be applied to understand the role of the ribosome-associated Nat complexes in determining the proteostasis of their substrates. In addition, we will apply a combination of forward-genetics screens and peptide-pulldown approaches to identify the components of the ubiquitin-proteasome system that target non-acetylated proteins for degradation.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 33, Number of peer-reviewed articles as first or last/corresponding author 2017-2021: 8, Number of citations 2017-2021: 3659 (according to Google Scholar)

Huber, M., Bienvenut, W.V., Linster, E., Stephan, I., Armbruster, L., Sticht, C., Layer, D., Lapouge, K., Meinel, T., Sinning, I., Giglione, C., Hell, R., and **Wirtz, M.** (2020). NatB-Mediated N-Terminal Acetylation Affects Growth and Biotic Stress Responses. *Plant Physiology* 182, 792-806.

Linster, E., Layer, D., Bienvenut, W.V., Dinh, T.V., Weyer, F.A., Leemhuis, W., Brünje, A., Hoffrichter, M., Miklankova, P., Kopp, J., Lapouge, K., Sindlinger, J., Schwarzer, D., Meinel, T., Finkemeier, I., Giglione, C., Hell, R., Sinning, I., and **Wirtz, M.** (2020). The Arabidopsis N α -acetyltransferase NAA60 localizes to the plasma membrane and is vital for the high salt stress response. *New Phytologist* 228, 554-569.

Zhang, H., Linster, E., Gannon, L., Leemhuis, W., Rundle, C.A., Theodoulou, F.L., and **Wirtz, M.** (2019). Tandem fluorescent protein timers for non-invasive relative protein lifetime measurement in plants. *Plant Physiology* 180, 718-731.

Batool, S., Uslu, V.V., Rajab, H., Ahmad, N., Waadt, R., Geiger, D., Malagoli, M., Xiang, C.B., Hedrich, R., Rennenberg, H., Herschbach, C., Hell, R., and **Wirtz, M.** (2018). Sulfate is incorporated into cysteine to trigger ABA production and stomatal closure. *Plant Cell* 30, 2973-2987.

Dong, Y., Silbermann, M., Speiser, A., Forieri, I., Linster, E., Poschet, G., Allboje Samami, A., Wanatabe, M., Sticht, C., Teleman, A.A., Deragon, J.-M., Saito, K., Hell, R., and **Wirtz, M.** (2017). Sulfur availability regulates plant growth via glucose-TOR signaling. *Nature Communications* 8, 1174.





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

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Fields of Interest

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



Summary and outlook

To understand the origin and evolution of key regulators in animal development, our lab is analyzing cnidarians, simple diploblastic animals with a gastrula-like body plan and an ancient nervous and stem cell system. We are working with the freshwater *Hydra*, famous for its almost unlimited life span and regeneration capacity, and with embryos of the sea anemone *Nematostella*. We discovered cnidarian Wnt signaling and we were major players in sequencing the *Hydra* and *Nematostella* genomes. Our work revealed an unanticipated genomic complexity of these ancient organisms with intriguing similarities to vertebrates. It also indicated that the genetic repertoire required for setting up the bilaterian body plan was already present in the common cnidarian/bilaterian ancestor. This finding is also supported by our recent collaborative work on the genome of the moon jelly *Aurelia*, which revealed that the earliest eumetazoans were more physically complex than previously hypothesized (Gold et al., 2019).

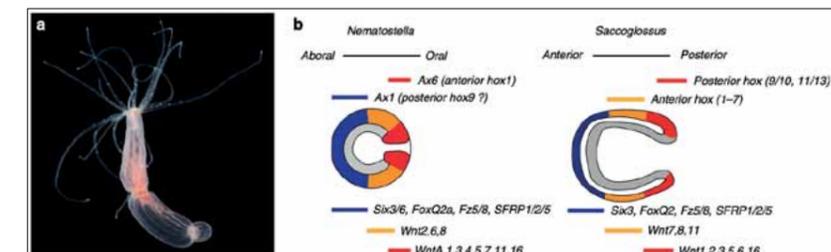
Research highlights since 2017

Molecular evolution and genomics of prebilaterian animals. A goal of our comparative genomics is to decipher the causal connections between genome composition and animal form. The phylum Cnidaria (sea anemones, corals, hydroids and jellyfish) holds a pivotal place in such studies, because Cnidaria are the sister clade to Bilateria (protostomes and deuterostomes), the clade that encompasses 99% of extant animals. Our molecular phylogenetic analyses on the freshwater polyp *Hydra* and the sea anemone *Nematostella* have provided fundamental insights into the molecular organization of the primary body axis in metazoan evolution. It is realized by an evolutionarily highly conserved cluster of Wnt gene families that precede the Hox gene cluster specifying body regions along the AP axis in Bilateria, as confirmed in new work on hemichordates (Fig. 1).

We have now extended our molecular-phylogenetic analyses to the medusae-forming Cnidaria and examined the genome and transcriptome in the moon jellyfish *Aurelia* (Gold

Figure 1

Nematostella and the evolution of axial patterning systems. A photograph of an adult *Nematostella vectensis* polyp. A ring of tentacles that are used for capturing prey surrounds the oral opening (oriented to the top). The "oral" opening serves as both mouth and anus and derives directly from the blastopore (to the right in b). b Schematic drawing of gastrula stages of *Nematostella vectensis* and the hemichordate *Saccoglossus kowalevskii*, which serves as an example for a bilaterian embryo. The blastopore is oriented to the right. Relative gene expression domains are indicated to illustrate the support for different scenarios in the evolutionary relation of the primary body axis. Note that the gene expression domains are simplified and that some of the *Nematostella* Wnts are expressed in the endoderm. From: Rentzsch & Holstein (2018). Making head or tail of cnidarian hox gene function. *Nature communications* 9, 2187-2190

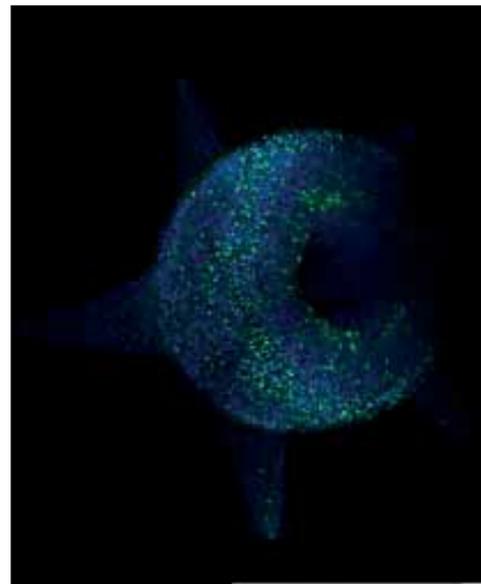


et al., 2019). Our analyses on the genome of *Aurelia* suggest that gene gain and loss in *Aurelia* is comparable to what has been found in its morphologically simpler relatives—the anthozoan corals and sea anemones. RNA sequencing analysis does not support the hypothesis that taxonomically restricted (orphan) genes play an oversized role in the development of the medusa stage. Instead, genes broadly conserved across animals and eukaryotes play comparable roles throughout the life cycle. All life stages of *Aurelia* are significantly enriched in the expression of genes that are hypothesized to interact in protein networks found in bilaterian animals. Collectively, our results suggest that increased life cycle complexity in *Aurelia* does not correlate with an increased number of genes. This leads to two possible evolutionary scenarios: either medusozoans evolved their complex medusa life stage (with concomitant shifts into new ecological niches) primarily by re-working genetic pathways already present in the last common ancestor of cnidarians, or the earliest cnidarians had a medusa life stage, which was subsequently lost in the anthozoans. While we favor the earlier hypothesis, the latter is consistent with growing evidence that many of the earliest animals were more physically complex than previously hypothesized (Gold et al., 2019).

Evolution and Function of Wnt signaling. Wnt signaling is an evolutionary old and animal-specific pathway. We have discovered this pathway in cnidarians, gastrula-shaped organisms at the base of eumetazoan evolution and sister group to bilaterians. Cnidarians exhibit the complete repertoire of Wnt ligands essential for the formation of the blastopore-like signaling center of polyps. This developmental organizer guides pattern formation and stem cell differentiation along the animal's oral-aboral body axis, which is reminiscent to the gradient of Wnt/ β -Catenin signaling regulating anteroposterior patterning in vertebrates.

Figure 2

MAPKs transmit injury-response signals to the initial steps of patterning, which in *Hydra* includes Wnt signaling. The three MAPKs, p38, c-Jun N-terminal kinases (JNKs), and extracellular signal-regulated kinases (ERK), are essential to initiate regeneration in *Hydra* in response to an injury signal, which is constituted of calcium and reactive oxygen species (ROS) signaling. The image shows phosphorylated p38 during foot regeneration. Note the opened site of foot removal that permits a view into the gastric cavity. Our data show that the activation of MAPKs is the essential part of the wound response transmitting the injury signal to the patterning system by specifically inducing different Wnts that in turn are fundamental for patterning the lost body part (Image © Tursch & Holstein)



Our work on Wnt signaling is a core project of the DFG Collaborative Research Center (CRC) 1324 (A5) on “Mechanisms and functions of Wnt signaling” (1st funding period July 2017 – June 2021, 2nd funding period July 2021 – June 2025). In our project we studied receptor-ligand interactions and functions of specific Wnt ligands during regeneration. This work was based on our previous integrative transcriptome and SILAC proteome study on *Hydra* head regeneration, where we used a combined transcriptomic and stable isotope labelling by amino acids in cell culture proteomic/phosphor-proteomic approach (Stefanie Höger, Hendrik Petersen, and Oleg Simakov). Our global analysis revealed two distinct molecular cascades: an early injury response and a subsequent, signaling driven patter-

ning of the regenerating tissue. A key factor of the initial injury response where MAPKs and a general stabilization of proteins, which was followed by a subsequent activation cascade of signaling molecules including Wnts (Fig. 2). By interfering with Wnt functions by loss-of-function and gain of function approaches, we found highly specific “morphants” for *Wnt2*, *-3*, *-5*, *-7*, *-9/10a*, *-9/10b*, and *-16* during head regeneration (A5, Mark Lommel). They indicate a different response of different Wnts to the regeneration stimulus within the Wnt cascade. When we studied the role of the injury signal on the activation of Wnt genes we found that MAPK signaling activates *Wnt3* and *Wnt9/10a* (i.e., the earliest Wnt genes) in a specific and antagonistic manner (A5, Anja Tursch). These data demonstrate how “position-independent” injury signals can be transmitted to position-specific patterning signals, which is fundamental to any regeneration process in animals.

On the origin of the germline in animals. In this work, we addressed a more than 100 years old topic in biology. Based on studies on hydrozoan polyps, August Weismann postulated that germline cells are formed by an early segregation of somatic cells and maintain their undifferentiated state until gametogenesis. This concept had a major influence on the modern synthesis of evolutionary biology, since heredity was limited to the germline, which can form a barrier to prevent mutations that accumulate in somatic cells from entering the germline. However, the significance of this concept was repeatedly questioned. At the heart of the arguments against the germline were observations in basal metazoans – including hydrozoans – that suggested that germline stem cells can be formed from somatic stem cells throughout the life of an animal, similar to plants. In our project on *Hydra* germline stem cells, we re-investigated this important question. – In our project of the CRC 873 on “Maintenance and Differentiation of Stem Cells in Development and Disease” we have reinvestigated this fundamental biological issue by analyzing adult polyps and embryos (A1, Chiemi Nishimiya-Fujisawa, Stephanie Kuen). By using transgenic strains for germ cell and somatic stem cell markers, we performed a long-term study in which we clonally traced putative germline and somatic stem cells; this study was combined with RNA-seq and single cell RNA transcriptomics. To our knowledge, this is to the first large-scale transcriptomic analyses of germline cells from any prebilaterian animal. Our study revealed the existence of a genuine germline in *Hydra*, which is separated from all somatic cells over the animal's entire life-span; somatic stem cells never give rise to germline under normal conditions. Germline and somatic stem cell lineages in *Hydra* largely differ from each other on the transcriptomic level, but they also share an archaic core of stemness-related epigenetic and transcription factors that originates in unicellular eukaryotes and is preserved throughout metazoan evolution. We propose that this sophisticated division of labor between germline and somatic stem cells did not evolve repeatedly in animal evolution, but was already linked to the emergence of the first animals. While it may have been lost in colonial forms, it is unique for most other animals in that it ensures the versatile differentiation capacities of somatic stem cell lineages building up the animal body plans.

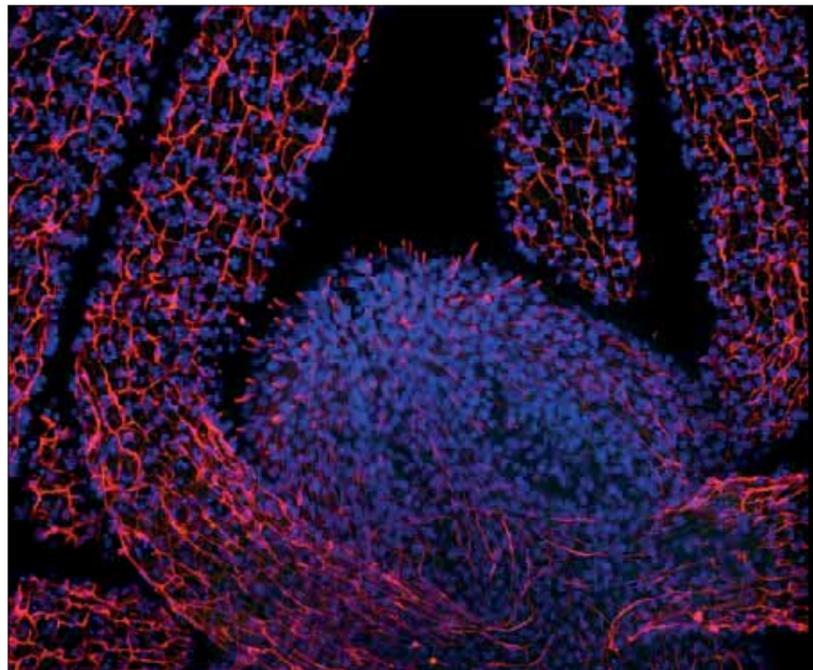
Origin and evolution of the nervous system and nematocytes. The emergence of nerve cells is another key novelty in animal evolution that was closely related with the emergence of cnidarians. The general view holds that the bilaterian central nervous system (CNS) can be traced back through evolution to a nerve net in a cnidarian-like ancestor. In our previous work we could demonstrate that patterning of the Cnidarian nervous system is highly linked to the patterning of the body axis. Cnidarian neurogenic TFs and neuropeptide-positive neurons exhibit a clear position dependency along the oral-aboral body axis, which is highly reminiscent to bilaterian embryos (Fig. 3). Based on our discovery that β -catenin signaling is important for early induction of the embryonic nervous system in *Nematostella*, we have extended our analyses to *Hydra*, which has a number of different neuronal subpopulations responsible for *Hydra*'s distinct behavioral patterns (Hendrik Petersen, Tracy Chih-Ting Koubkova-Yu).

We also addressed the long-standing question of the emergence of nematocytes, a neuronal cell type in cnidarians containing just one giant secretory vesicle (5-20 μ m in diameter) called the nematocyst (cnidocyst). Nematocytes (cnidocytes) represent one of the most

evolved and sophisticated cell-type in animal evolution and are the name-giving cell type of the cnidaria. Nematocytes have also been a long-standing topic of our research, so we have been able to show that this is the fastest process in biology that has been documented at the cellular level, and we have also been able to resolve the major steps in morphogenesis of this organelle and its molecular composition. Nevertheless, it is still an enigma, whether nematocytes are (i) a product of cnidarian evolution, (ii) an inheritance from the unicellular ancestors of metazoans, or (iii) a product of lateral gene transfer. In a collaborative project we analyzed the extrusive organelles in dinoflagellate protists, which have been hypothesized to be homologous to the nematocytes (cnidae) in cnidarians (Gavelis et al, 2017). Both types of extrusive organelles (extrusomes) share intriguing structural similarities suggesting a common evolutionary origin. But we could show, using structural, functional, and phylogenomic data, that nematocytes evolved independently in both lineages, i.e., in dinoflagellates and cnidarians. This excludes the hypothesis according to which cnidarian cnidae are a product of gene transfer from dinoflagellate-like unicellular ancestors. Different from modern cnidarians, dinoflagellates (and other protists) have a large arsenal of extrusomes, ranging from simple defensive organelles (e.g., mucocysts and trichocysts) to elaborate organelles for predation (e.g., taeniocysts and nematocysts). This diversity of traits in the extrusive organelles of dinoflagellates suggests how – within one clade – a stepwise route from simple secretory structures could lead to elaborate sub-cellular weapons (Gavelis et al, 2017). It opens up a scenario according to which nematocysts evolved within the cnidarian clade, likely inherited from an extinct uni- or multicellular ancestor cnidarian ancestor that already used such sophisticated subcellular weapons in the arms race between different unicellular eukaryotes.

Figure 3

A diffuse nervous network without brain and ganglia, like the nervous system of cnidarians, probably was at the beginning of the evolution of nervous systems. The nerve net of the freshwater polyp *Hydra vulgaris*, shown by expression of a nerve-specific membrane protein (red) and nuclei stained with a DNA dye (DAPI) (blue). A high density of sensory neurons is established around the mouth opening (hypostome) of the polyp, which are important for the act of capture and feeding. There is a wide spectrum of different neurotransmitters expressed in subpopulations of neurons. Such simple neural networks are capable of complex behavioral patterns, including the act of prey capture with nematocytes (cnidocytes), or the various forms of locomotion and are currently under cellular and neurobiological investigation. (Image © Bertulat & Holstein)



Future directions

We will address three main questions that are currently in progress:

- 1) Stem cell plasticity in *Hydra* (DFG SFB873-A1)
- 2) Impact of transposable elements during animal regeneration (DFG D.A.CH. HO1088-10-1)
- 3) The Wnt code: Evolutionary origin of Wnt ligand-receptor interactions (DFG SFB1324-A5)

1) Stem cell plasticity in *Hydra*. Similar to plants, *Hydra* exhibits an almost unlimited life-

time and regeneration capacity. On the cellular level, these properties are based on three distinct somatic stem cell systems, two epithelial and one interstitial stem cell system. Here we focus on the interstitial stem cell population to identify mechanisms guiding the transition of somatic stem cells to germline precursors. This stem cell population is special, as it gives rise not only to many somatic cells, including neurons, but more remarkably also to germline precursors. However, the molecular mechanisms of this soma-to-germline transition are still enigmatic. Based on cell type-specific RNA-Seq profiling, which revealed the existence of genes specifically expressed in interstitial cell lines that give rise to the egg or sperm lineage, we have established tools to reliably induce the switch from somatic interstitial stem cells to germline precursors and to visualize this process by genetically labelling somatic stem cells and germline precursors. Using this experimental set-up, we expect to identify relevant genes and molecular mechanisms that control and maintain the somatic and the germline lineage (see also above). Unravelling the function of the underlying genetic networks will also open novel approaches to identify evolutionary conserved mechanisms that are common to somatic stem cells and the germline and may form the genetic essence for longevity.

2) Impact of transposable elements during animal regeneration. This project is based on our previous discovery of an activation of transposon during *Hydra* regeneration and might explain why the regeneration capabilities can vary significantly across multicellular organisms (Petersen et al 2015). – Studies on the underlying molecular mechanisms and their evolution have largely focused on deciphering the coding gene complement in various model systems with studies of the non-coding genome recently coming into focus. Transposable elements (transposons) comprise the largest portion of the non-coding sequence. Our recent data showed that specific transposons are activated during regeneration. Interestingly, on an evolutionary time-scale, transposons can contribute to the characteristic large genome size of many species with extraordinary regeneration capabilities. This is a result of both elevated activity and insertion rate of transposons, cellular defenses against their insertions or deletions, as well as the given population structure and selective pressures that in the long time-scale balances their maintenance or deletion. Combined with their known role in genome stability and generation of regulatory novelty those insights pose key yet still unanswered questions regarding the role of transposons both during the actual process of regeneration as well as a potential evolutionary drive to evolve sophisticated regenerative capabilities. In a collaborative project with Oleg Simakov (University Vienna) and Ely Tanaka (Institute for Molecular Pathology, Vienna), we compare the transcriptional dynamics of transposons during regeneration and their effect on the genome architecture in two key, phylogenetically informative model systems for regeneration, the cnidarian *Hydra magnipapillata* and the vertebrate, salamander, *Ambystoma mexicanum* (axolotl). Here we characterize the shared and derived transcriptionally active transposable elements among those two species, providing the first complete overview of regeneration-active elements at the sub-family resolution level. We study cellular-level activity of transposons in regenerating tissues and functionally test their role *in vivo*. We also study genome-wide transposon insertion dynamics in consecutively regenerating tissues, such as in the repeated cycles of regeneration during dissociation-reaggregation experiments in *Hydra* or consecutive limb regeneration in *Ambystoma*. This experiment allows us to study, on the genome-wide scale, transposon insertion patterns and their effect on core genes involved in injury response and tissue regeneration. Our evolutionary comparison of genomic responses during vertebrate and cnidarian regeneration will unravel common and unique patterns of how metazoan genome architecture is utilized to enable the extraordinary regenerative capabilities in those clades.

3) The Wnt code: Deciphering early Wnt interactions in *Hydra*. *Wnt* genes are deeply embedded in metazoan genomes where they act in embryonic patterning and cell differentiation. We have previously shown that cnidarians, the sister group of bilaterian animals, exhibit already a complete repertoire of *Wnt* gene subfamilies that is only known from vertebrates. We also found that a cascade-like activation of *Wnt* genes is preceding the patterning of the animal's body axis (the "Wnt- code") during embryogenesis and regeneration.

By interfering with Wnt functions by loss-of-function and gain of function approaches, we found highly specific “morphants” for Wnt2, 3, -5, -7, -9/10a, -9/10b, and -16 during head regeneration. They indicate a different response of different Wnts to the regeneration stimulus within the Wnt cascade. Biophysical data together with Motomu Tanaka (Institute of Physical Chemistry) indicated that the stiffness of the extracellular matrix was dramatically lowered in *Hydra* wildtype polyps at the sides of head organizer formation and in animals with activated canonical Wnt signaling. This was the starting point of a collaboration of the Holstein and Tanaka labs on the basis of which we apply for this joint project. – Now, we will study the hierarchy of Wnts (“Wnt code”) in the *Hydra* head organizer and their interplay with physical cues. To understand the activation of the Wnt-network of the *Hydra* head organizer in steady state animals and during regeneration we analyze the activation range of Wnts with an emphasis on antagonistic Wnts (Wnt3 and -9/10a; Wnt5 vs -8). We currently combined these studies with an analysis of the transcriptomic and proteomic landscape of the emerging head organizer together with Jeroen Krijgsveld (Division of proteomics of stem cells and cancer, DKFZ). To understand how biophysical (mechanical) and biochemical (Wnt signaling) cues interact, we will study the symmetry break in regenerates and in reagggregates under conditions of de novo pattern formation. By mathematical modelling with Anna Marciniak-Czochra (Institute of Applied Mathematics) we test the putative feedback loop between morphogen patterning and tissue mechanics. Our main goal here is to generate a generalized model that integrates the molecular network underlying *Hydra*'s Wnt code with biophysical dynamics during pattern formation. This will be crucial for the analysis of more complex bilaterian models.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 11, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 2, Number of citations 2017-2021: 1655

Ziegler, B., Yiallourous, I., Trageser, B., Kumar, S., Mercker, M., Kling, S., Fath, M., Warnken, U., Schnolzer, M., **Holstein, T.W.**, et al. (2021). The Wnt-specific astacin proteinase HAS-7 restricts head organizer formation in *Hydra*. *BMC Biol* 19, 120.

Holstein, T.W. (2019). What makes flatworms go to pieces. *Nature* 572, 593-594.

Bandmann, V., Mirsanaye, A.S., Schafer, J., Thiel, G., **Holstein, T.**, and Mikosch-Werschling, M. (2019). Membrane capacitance recordings resolve dynamics and complexity of receptor-mediated endocytosis in Wnt signalling. *Sci Rep* 9, 12999.

Wong, W.Y., Simakov, O., Bridge, D.M., Cartwright, P., Bellantuono, A.J., Kuhn, A., **Holstein, T.W.**, David, C.N., Steele, R.E., and Martinez, D.E. (2019). Expansion of a single transposable element family is associated with genome-size increase and radiation in the genus *Hydra*. *Proc Natl Acad Sci U S A* 116, 22915-22917.

Gold, D.A., Katsuki, T., Li, Y., Yan, X., Regulski, M., Ibberson, D., **Holstein, T.**, Steele, R.E., Jacobs, D.K., and Greenspan, R.J. (2019). The genome of the jellyfish *Aurelia* and the evolution of animal complexity. *Nat Ecol Evol* 3, 96-104.

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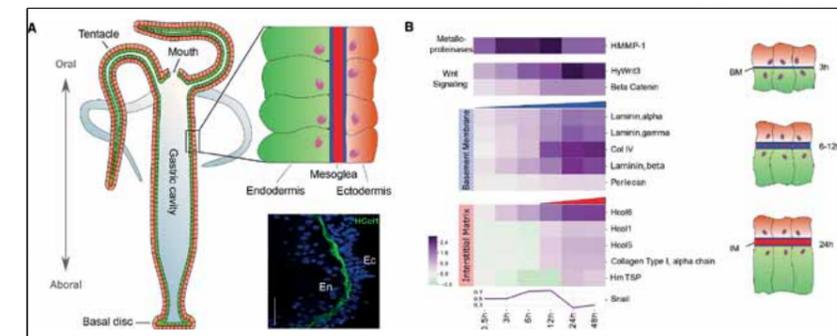
Fields of Interest

Molecular evolution in cnidarians;
protein biochemistry and evolution of Wnt
signaling and ECM molecules; organelle
morphogenesis; nematocyst biology;
proteomics; imaging.



Summary and outlook

Our research is focused on evolutionary, developmental and structure-function aspects of Wnt signaling and extracellular matrix (ECM) in cnidarians (Fig. 1). Our model organisms are the cnidarians *Hydra* and *Nematostella*, which represent early branching metazoan animals and a sister group to the bilateria. In the last years, we identified by several proteomic screens novel ECM factors, which are closely linked to Wnt-regulated pattern and axis formation in *Hydra*. Our future research includes a comparative molecular analysis



a hitherto unknown Thrombospondin superfamily which we characterized in a separate paper by Shoemark et al. (2019).

A recent report by Ziegler et al. (2021) presented HAS-7 as a Wnt-specific astacin protease (Fig. 2). By a proteomic screen of Hydra's head lysate, HAS-7 was identified as a proteolytic factor that cleaves and thereby inactivates Wnt3. HAS-7 siRNA knockdown abrogated Wnt3 proteolysis in the head tissue and induced a double axis phenotype in steady-state polyps. Importantly, Wnt3-induced double axes in *Xenopus* embryos could be rescued by co-injection of HAS-7 mRNA. In summary, our study suggested a negative regulatory function of a Wnt processing metalloproteinases in the global patterning of the oral-aboral axis in Hydra.

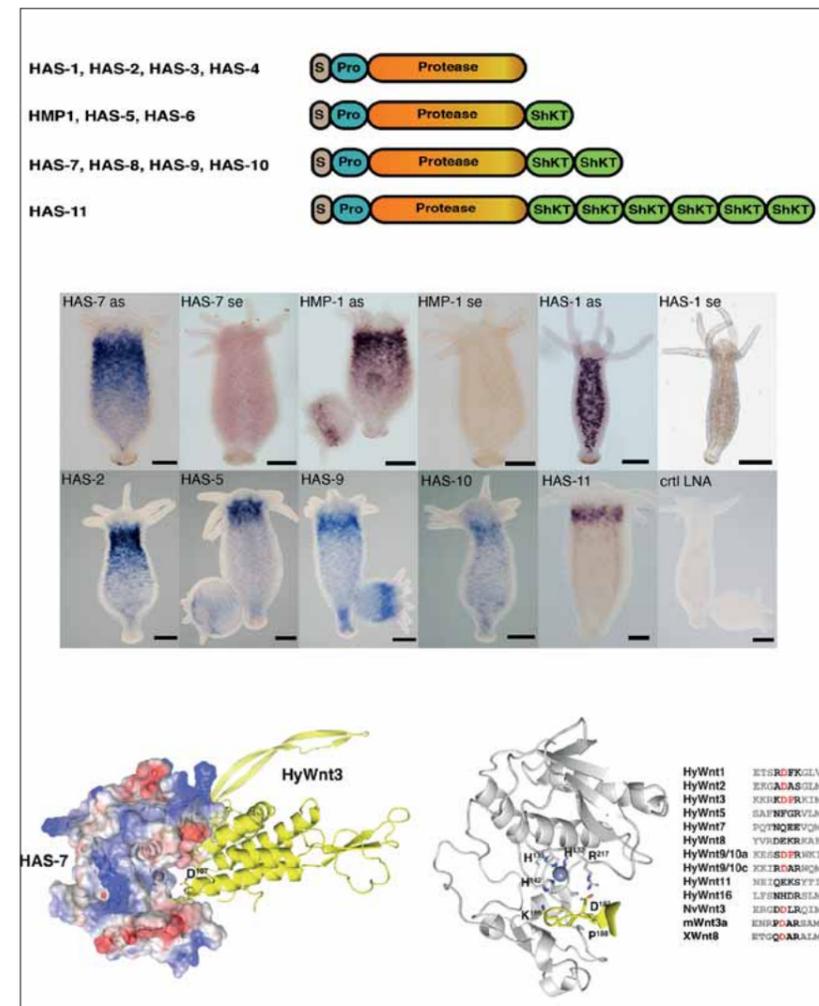


Figure 2
In Ziegler et al. (2021) we have identified by a proteomic screen a Wnt-specific protease of the astacin family involved in the maintenance of a single head organizer. A structural model of the putative HyWnt3:HAS-7 complex was used to predict the putative cleavage motif in Wnt3.

Nematocysts, the stinging organelles of cnidarians, have remarkable mechanical properties. They undergo volume changes of 50% during their explosive exocytosis and withstand osmotic pressures of beyond 100 bar. Recently, two novel protein components building the nematocyst capsule wall in Hydra were identified: CPP-1 characterized by a rigid polyproline motif and the elastic Cnidoin possessing a silk-like domain were shown to crosslink via disulfide bonds. In a recent study by Bentele et al. (2019), recombinant Cnidoin and CPP-1 were expressed in *E. coli* and uniform protein nanofibers were fabricated

by electrospinning. Following a quantitative assessment of mechanical properties by AFM, the potential of Cnidoin and CPP-1 nanofibers was examined towards the maintenance of human mesenchymal stem cells.

Future directions

(i) We plan to unravel, applying a proteomic analysis, the molecular composition of the mesoglea in the starlet sea anemone *Nematostella vectensis*. In particular, we are interested in determining quantitative and qualitative adaptations of the ECM during the different life stages (larva, primary polyp, adult polyp) of this anthozoan species. Using dsRNA injections into embryos we will test candidate genes as potential gatekeepers of morphogenetic processes.

(ii) In a follow-up project on the HAS-7 astacin protease we will attempt to elucidate the crystal structure of the Wnt-protease complex in cooperation with Irmis Sinning (BZH). In addition, we will examine the role of further astacin family members in axis patterning. This work will be funded by the SFB 1324.

(iii) We will also proceed with the molecular analysis of the cnidarian stinging organelle. We have identified a myosin II homolog, which is exclusively expressed in developing nematocytes. By genetic knockdown studies, inhibitor treatments and life imaging of transgenic lines we will study the role of this myosin molecule in the morphogenesis of the nematocyst capsule.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 10, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 5, Number of citations 2017-2021: 1163

Ziegler, B., Yiallourous, I., Trageser, B., Kumar, S., Mercker, M., Kling, S., Fath, M., Warnken, U., Schnolzer, M., Holstein, T.W., et al. (2021). The Wnt-specific astacin proteinase HAS-7 restricts head organizer formation in Hydra. *BMC Biol* 19, 120.

Bergheim, B.G., and **Ozbek, S.** (2019). Extracellular matrix and morphogenesis in cnidarians: a tightly knit relationship. *Essays Biochem* 63, 407-416.

Shoemark, D.K., Ziegler, B., Watanabe, H., Strompen, J., Tucker, R.P., **Ozbek, S.**, and Adams, J.C. (2019). Emergence of a Thrombospondin Superfamily at the Origin of Metazoans. *Mol Biol Evol* 36, 1220-1238.

Bentele, T., Amadei, F., Kimmle, E., Veschgini, M., Linke, P., Sontag-Gonzalez, M., Tenigkeit, J., Ho, A.D., **Ozbek, S.**, and Tanaka, M. (2019). New Class of Crosslinker-Free Nanofiber Biomaterials from Hydra Nematocyst Proteins. *Sci Rep* 9, 19116.

Lommel, M., Strompen, J., Hellewell, A.L., Balasubramanian, G.P., Christofidou, E.D., Thomson, A.R., Boyle, A.L., Woolfson, D.N., Puglisi, K., Hartl, M., et al. (2018). Hydra Mesoglea Proteome Identifies Thrombospondin as a Conserved Component Active in Head Organizer Restriction. *Sci Rep* 8, 11753.



2.14 PROF. DR. MARCUS KOCH BIODIVERSITY AND PLANT SYSTEMATICS

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Fields of Interest

Evolutionary biology spanning a wide range of dimensions (time, space, complexity) and bridging various disciplines: taxonomy, systematics, micro- and macroevolution, environmental science, in-situ biodiversity research and collection-based approaches, and increasingly combining interdisciplinary research with economy, geo-sciences, and politics.



Summary and outlook

The past five years reflect a successful transition from research fields focusing on systematics and taxonomy towards unravelling principles and processes of plant evolution studied at high spatio-temporal resolution. We are now successfully combining and integrating our expertise in taxonomy and systematics with NGS methods and genome-aided evolutionary analyses to unravel aspects and principles of plant evolution such as parallel and convergent evolution of traits and characters in various target species on different taxonomic levels. The Brassicaceae family is our model system to address fundamental questions of plant evolution and adaptation spanning millions of years of evolutionary history. Temporal perspectives of the more recent past and linking micro- and macroevolutionary are exemplified by projects focusing on substrate adaptation (*Dianthus gratianopolitanus*, Cheddar Pink) or interspecies gene flow mediated differentiation and adaptation processes (*Hypericum perforatum*, St. John's Wort).

The past years also demonstrated continued undiminished loss of biodiversity on all scales. Consequently, we continue to develop various research projects to contribute to biodiversity protection and respective transformations of society and human activities. These projects range from locally and regionally species protection actions to landscape genomics. With AgroBioDiv we have started an interdisciplinary (biology, politics, economy) long-term project to contribute scientifically to a successful transformation of agriculture, and this project might serve as an example that we actively and increasingly seek to help stopping biodiversity loss – our primary target in our basic research focusing on species, genera and families.

Research highlights since 2017

During the past five years a major achievement has been the successful implementation of the Brassicaceae family as a model system for a range of evolutionary questions bridging biological systems, levels of complexity and disciplines. The knowledge database system *BrassiBase* is widely recognized and acknowledged. The knowledge database does not only include tools such as barcode aided determination, character and trait examination, cytogenetic information, but provides also information on literature and as key component a worldwide and curator species checklist. Among our more recent contributions this is demonstrated with Nature Comm. (Walden et al. 2020) highlighting this long-term exercise. Angiosperms have become the dominant terrestrial plant group by diversifying for ~145

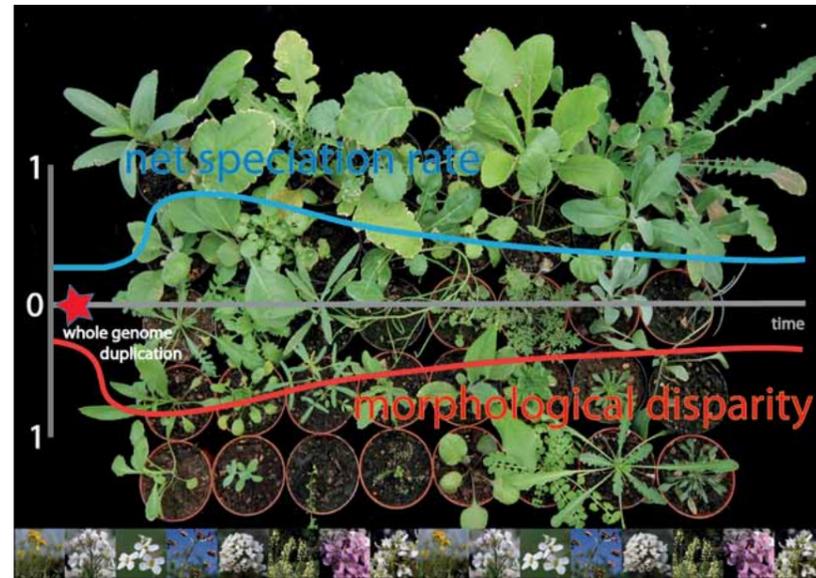


Figure 1
Principles of Crucifer Evolution
Whole genome duplications provide in concert with shifts in species diversification rates a pump towards increased biodiversity while realizing the adaptive potential under changing environments and thereby exploring successfully the relevant morpho-space since more than 30 million years of evolution.

million years into a broad range of environments. During the course of evolution, numerous morphological innovations arose, often preceded by whole genome duplications (WGD). The mustard family (Brassicaceae), a successful angiosperm clade with ~4000 species, has been diversifying into many evolutionary lineages for more than 30 million years. Here we develop a species inventory, analyze morphological variation, and present a maternal, plastome-based genus-level phylogeny. We show that increased morphological disparity, despite an apparent absence of clade-specific morphological innovations, is found in tribes with WGDs or diversification rate shifts. Both are important processes in Brassicaceae, resulting in an overall high net diversification rate. Character states show frequent and independent gain and loss, and form varying combinations. Therefore, Brassicaceae pave the way to concepts of phylogenetic genome-wide association studies to analyze the evolution of morphological form and function. The importance of whole-genome duplication for the evolution of all green life has been demonstrated convincingly, and we were able to contribute with our particular knowledge on order Brassicales accordingly (One Thousand Transcriptome Initiative 2019).

This work has provided the base for our ongoing projects unravelling the genomic basis and consequence of convergent and parallel evolution of traits and characters and thereby addressing the question to what extent phenotypic disparity and eventually plasticity may contribute to adaptative trait evolution. The project is actually being funded by DFG as continuation of our projects embedded in the meanwhile finished DFG priority program SPP1529 (Evolutionary Plant Solutions to ecological challenges) and is well connected with collaborative research efforts e.g. presenting new approaches using inter-species genome-wide association mapping (Kiefer et al. 2019). With Korbinian Schneeberger (Cologne, Munich), heading this association mapping project, we were able to confirm the functionality of this new approach by finding a known key regulator that is responsible for the complexity of leaf shape in plants. We detected an unusual connection between an unexpectedly high mutation of the sequence motif 'CG' to 'TG' and the absence of the gene chromomethylase 3 (CMT3). The absence of this gene leads to missing modifications of the genome – complex, genome-wide methylation patterns–, an important element in the mechanism for adaptation to the environment but also in evolutionary processes such as gene silencing of previously duplicated genes.

We are continuing to add knowledge on breeding system evolution, and we are in particular focusing on interspecies gene flow and poly/diploidization cycles and processes. Among those studies we have shown that asexual reproduction is not necessarily an evolutionary dead end, but that there is often unidirectional and gene-pool dependent evolutionary dynamics allowing diploid persistence, while apomicts are trapped on polyploid level serving potentially as gene-pool reservoir under changing environmental conditions (genus *Hypericum*). Similarly, uni-directional gene flow among *Arabidopsis* species has been further explored and showing the complexity of the genetic basis of breeding system evolution and diversification of the SRK genes and its alleles (S-receptor kinase) shaping the sporophytic incompatibility system in Brassicaceae (Mable et al. 2018). Our results suggest that the highly polymorphic SRK alleles are useful for interpreting evolutionary patterns of gene flow among populations, species and ploidy levels. We have demonstrated that tetraploids show no apparent advantage in terms of allelic or haplotypic repertoire due to more relaxed selection than diploids but that there is increased evidence for introgression among tetraploids from suspected hybrid populations.

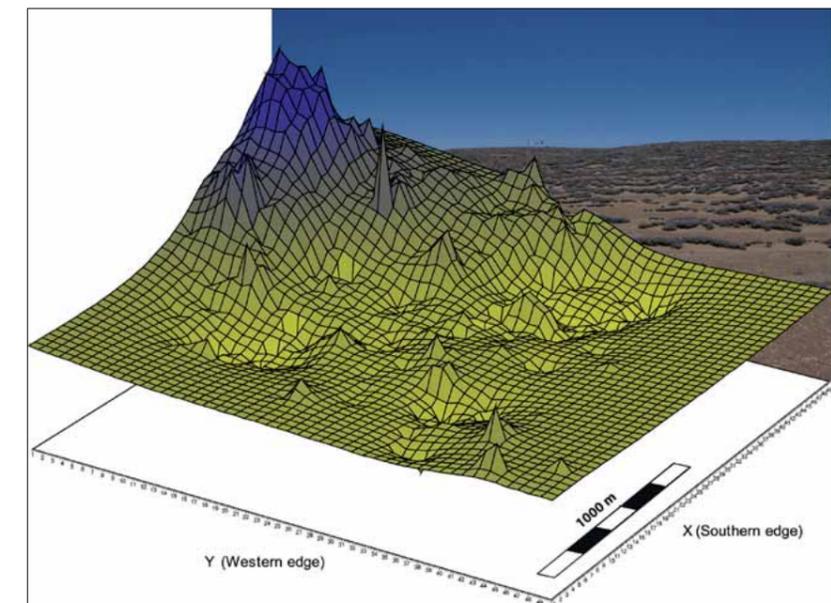


Figure 2
Life at its Dry Limits
Genetic landscape of the terrestrial bromeliad *Tillandsia landbeckii* for the Oyarbide study area in the Atacama Desert in Northern Chile. The background image is a view toward the north-east (increasing altitude and genetic diversity)

We developed our studies in the Atacama Desert further into a major research theme and are contributing since 2020 to the CRC1211 (Earth – Evolution at the dry limit). The unique ecosystem of *Tillandsia lomas* (fog oasis vegetation) is analysed from landscape to individual scale, and the first results showed that metapopulations of *Tillandsia landbeckii* are genetically connected over many hundreds of square kilometers, and despite having a large potential for clonal propagation, genetic diversity is regionally and locally structured. At the landscape level, genetic diversity correlates well with fitness parameters such as growth, flowering, and vegetation density. We also observed fine-scale correlation with a 3-D landscape model indicating a positive feedback with seasonal fog occurrence and availability (Koch et al. 2020), and the project is actually being further developed in a multidisciplinary context.

Future directions

Among our main future research there are three main schemes. Comparative evolutionary genomics in tribes Arabideae and Cochlearieae (DFG) should contribute to our knowledge on parallel and convergent evolution. Life in general, and plants in particular, adapt to a new environment on evolutionary time scales. Studying adaptation is essential if we aim at understanding how life persists in the face of changing environments, a topic of immediate relevance in today's world. The study of adaptation is often limited by a lack of replication and without independent replication it is impossible to determine how much of what we see in nature is particular to a certain example and how much can be regarded as a general result or rule. However, convergent evolution allows us to overcome this fundamental limitation, as this process is nature's own replicate experiment in generating a phenotype and this leads very often to convergent evolution.

On landscape scale we explore the evolutionary dynamics of adaptation in two different settings. Water is the defining feature of the habitable Earth; it is essential for all life as we know it. Evolution of life in extremely water limited environments, which cover significant portions on of the Earth, is not well understood. Akin to life, water-driven processes leave unique marks on the Earth's surface. The Atacama Desert (CRC1211) provides a unique natural laboratory, which we use to explore in-depth together from groups from very different disciplines and focusing on unique ecosystems called fog oasis and members from the bromeliad family. Two projects explore large-scale evolutionary differentiation and adaptation processes in Europe and focusing on the orchid genus *Platanthera* and Cheddar Pink from the carnation family unravelling the importance of various fitness traits (Stiftung Naturschutzfond Baden-Württemberg).

The third major pillar is AgroBioDiv as an interdisciplinary, participatory project, blending expertise in the fields of biology and political science to foster biodiversity within larger landscapes. The project aims to develop integrated strategies and political instruments for increasing biodiversity in Baden-Württemberg through organic agriculture for long-term sustainability. The biodiversity of agricultural fields will be measured and observed with farmers and conservationists in various case study regions. Applying discourse analysis, the problem definition of biodiversity loss will be examined within local political processes through observation of meetings and interviews with decision-makers. The coalitions supporting biodiversity conservation and the strategies they implement to assert its importance in discussion, will provide insight into how biodiversity loss is represented and supported at the political level. Social network analysis will be used to describe and capture key characteristics of these coalitions. With this knowledge, policy packages will be designed to support transition toward organic agriculture in Baden-Württemberg and be presented to stakeholders for reflection and further refinement.



Figure 3

Farmland displaying highest Biodiversity

Arable fields in Southwestern Germany exhibiting extraordinary rich plant biodiversity, which elsewhere most often disappeared and went lost such *Agrostemma githago* (Common corn-cockle) flowering in pink in the foreground (©M.A. Koch, June 2021).

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 52, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 23, Number of citations 2017-2021: 3348 (WOS)

Walden N., German D.A., Wolf E.M., Kiefer M., Rigault P., Huang X-C., Kiefer C., Schmickl R., Franzke A., Neuffer B., Mummenhoff K., **Koch M.A.** (2020) Nested whole-genome duplications coincide with diversification and high morphological disparity in Brassicaceae. *Nature Communications* 11, 3795, doi: 10.1038/s41467-020-17605-7

Koch, M.A., Stock, C., Kleinpeter, D., Del Rio, C., Osses, P., Merklinger, F.F., Quandt, D., and Siegmund, A. (2020) Vegetation growth and landscape genetics of *Tillandsia lomas* at their dry limits in the Atacama Desert show fine-scale response to environmental parameters. *Ecology & Evolution* 10:13260-13274, doi.org/10.1002/ece3.6924.

One Thousand Plant Transcriptomes Initiative (2019) One thousand plant transcriptomes and the phylogenomics of green plants. *Nature* 574: 679-685.

Kiefer, C., Willing, E.-M., Jiao, W.B., Sun, H., Piednoel, M., Hümann, U., Hartwig, B., and **Koch M.A.**, Schneeberger K. (2019) Inter-species association mapping links reduced CG-TG substitution rates to the loss of gene body methylation. *Nature Plants* 5:846-855, doi: 10.1038/s41477-019-0486-9

Mable, B.K., Brysting, A.K., Jørgensen, M.H., Carbonell, A.K.Z., Kiefer, C., Ruiz-Duarte, P., Lagesen, K., and **Koch M.A.** (2018) Adding complexity to complexity: gene family evolution in polyploids. *Frontiers in Ecology and Evolution* 6, e114, doi: 10.3389/fevo.2018.00114.

PROJECT LEADER

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Fields of Interest

Our research is focused on floral developmental and morphological features for understanding relationships between structure, function, systematics, and phylogeny as well as on plant-flower-interactions.

Key words: Flower ontogeny, flower morphology, flower ecology, angiosperm systematics, evolution of the angiosperms, biodiversity, evolutionary



Summary and outlook

As a special item, the sex- and breeding-behaviour of the Sicilian snail-shell bee (*Rhodanthidium siculum*) and their flower visits were studied during several field trips (Erbar & Leins 2017, APIS 11, 317–328). Projects that have been pursued in detail are style diversity in Asteraceae (see under “research highlights”) and diversity of nectaries in angiosperms. The function of nectar is seen in attracting and rewarding insects in the process of pollination. In fly-deceptive pitcher-trap blossoms of *Aristolochia*, however, nectaries on the inner wall of the kettle which provide a small quantity of nectar can be interpreted as an adaptation directing the trapped flies to the exit after pollination and fresh pollen loading (Erbar et al. 2017, Flora 232, 128–141).

Research highlights since 2017

The in-depth research on style morphology and mode of secondary pollen presentation in the family Asteraceae (with regard to function and phylogeny) has been finished in an atlas-like book (Erbar & Leins 2021). The most species-rich plant family is characterized by particularly small pollen portions, which without exception are provided by secondary pollen presentation – obviously one of the key factors for the great evolutionary success of this family in terms of biodiversity (species richness) and distribution in different habitats nearly all over the world. The most important player within the different mechanisms of secondary pollen presentation is the style of the individual flowers.

Firstly, by SEM studies and histological sections, we presented a dataset of style characteristics (detailed morphological descriptions) for altogether 346 genera and more than 580 species, covering all presently accepted subfamilies (14) and tribes (44) within the family. Overall, we identified 49 different style types in the whole of Asteraceae. States of characters are plotted onto up-to-date phylogenetic trees to illustrate and discuss possible evolutionary trends, including ancestral vs. derived states, levels of homoplasy and possible instances of parallel character evolution.

Secondly, we categorized the different possibilities of secondary pollen presentation found in Asteraceae into eight mechanisms: deposition/simple brushing mechanism, brushing mechanism, pump mechanism (with blocking hairs), pump mechanism (with apical thickening), special pump mechanism, special brushing mechanism, combination of pump and brushing mechanism, combination of pump and slightly brushing mechanism.

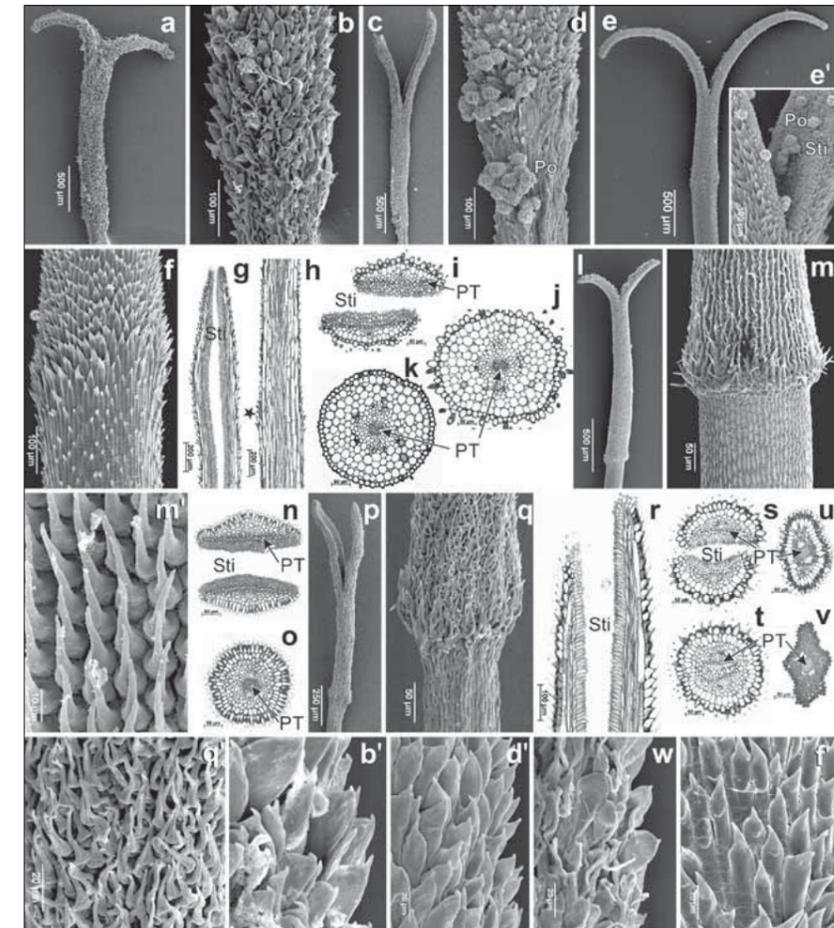


Figure 1
Fig. 36 from Erbar & Leins 2021: Style apices (SEM) and histological sections in Vernoniaceae-Arctotideae-Gorteriinae. Arctotis-style type. a–b: *Berkheya armata*; c–d: *B. cuneata*; e–k: *B. purpurea*; star marks the less conspicuous collar of hairs; l–o: *Gazania splendens*; p–v: *Gorteria corymbosa*; w: *Berkheya decurrens*. Po = pollen grains, PT = pollen tube transmitting tissue, Sti = stigma.

Asteraceae have a large spectrum of different pollinators such as beetles, bees, wasps, flies, butterflies, and birds. Insect pollinators of *Asteraceae* seem not to be very selective, since they may run across the capitula and appear to pollinate indiscriminately. Correlated with this behaviour, the high number of pollen application sites to the pollinator leads to most species of *Asteraceae* being generalists (in terms of flower/pollination ecology). This can lead to a considerable loss of pollen, since some of these flower-visiting insects may be unreliable concerning the repeated visit of the same plant species. Nevertheless, loss of pollen through „vagabonds“ can be reduced by limiting the amount of pollen offered by a flower at a time by the mechanisms of secondary pollen presentation (pollen portioning). The atlas finishes-off with two colourful plates of *Asteraceae* capitula and their various insect pollinators.

Future directions

Nectar secretion already early in angiosperm evolution raises the question about the driving force behind the development of nectaries. Since nectar-secreting tissues show some variation in location and histological structure, these characters can be used in systematics. Research is almost finished in Rosaceae (a morphologically heterogeneous family with a uniform nectary, namely a hypanthial one, though quite diverse as regards size, proliferation, and cuticular striation of the epidermis). In the near term, our research on nectaries will concentrate on Brassicaceae. The insect-pollinated flowers of Brassicaceae exhibit a receptacular nectary of the mesophyllary type. In Brassicaceae, the receptacular nectaries show a high diversity as regards number and position as well as shape and size. We want to characterize the tribes by their nectaries (SEM). On the other hand, we want to trace some possible trends, which occurred several times in parallel and for the underlying evolutionary constraint.



Figure 2
Fig. 113 from Erbar & Leins 2021: A selection from the broad spectrum of insect pollinators (bees, wasps, butterflies, moths) on the capitula of Asteraceae.

Another focus is a book on vegetables. With the question of what we eat, the morphology and anatomy of vegetables (roots, shoots, leaves) will be analysed, the whole garnished with cooking recipes (Leins & Erbar).

Finally, the “Hühnerstein project: colonisation of a seed-sterile area” (initiated by Peter Leins) will be summarized after 20 years.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 10, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 10, Number of citations 2017-2021: 364

Erbar, C., and Leins, P. (2021). Style diversity in Asteraceae: morphology, anatomy, phylogeny, and function. *Bibliotheca Botanica* 163, 1–260.

Erbar, C. (2020). *Pelucha trifida* – a case study in Asteraceae-Astroideae on the value of stylar characters analysed in detail. *Plant Div. Evol.* 132, 57–86.

Erbar, C., Eichelser, C., and Mögerle, S. (2018). Styles in Carduoideae (Asteraceae) - diversity in the uniformity. *Plant Div. Evol.* 132, 1–42.

Leins, P., Fligge, K., and **Erbar, C.** (2018). Silique valves as sails in anemochory of *Lunaria* (Brassicaceae). *Plant Biol.* 20, 238–243.

Erbar, C., Heiler, A., and Leins, P. (2017). Nectaries in fly-deceptive pitcher-trap blossoms of *Aristolochia*. *Flora* 232, 128–141.

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Fields of Interest

The focus of my scientific and research interest lies on an understanding of the gene regulatory programs underlying plant sexual and asexual reproduction through seeds (apomixis), with an emphasis on the roles of RNA helicases and aspects of their evolution.



Summary and outlook

Reproduction and seed development are important steps in the live cycle of higher plants. Apart from sexual reproduction, also asexual reproduction through seeds (apomixis) has been described in ~400 species. In contrast to sexual reproduction, apomixis leads to formation of clonal offspring. This is scientifically greatly interesting, and it generates a high potential for agricultural applications. However, for targeted manipulation of reproduction and for engineering of apomixis in sexual crop species, a precise understanding of the underlying regulatory mechanisms is required. Increasing evidence suggests that RNA helicases are crucial regulators acting during formation and development of reproductive lineages (germlines). A detailed understanding of their roles can give fundamentally new insights into the molecular mechanisms governing germline development and seed formation.

Research Highlights since 2017

To gain new insights into the gene regulatory programs controlling plant sexual and apomictic reproduction, we combine different approaches to study (I) gene expression in reproductive cell and tissue-types at high spatial and temporal resolution (Zühl et al., 2019), (II) functional roles of regulators of germline development, in particular selected RNA helicases (Stein et al., 2021), and (III) aspects of their evolution (Kiefer et al., 2020).

Investigations on gene regulatory programs controlling development of the female germline in higher plants are especially challenging, as the cells of interest are small, rare abundant and tightly enclosed by sporophytic flower tissues (reviewed in Schmidt, 2020). To overcome this technical limitation, cell and tissue type-specific analysis by combining laser assisted microdissection (LAM) with transcriptional profiling is a state of the art approach. Using LAM combined with RNA-Sequencing, we recently conducted a comprehensive transcriptome analysis of reproductive tissues from different sexual and apomictic *Boechera* accessions (Zühl et al., 2019). This allowed us to propose new candidates for germline specification in apomicts. In agreement with previous findings, activities of RNA helicases were found to be enriched during germline specification, suggesting their importance for reproductive development. In this line, using targeted sequencing of ~60 RNA helicase from 24 sexual, facultative apomictic, or apomictic *Boechera* accessions, we demonstrated that coding sequences of RNA helicases remain largely conserved even in apomicts (Kiefer et al., 2020). This observation challenges the longstanding hypothesis

on accumulation of deleterious mutations in apomicts and further points to crucial roles of RNA helicases in regulation of fundamental cellular and developmental processes (Kiefer et al., 2020). Strikingly, we could demonstrate that mutant alleles of a single RNA helicase are sufficient to lead phenotypes resembling different elements of apomixis in sexual *Arabidopsis*, including aspects of autonomous seed development (Stein et al., 2021). Based on differential expression in reproductive tissues from *Boechera*, we identified additional RNA helicases as promising candidates to determine aspects of sexual versus apomictic germline development (unpublished). Interestingly, these include homologs of genes previously identified as highly enriched during reproduction in *Arabidopsis thaliana* (Figure 1; Wüst et al., Curr. Biol. 2010). Taken together, this provides new insights into the gene regulatory control underlying plant reproduction and the importance of RNA helicases in these processes.

Future directions

In future, I aim to continue my line of research and to focus on the gene regulatory pathways and molecular mechanisms governing plant reproduction. A major focus will remain on the investigation of RNA helicases. Detailed investigations on the regulatory processes and molecular machineries involving selected members of this gene family are foreseen. This is of great interest not only to contribute to an understanding of apomixis, but also to elucidate the impact of environmental conditions on reproductive success and seed set in plants. Apart from being crucial for reproductive development, RNA helicases are known to mediate responses to stress conditions. As RNA helicases represent an ancient and evolutionary conserved gene family, the investigations will further allow to gain novel

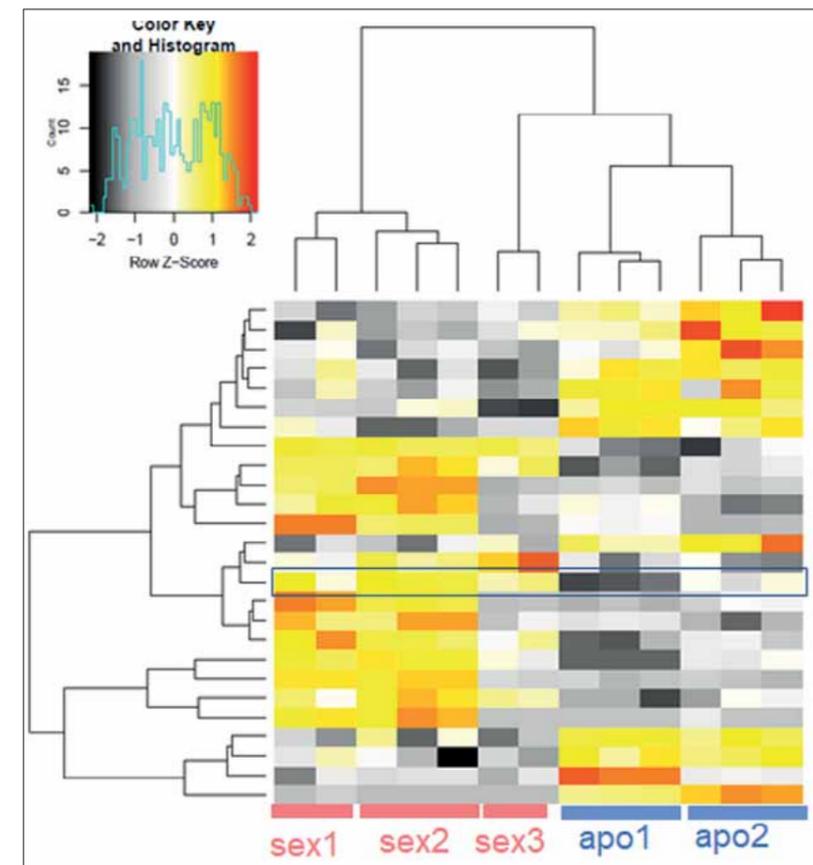


Figure 1
Heatmap of log₂-scale normalized read counts. Genes differentially expressed in mature ovules from 3 sexual and 2 apomictic *Boechera* accessions. Homologs of genes enriched in *Arabidopsis* mature gametophytes are shown (Wüst et al., Curr. Biol. 2010). The box designates a candidate RNA helicase. Hierarchical clustering of samples and genes was based on euclidean distance and hierarchical agglomerative clustering. Colors are scaled per row.

insights into fundamental mechanisms involved in the regulation of gene activity and the maintenance of genome integrity. New directions planned aim at the identification of interaction partners and targets of candidate genes to improve our understanding of the regulatory machineries and networks controlling reproduction. In longer terms, this can provide a basis for targeted manipulation of reproductive development and improvement of crop seed formation.

Selected publications since 2017

Number of peer reviewed articles 17-21: 5, First, last or corresponding author: 3, Numbers of citations 17-21: 422

Stein RE, Nauwerth BH, Binmöller L, Zühl L, Loreth A, Reinert M, Ibberson D, **Schmidt A.*** (2021). RH17 acts in repression of reproductive fate and autonomous seed coat development in Arabidopsis. *Development*, dev.198739.

Kiefer, M., Nauwerth, B.H., Volkert, C., Ibberson, D., Loreth, A., and **Schmidt, A.** (2020). Gene function rather than reproductive mode drives the evolution of RNA helicases in sexual and apomictic *Boechera*. *Genome Biol Evol.* 12, 656-673.

Schmidt A. (2020). Controlling apomixis: Shared features and distinct characteristics of gene regulation. *Genes* 11, 329.

Zühl, L., Volkert, C., Ibberson, D., and **Schmidt, A.** (2019). Differential activity of F-box genes and E3 ligases distinguishes sexual versus apomictic germline specification in *Boechera*. *J. Exp. Bot.* 70, 5643-5657.

Zhao, X., Bramsiepe, J., Van Durme, M., Komaki, S., Prusicki, M.A., Maruyama, D., Forner, J., Medzihradzky, A., Wijnker, E., Harashima, H., Lu, Y., **Schmidt, A.**, Guthörl, D., Logroño, R.S., Guan, Y., Pochon, G., Grossniklaus, U., Laux, T., Higashiyama, T., Lohmann, J.U., Nowack, M.K., and Schnittger, A. (2017). RETINOBLASTOMA RELATED1 mediates germline entry in Arabidopsis. *Science* 28(356), 6336.

Kotlinski, M., Knizewski, L., Muszewska, A., Rutowicz, K., Lirski, M., **Schmidt, A.**, Baroux, C., Ginalski, K., and Jerzmanowski, A. (2017). Phylogeny-based systematization of Arabidopsis proteins with histone H1 globular domain. *Plant Physiol.* 147(1): 27-34.





2.15 PROF. DR. URSULA KUMMER MODELLING OF BIOLOGICAL PROCESSES

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Fields of Interest

Developing and applying computational methodology to study regulation, information processing and dynamics of cell biological processes.



Summary and outlook

The department "Modeling of Biological Processes" focuses on the development of methods for the modeling, simulation and computational analysis of biological systems, as well as the application of these methods to answer biological questions. Among the biological questions core to the department are the processing of information in the cell and the importance of dynamics in individual processes. Both questions are often interlinked, e.g. as in the prominent example of calcium oscillations in calcium signal transduction. The developed methods are integrated in the software COPASI which is of widespread use in the international community (see Figure) and is developed between the groups of Pedro Mendes and Stefan Hoops (both USA), as well as the two groups of the department "Modeling of Biological Processes" - Sven Sahle's and Ursula Kummer's.



Figure 1
Location of authors publishing research results performed with the aid of COPASI.

Research Highlights since 2017

Within the SFB 1101 and in collaboration with the group of Karin Schumacher, a first integrative computational model of the pH homeostasis in plants has been developed. The model allowed to falsify or verify potential hypothesis for additional mechanisms playing a role in vacuolar pH beyond the V-ATPases (Holzheu et al., 2021). In an additional project in the same SFB and in collaboration with the group of Klaus Harter (University of Tübingen) we developed a computational model to study the dynamics and regulation of the fast brassinosteroid signalling pathway. For this purpose we first clarified the interaction partners of BIR3 using molecular modeling (Großholz et al., 2020, see Figure). The re-

sults were integrated in the computational pathway model consisting of ordinary differential equations comprising the brassinosteroid induced hyperpolarization of the membrane as well as the acidification and subsequent swelling of the cell wall. Utilizing this model we demonstrated that the signaling output of the fast brassinosteroid signaling pathway depends on the concentration of H⁺-ATPases in the cell membrane, which we verified experimentally by measuring the response to brassinosteroid stimulation in the meristematic zone. We further showed that an influx of cations is required to balance the shift of charges caused by the acidification of the cell wall. Finally, we further specified the role of the negative regulator BIR3, which can fine-tune the signaling output in silico (Großholz et al., 2021).

In another collaboration within COS, we studied the distribution of metabolic control in sulfur assimilation in *Arabidopsis*. With the aid of computational modeling we were able to show that the metabolic control is not statically distributed in this pathway, but depends on the environmental conditions (Feldman-Salit et al., 2019).

Following the earlier observation that calcium oscillations in fish liver cells are amplitude encoded (collaboration with Thomas Braunbeck, COS) which is in contrast to basically all known mammalian systems which are frequency encoded, we systematically studied and characterized the properties of amplitude encoded vs frequency encoded calcium oscillations with respect to properties of information processing. We found that frequency encoding is more versatile, but also more sensitive e.g. to temperature changes of the system (Aguilera et al., 2019).

In addition, we developed methods for integrating cellular models of e.g. signal transduction with whole-body pharmacokinetic models and applied this methodology to the medical use of interferon injection (during hepatitis C infection) predicting the intracellular interferon signalling response in hepatocytes. Thus, we were able for the first time to integrate these different layers successfully (Kalra et al., 2019)

Further on the method side, we developed new algorithms to fit data distributions which allows to simulate cell-cell variability and the usage of e.g. FACS data in a straightforward manner. This approach was used e.g. when deciphering the mechanism behind the bi-modal IRF7 distributions often observed in interferon signalling (Aguilera et al., 2017). An API for the software COPASI has been developed, among other features, that allows easy integration of software features into workflows etc.

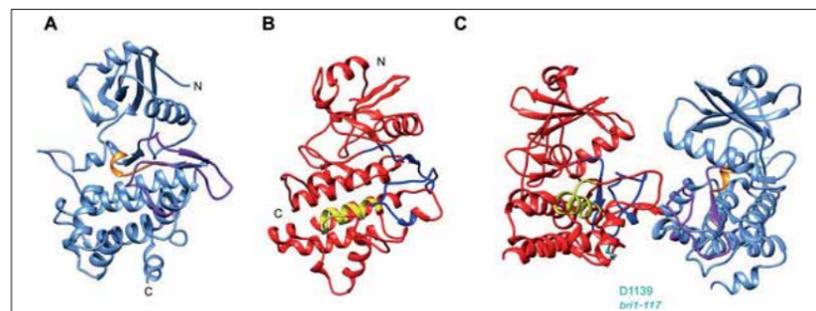


Figure 2
BIR3 interacts with BAK1 and BRI1 (A) Modeled structure of BIR3. (B) Favored complex between the BIR3 structure and BAK1 calculated by webSDA. (C) Favored complex between BIR3 and BRI1 calculated by webSDA. Color code: BIR3 (green); BAK1 (blue), catalytic domain (orange) (adapted from Großholz et al., 2020).

Future directions

Within the SFB 1101 we want to extend the computational model of the brassinosteroid response to directly compute cellular elongation. In addition, an agent-based spatial model of the elongating root is currently finalized. This model will then be able to represent brassinosteroid signalling in each root cell and depending on the cellular location within the root, swelling and elongation of the individual cells which results in overall elongation. In the project with Karin Schumacher and following the modeling of the pH homeostasis of the vacuole, we are now aiming at also modeling the pH homeostasis of the TGN.

On the method side, we are currently developing methods to optimize parameters of kinetic models in such a way that certain dynamic states of the model (e.g. oscillations, complex dynamics) can be found easily in parameter space. In addition, we are developing methods to integrate proteomic and transcriptomic data into whole-genome scale metabolic models and investigate the solution space of these models.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 15, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 9, Number of citations 2017-2021: 1336 (Web of Science)

Grigaitis P, Starkuviene V, Rost U, Serva A, Pucholt P and **Kummer U.** (2020) miRNA target identification and prediction as a function of time in gene expression data. *RNA Biol.* 17(7):990-1000.

Großholz R, Feldman-Salit A, Wanke F, Schulze S, Glöckner N, Kemmerling B, Harter K and **Kummer U.** (2020) Specifying the role of BAK1-interacting receptor-like kinase 3 in brassinosteroid signaling. *J Integr Plant Biol.* 62(4):456-469.

Aguilera L, Bergmann FT, Dalmaso G, Elmas S, Elsässer T, Großholz R, Holzheu P, Kalra P **Kummer U,** Sahle S and Veith N. (2019) Robustness of frequency vs. amplitude coding of calcium oscillations during changing temperatures. *Biophys Chem.* 245:17-24.

Feldman-Salit A, Veith N, Wirtz M, Hell R and **Kummer U.** (2019) Distribution of control in the sulfur assimilation in *Arabidopsis thaliana* depends on environmental conditions. *New Phytol.* 222(3):1392-1404.

Kalra P, Brandl J, Gaub T, Niederal C, Lippert J, Sahle S, Küpfer L, and **Kummer U.** (2019) Quantitative systems pharmacology of interferon alpha administration: A multi-scale approach. *PLoS One.* 14(2):e0209587.

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Fields of Interest

The group focuses on methodological research for mathematical modeling of biological processes. Areas of expertise are kinetic modeling with stochastic simulations and ODEs, spatial kinetic modeling using PDEs, optimization techniques including parameter estimation, and constraint based modeling.



Summary and outlook

Quantitative modelling of biological processes is the core methodological approach in systems biology. Methodological research in this area comprises the development of numerical methods, workflows that make the mathematical methods accessible for researchers with biological rather than computational expertise, and finally software support for these methods.

One of the main long term projects of the group is the software COPASI, a tool for creating, simulating and analysing kinetic models that is developed in an international cooperation. COPASI provides access via a graphical user interface to state-of-the-art methods for stochastic and deterministic simulation, parameter estimation, optimisation, time scale analysis, sensitivity analysis, etc. It is free and open software, and new releases are typically downloaded thousands of times.

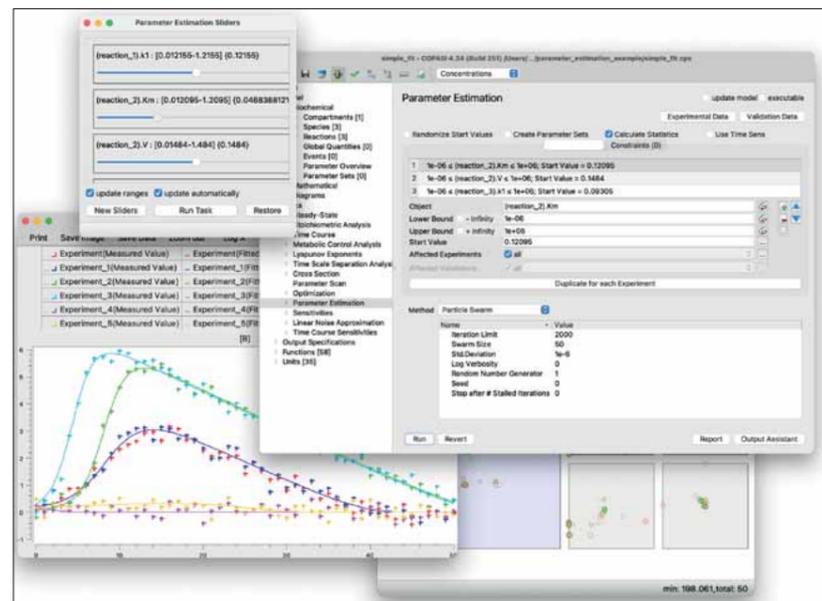


Figure 1
Graphical user interface of COPASI, showing the result of a parameter estimation run and sliders that allow the manipulation of model parameter values.

Research Highlights since 2017

New technological developments in experimental techniques that lead to the availability of new kinds of data imply a need for a corresponding development of new modeling techniques and tools. One such development is that fluorescence imaging has now become a routinely used method that can supply time resolved quantitative data about the spatial distribution of various biochemical compounds, on the tissue level, but also on a single cell level. A possible corresponding modeling framework is kinetic modeling of reaction-diffusion systems using partial differential equations. While the theoretical background of this framework is well established, work flows and software support for biological applications was so far very limited. Supported by a BMBF grant and in collaboration with Prof. P. Bastian (IWR, Heidelberg University) we have, using our experience in the development of widely used and user friendly software, created a simulation tool for spatial models on the single cell level. The tool is open source, provides a graphical user interface, and employs state-of-the-art numerical solvers for partial differential equations. It is publically available (<https://github.com/spatial-model-editor/spatial-model-editor>), was already successfully used in trial projects within our department. The tool allows to either set up spatial models from scratch or to import and convert a non-spatial model as a starting point. The geometrical features can be extracted from microscopy images, and initial condition can also be taken from quantitative fluorescence images. Simulation of the model can be performed with a number of different numerical solvers. More complex workflows can be implemented using an API for scripting languages. From our long standing experience with non-spatial simulation software we anticipate that the availability of userfriendly and powerful software will have a big impact in how imaging data is used in computational modeling of biological processes.

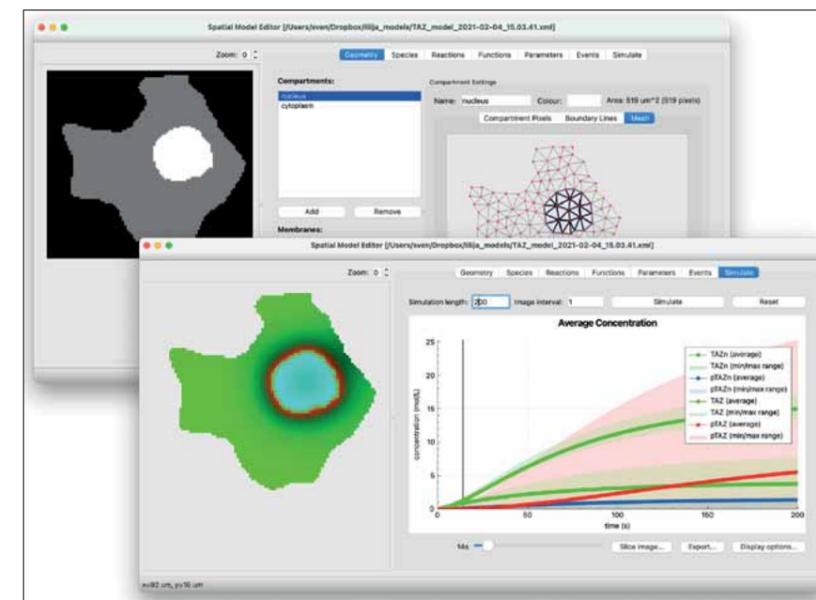


Figure 2
User interface of the spatial model editor, showing the generation of a mesh from a bitmap that was generated from a microscopy image (top) and the display of simulation results (below).

Future directions

There is a continuing need for methods research and software support for new modeling approaches. Building on our existing work for spatial model simulation a workflow for estimating model parameters by comparing simulation results with time resolved imaging data, including software support, will be developed. The aim is to make the full modeling workflow (i.e. encoding a hypothesis in a model, parameterising the model using experimental data, testing and validating the model using experimental data, testing the original hypothesis) available for spatial models in a user friendly way for researchers with biological expertise.

In more general terms, one of the key aims of computational modeling in biology is the creation of more comprehensive models of biological systems that span wide ranges of time scales, complexity, and spatial scales. Examples would be models that combine signaling, gene expression and metabolic networks of cells, or models that describe interactions of internal cell processes with organ/organism level communication processes. These comprehensive models require the coupling of submodels using different modeling frameworks (multiscale modeling). Methodological and software support for these scenarios, e.g. the coupling of constraint based models with kinetic models is a very challenging topic for future research.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 13, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 3, Number of citations 2017-2021: 927

Wehling, L., L. Keegan, J. Schmitt, **S. Sahle**, P. Schirmacher, U. Kummer, and K. Breuhahn (2021). Mathematical modeling of YAP and TAZ nuclear/cytoplasmic shuttling in liver cancer cells. *Zeitschrift für Gastroenterologie* 58(01).

Keating, S.M., Waltemath, D., König, M., Zhang, F., Dräger, A., Chaouiya, C., Bergmann, F.T., Finney, M. et al., (2020). SBML Level 3: an extensible format for the exchange and reuse of biological models. *Molecular Systems Biology* 16(8)

Kalra, P., Brandl, J., Gaub, T., Niederalt, C., Lippert, J., **Sahle, S.**, Küpfer, L., and Kummer, U. (2019). Quantitative systems pharmacology of interferon alpha administration: A multi-scale approach. *Plos One* 14(2).

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

Bergmann, F., S. Hoops, B. Klahn, P. Mendes, J. Pahle, **S. Sahle**, U. Kummer (2017). COPASI and its Applications in Biotechnology. *Journal of Biotechnology* 261, 215–220.





2.16 JUNIOR PROF. DR. STEFFEN LEMKE INDEPENDENT RESEARCH GROUP

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Fields of Interest

Mechanisms of Biological Innovation,
Epithelial Morphogenesis,
Gastrulation



Summary and outlook

Biological innovation has long been of general interest; in the past, it has been mostly associated with a re-wiring of existing developmental transcription factors through the gain or loss or regulatory DNA elements. Using a comparative approach in flies, we have previously shown that similar mechanisms also account for innovation during the first shape-giving stages of development. In the past years we have identified examples of developmental innovation that are driven by alternative mechanisms: we could illustrate how innovation of tissue architecture was established from scratch through the rise of a novel developmental program, or how a new tissue type emerged with changes in cell and tissue mechanics. Our findings illustrate how evolution employs mechanical capacities of cells and tissues that are similarly operational in today's embryos to serve as noise-cancelling mechanisms for imprecise molecular patterning.

Research Highlights since 2017

A key step in the development of metazoan animals is the generation of a simple epithelium, the blastula or blastoderm. For every animal, this epithelium provides the starting material to build the body plan. And for its simplistic yet universal properties it is often used as a simplified model of epithelia in general. The insect blastoderm as well as other epithelia are characterized by notable diversity in cell architecture, which can range from thin and spread-out to tall and slim. These differences are often used characters of tissue types, but little is known about how such distinct epithelial architectures form, function, or evolve. Because the process of epithelia development is particularly well characterized for the blastoderm of the *Drosophila* embryo, we used flies to address innovation in the programs that define epithelial architecture. Very surprisingly, whole-taxon phylogenomics of 172 fly species revealed that the transition from short to tall tissue architecture can be associated with the appearance of a single, newly originated gene, *slam* (A). Novel genes have long been associated with evolutionary innovation. It has been challenging, however, to mechanistically link their appearance to a biological process and an observable phenotypic output. Our access to fly species that represent either a short-cell blastoderm (the midge *Chironomus*, B) or a tall-cell blastoderm (the fruit fly *Drosophila*, C), provided a unique opportunity to experimentally address the cell-biological link between the appearance of a new gene and qualitative differences in cell shape (D). We first asked what would happen to the short cells in *Chironomus* embryos if *slam* was present. We expressed *Drosophila slam* in *Chironomus* and could observe a blastoderm with cells of similar height as in *Drosophila* (E). This indicated that the activity of a single gene alone was sufficient to instruct tissue architecture. To understand how, we turned to the cell biology of actin polymerization (using *Diaphanous* as a reporter, F) and cell-cell adhesion (revealed through E-cadherin, G) and asked how they were re-purposed by the origin of *slam*. Our results

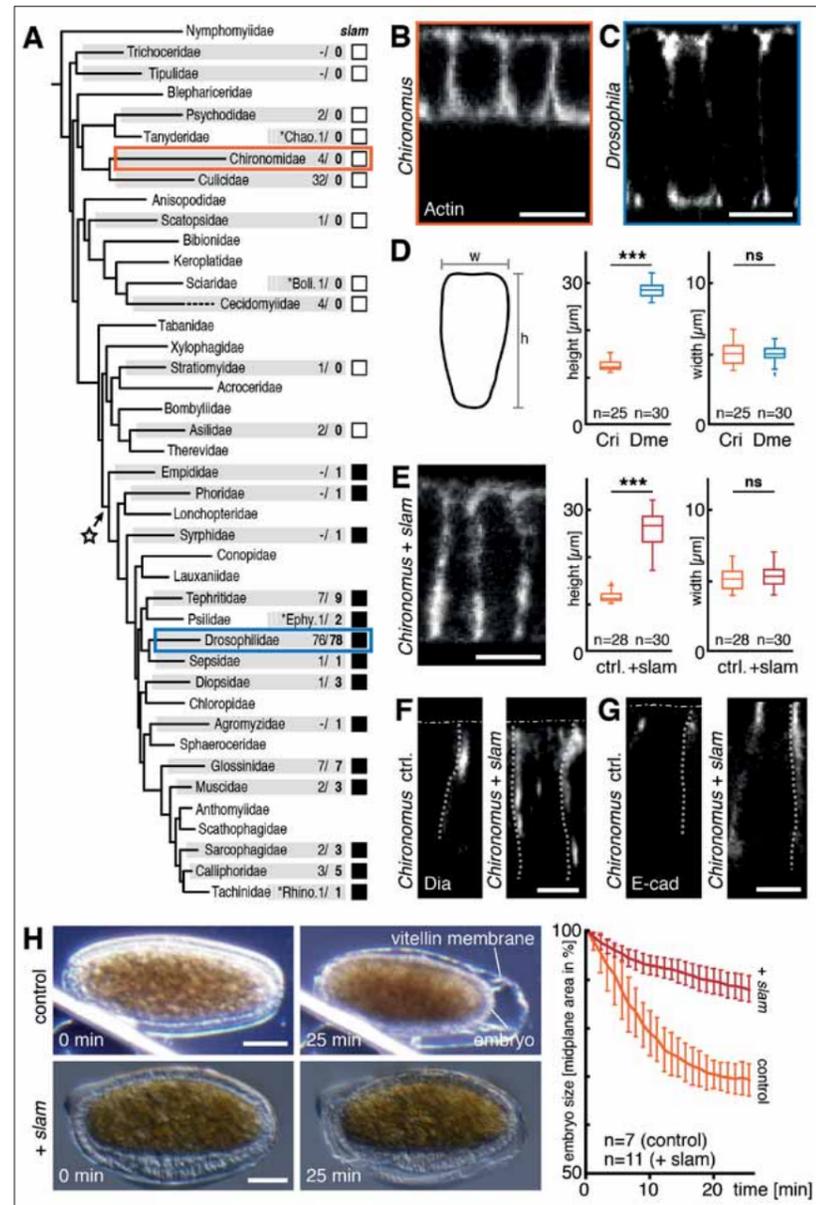


Figure 1
A single new gene transforms
tissue architecture and function

show that *slam* directs basal enrichment of F-actin and E-cadherin and thus drives cell columnarization. These results indicate new uses of an ancient cellular machinery and allowed us to infer a very plausible and general mechanism of how evolution takes advantage of novel genes. This work was concluded by addressing possible adaptive advantages associated with the evolutionary switch from short- to tall-cell epithelia. For this, we tested the function of the blastoderm as a chemical barrier, and we found that tall cells decrease water permeability and thus can serve as protection against embryo desiccation (H), indicating that tissue architecture has a functional importance and provides a basis for selection and adaptation. We have interpreted the surprisingly simple mechanism underlying the origin of a tall-cell blastoderm as an evolutionary ratchet (*slam* and columnar epithelia have been gained once and never lost), with major consequences on how we think more broadly about the evolution of tissue architecture and function. Our work thus provides a

first example that traces the origin of a developmental program across scales and demonstrates how novel phenotypes and functions can arise through novel genes that effectively repurpose an ancient molecular effector machinery.

In a separate line of research, we addressed modulations in tissue-tissue interactions as cause for innovation in tissue properties. Here we took advantage of access to fly species that differ in the formation of a supportive epithelium during embryo development. In the scuttle fly *Megaselia abdita*, such extraembryonic tissues separate from the underlying yolk membrane and spread over the embryo to enclose it in a protective envelope. In *Drosophila*, the extraembryonic tissue is reduced and does not exhibit the ability to spread over the embryo. To address the cell-biological basis for these differences in tissue behavior, we used light sheet microscopy for whole-embryo in toto time lapse recordings, cell- and tissue tracking, and identified a previously unknown level of regulation underlying extraembryonic tissue spreading in *Megaselia*. We could show that proper tissue spreading relied on the activity of a matrix metalloproteinase (MMP-1) in the yolk sac, which severs the connection between yolk sac and extraembryonic epithelium. This matrix metalloproteinase is not expressed in the *Drosophila* embryo. When we mimicked this condition by RNAi knocked down in *Megaselia*, we found extraembryonic tissue spreading impaired, suggesting that altered tissue interaction through loss of MMP-1 activity contributed to the innovation of a non-spreading extraembryonic tissue in *Drosophila*.

Future directions

We have built a simple experimental framework to study mechanical, chemical, and organizational properties of epithelia with various degrees of self-organization. Our experimental setup is based on the natural diversity in the simple and naive epithelial monolayer of early insect embryos, the blastoderm. Using the *Drosophila* blastoderm as a reference of a highly sealed, extremely robust and snap-quick folding epithelium, we have established various blastoderm systems in related fly species as in vivo test tubes with considerable variation in cellular and epithelial self-organization. Our main findings are that local modulation of signal transduction and cytoskeletal organization are prime parameters to change epithelial properties and support the transition from cellular self-organization to the emergence of higher-level tissue properties.

Our work has since continued to confirm the generality of simple switches in the origin of developmental novelty and indicated signaling cascades (GPCR, Wnt, BMP, FGF) and intracellular trafficking (local activation of Rho1, anchors of GAPs and GEFs) as genetic variables to innovate tissue organization and morphogenesis through cytoskeletal remodeling (E-Cadherin, Integrins, F-actin, Microtubules, MyoII). We now know that the formation of the blastoderm is influenced by mechanical and environmental parameters (stiff egg shell, temperature), which appear to be buffered more effectively in *Drosophila* than in most other flies. This has allowed us to extend our initial approach by probing the robustness of epithelia formation and function.

In our planned work, we (1) consider the influence of mechanical crosstalk between epithelia or between epithelia and external scaffolds (egg shell), (2) reveal and quantify short-distance (cell scale) and long-distance (tissue scale) effects on co-occurring events of self-organization and tissue folding, (3) address mechanical effects of cell architecture on epithelial properties and folding dynamics, and (4) aim to distinguish molecular innovations that transform epithelial behavior from those that make existing properties more robust.

Selected publications since 2017

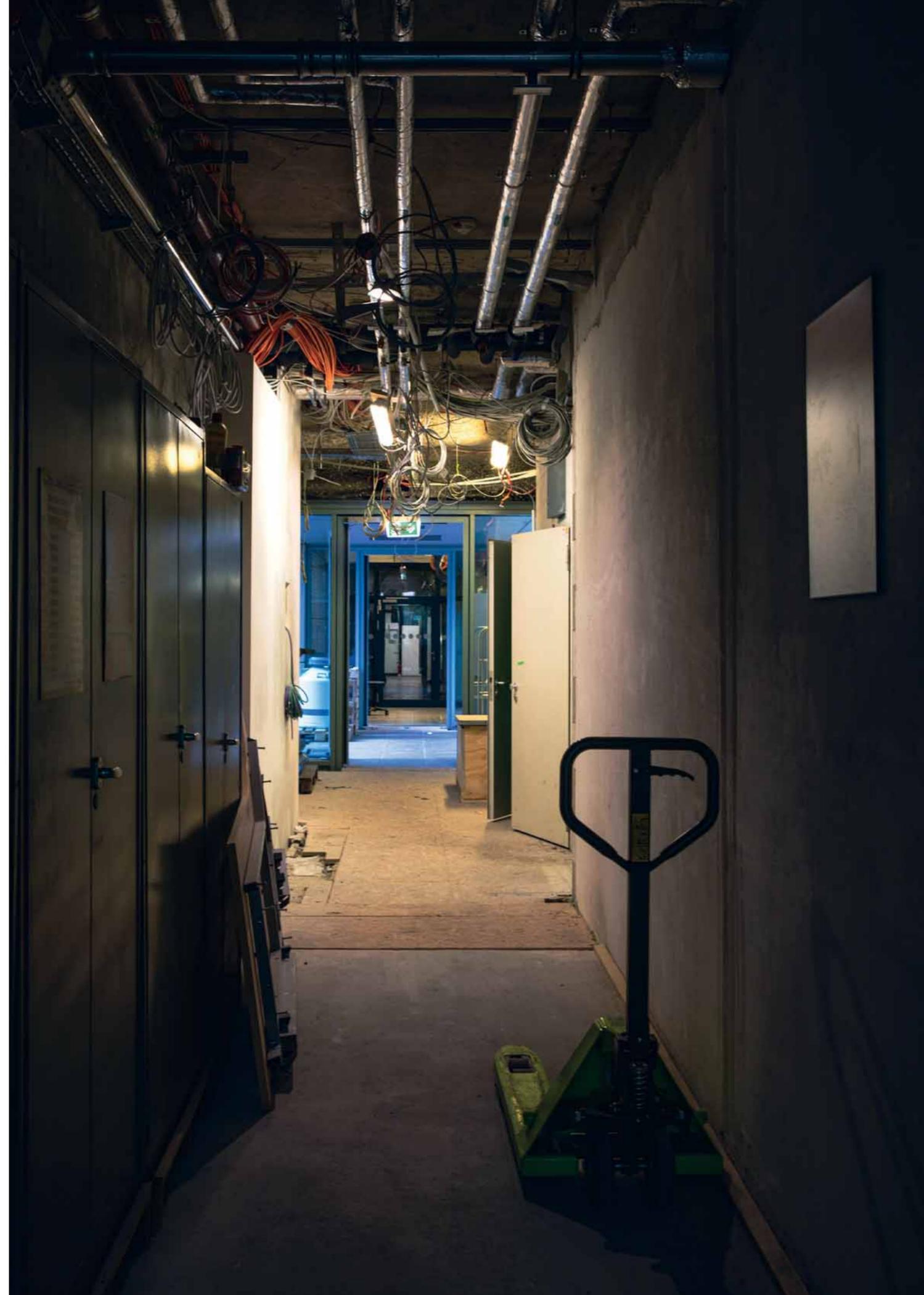
Number of peer-reviewed articles 2017-2021: 3, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 2, Number of citations: 507 (Google Scholar)

Noeske, V, Caglayan, E, **Lemke, S** (2021). Single gene initiates evolution of epithelial architecture and function. *bioRxiv*, <https://doi.org/10.1101/2021.05.04.442636>

Lemke, S, Kale, G, Urbansky (2020). Comparing gastrulation in flies: Links between cell biology and the evolution of embryonic morphogenesis. *MOD*, 164:103648.

Eritano, AS, Bromley, CL, Albero, AB, Schütz, L, Wen, F-W, Takeda, M, Fukaya, T, Sami, MM, Shibata, T, **Lemke, S**, Wang, Y-C (2020). Tissue-Scale Mechanical Coupling Reduces Morphogenetic Noise to Ensure Precision during Epithelial Folding. *Dev Cell*, 53:212.

Caroti, F, González Avalos, E, Noeske, V, González Avalos, P, Kromm, D, Wosch, M, Schütz, L, Hufnagel, L and **Lemke, S** (2018). Decoupling from yolk sac is required for extraembryonic tissue spreading in the scuttle fly *Megaselia abdita*. *eLife*, 7.





2.17 PROF. DR. INGRID LOHMANN DEVELOPMENTAL BIOLOGY

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Fields of Interest

Transcriptional control of development,
gene regulatory networks,
Hox transcription factors, cell plasticity
and maintenance, male Germline stem
cell system, Wnt signaling, Drosophila



Summary and outlook

The Lohmann lab studies on the one hand fundamental principles of transcriptional regulation using Hox transcription factors in the fruit fly *Drosophila* as a model to understand how this class of master-regulators controls cell fate decisions and maintenance during development. On the other hand, the Lohmann lab uses the *Drosophila* testis to elucidate how the balance between stem cell renewal and differentiation is controlled. A particular focus is here on the Wnt signaling pathway, which the lab has identified to be critical in the male germline stem cell system and controls the precise behaviour of the different cell populations in a lineage-specific manner. To tackle all of these questions, the group applies the power of *Drosophila* genetics, high-resolution imaging and approaches from developmental and evolutionary biology as well as genomic, proteomic and single cell methods to resolve the diverse molecular functions of Hox proteins in the embryos and the control of stem cell behaviour in the the male germline stem cell niche.

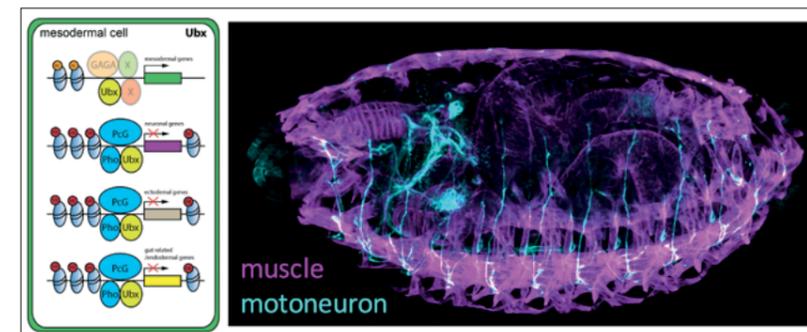


Figure 1
Left: Model of Hox-mediated restriction of cell fates. Right: Late *Drosophila* embryo with the muscles (pink) and motoneurons (blue) highlighted.

Research Highlights since 2017

The development of living organisms is the result of a fine-tuned spatial and temporal expression of genes, which is driven by transcription factors (TFs). Many TFs are expressed in several cell types, and control different transcriptional programs depending on the cell context. However, what multi-lineage TFs control precisely in each lineage and how they can function in such specific manner in different environments remains elusive. We use the Hox TFs, in particular Ultrabithorax (Ubx), to elucidate these fundamental questions in *Drosophila* embryonic mesoderm and nervous system development. Using this model, we

uncovered that Hox TFs, besides their well-described function in specifying regional identities along the anterior-posterior axis, play a major role in the development and diversification of lineages. We found that one of the mechanisms they instruct is the lineage-specific recruitment or stabilisation of the Polycomb complex to Hox chromatin sites for the repression of alternative fate and early cell lineage specification genes. Our results demonstrated that the Hox TF Ubx stabilizes lineage choice by suppressing the multi-potency encoded in the genome in a lineage-specific manner via its interaction with the repressive Polycomb complex. This mechanism may explain why the Hox code is maintained throughout the lifecycle, since it seems to set a block to transdifferentiation in many adult cells thereby ensuring organismal survival. One question arising from this finding is how individual TFs promote transcriptional diversity in different cell types. Using proximity-dependent Biotin Identification (BioID) in *Drosophila*, we identified interactomes of the Hox TF Ubx in three embryonic tissues. We found that Ubx interacts with largely non-overlapping sets of proteins with few having tissue-specific RNA expression. Instead most interactors are active in many cell types, controlling gene expression from chromatin regulation to the initiation of translation. Genetic interaction assays *in vivo* confirmed that they act strictly lineage- and process-specific. Thus, functional specificity of Ubx seems to play out at several regulatory levels and to result from the controlled restriction of the interaction potential by the cellular environment. Thereby, our findings challenge long-standing assumptions such as differential RNA expression as determinant for protein complexes.

Organismal life relies on stem cells, which remain undifferentiated and proliferative, while at the same time producing daughter cells that undergo differentiation. Due to this ability, stem cells are critical for growth and homeostasis, as they can build tissues and organs during development, and replace dying, lost or damaged cells in adult life. Stem cell niches critically control stem cell behavior, however its regulatory input at the whole genome level is poorly understood. We elucidated transcriptional programs of the somatic and germline lineages in the *Drosophila* testis and genome-wide binding profiles of two TFs

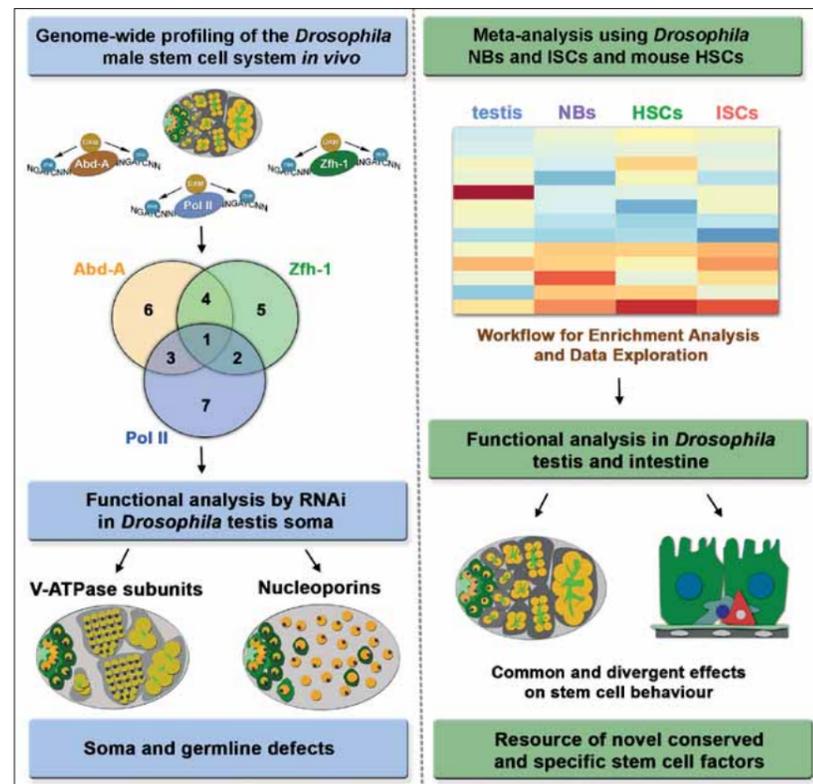


Figure 2
Workflow for the identification of lineage-specific stem cell regulators in the *Drosophila* male Germline stem cell system.

expressed in somatic support cells and crucial for fate acquisition of both cell lineages. We identified key roles of nucleoporins and V-ATPase proton pumps, and demonstrated their importance in controlling germline development from the support side. To make our dataset publicly available, we generated an interactive analysis tool, which uncovered conserved core genes of adult stem cells across species boundaries. We tested the functional relevance of these genes in the *Drosophila* testis and intestine and found a high frequency of stem cell defects. Our dataset and interactive platform represents a versatile tool for identifying novel gene networks active in diverse stem cell types.

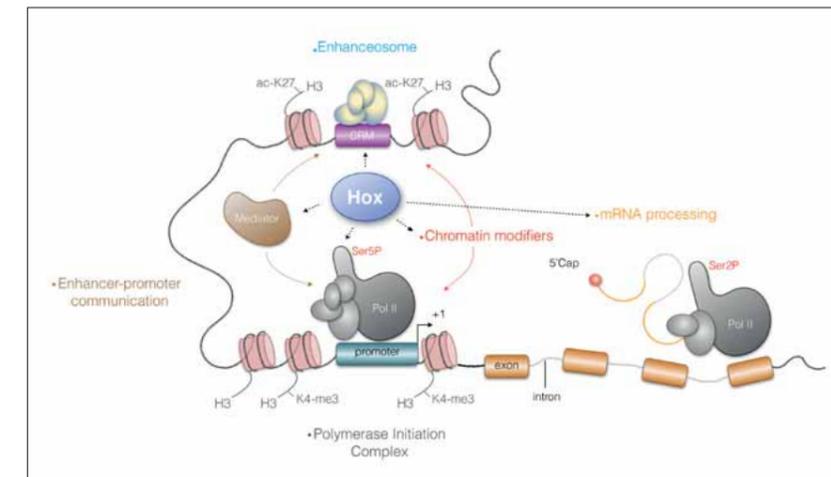


Figure 3
Hox TFs are multi-step players of gene expression acting at diverse levels to control cell specific behaviour and features.

Future directions

Our previous results have shown that the Hox-encoded restriction of cellular and temporal plasticity is required for stably maintaining cell fates throughout the lifetime of a cell. Thus, we hypothesize that the Hox-Polycomb mediated repression is one of the generic functions of Hox proteins to ensure long-term lineage stabilization and maintenance. One fundamental question arising from these findings is how an already committed lineage will develop in the absence of any functional Hox input and whether Hox-free cells are more plastic than Hox-expressing cells. We will tackle these questions by analyzing how a Hox-free cellular environment affects lineage development and whether cells “stripped” of their Hox code are more plastic and can thus more easily change their identity when stimulated than cells expressing the inherent Hox code.

Building on our recent findings that Ubx interacts in a lineage restricted fashion with components of the chromatin remodelling Brahma complex, we will now explore the possibility that Hox TFs pioneer chromatin. In order to rigorously test our hypothesis, we will resolve the modes and modalities of Hox binding, the sequence requirements and the role of other (pioneer) TFs. To this end, we will employ state of the art genomic techniques, which will allow us to simultaneously probe TF chromatin binding and the local chromatin environment *in vivo*, without the need of binding site predictions, as well as complementing biochemical and genetic approaches. We will use the Ubx and its lineage-specific interaction partners Tin and Grh as well as the generic interaction partner Exd, which has been shown to increase Hox binding to closed chromatin, as models, and will study their role in chromatin opening using *in vitro*, *in cellulo* and *in vivo* approaches.

And finally, we will test our hypotheses that Wnt signaling output in neighboring stem cell populations is controlled in a context-dependent manner by a specific Wnt code driving different responses in individual cells, a cross-talk of Wnt signaling activity with other signaling pathways modulating their activities and the induction of specific Wnt output effectors resulting in a differential behavior of (stem) cells.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 10, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 7, Number of citations 2017-2021: 213

Domsch, K., Schröder, J., Janeschik, M., Schaub, C. and **Lohmann, I.** (2021). The Hox transcription factor Ubx ensures myogenesis by suppressing the mesodermal master regulator Twist. *Cell Reports* 34(1):108577. doi: 10.1016/j.celrep.2020.108577.

Carnesecchi, J., Sigismondo, G., Domsch, K., Baader, C.E.P., Rafiee, M.R., Krijgsveld, J. and **Lohmann, I.** (2020). Multi-level and lineage-specific interactomes of the Hox transcription factor Ubx contribute to its functional specificity. *Nature Communications* 13;11(1):1388. doi: 10.1038/s41467-020-15223-x.

Sorge, S., Theelke, J., Yildirim, K., Hertenstein, H., McMullen, E., Müller, S., Altbürger, C., Schirmeier, S., **Lohmann, I.** ATF-induced Warburg metabolism drives over-proliferation in *Drosophila*. (2020). *Cell Reports* 9;31(7):107659. doi: 10.1016/j.celrep.2020.107659.

Domsch, K., Carnesecchi, J., Disela, V., Friedrich, J., Trost, N., Ermakova, O., Polychronidou, M. and **Lohmann, I.** (2019). The Hox Transcription Factor Ubx stabilizes Lineage Commitment by Suppressing Cellular Plasticity. *eLife*, doi: 10.7554/eLife.42675

Tamirisa, S., Papagiannouli, F., Rempel, E., Ermakova, O., Trost, N., Zhou, J., Mundorf, J., Brunel, S., Ruhland, N., Boutros, M., Lohmann, J. U., and **Lohmann, I.** (2018). Decoding the regulatory logic of the *Drosophila* male stem cell system. *Cell Reports* 24, 3072-3086.





2.18 PROF. DR. JAN LOHMANN STEM CELL BIOLOGY

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Fields of Interest

We are interested in various aspects of plant stem cell regulation and its plasticity, as well as how context dependent genetic networks drive cell function, development and morphogenesis.



Summary and outlook

The main thrust of the lab is to elucidate how stem cell containing tissues develop and respond to the environment, using *Arabidopsis* as a model. Plant stem are embedded in specialized structures called meristems, which provide a local environment that regulates the homeostasis between proliferation and differentiation. One major focus of our work is the homeodomain protein WUSCHEL (WUS), which induces stem cell fate in the shoot meristem. My lab has made important contributions to understanding WUS function, how it is connected to hormone signaling systems, most notably cytokinin and auxin and how the entire system is modulated by the environment. In recent years, my lab has focused on elucidating the mechanisms underlying WUS activity by studying the unusual DNA binding behavior and cell-to-cell mobility of WUS protein (Sloan et al, Nature Communications 2020; Fuchs et al. in preparation), and elucidating its connection with auxin signaling (Ma et al.; Nature Communications 2019). Another important line of research focused on the role of the environment for cell type specification using the HEC transcription factors and the TOR kinase as models (Gaillochet et al. eLife 2017; Gaillochet et al. Plant Journal 2018; Janocha et al. in preparation). Finally, we have initiated a new line of research with ERC funding aimed at elucidating context dependent genetic networks underlying tissue development and we were able to publish first results (Liu et al. Molecular Plant 2021).

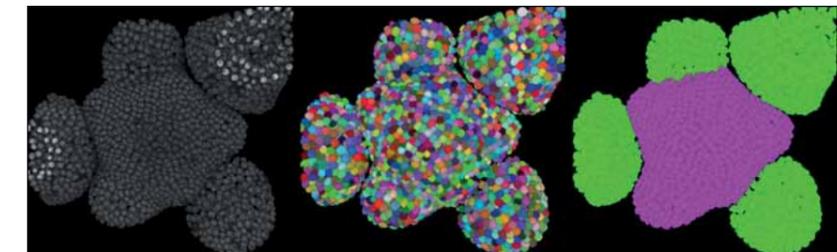


Figure 1
Computational image analysis of image stacks derived by live cell imaging of a shoot meristem. Left panel: Confocal image of fluorescently labelled nuclei. Central panel: output from automated nuclear segmentation. Right panel: Computational identification of shoot meristem (pink) and developing flowers (green).

Research Highlights since 2017

Plant meristems are home to continuously active stem cells, which are controlled by developmental and environmental signals and only daughter cells that exit the stem cell domain acquire the competence to differentiate. In the past years, work in our lab has shed light on the mechanisms involved in the specification of apical stem cells in *Arabidopsis*, the regulatory programs allowing them to communicate with their niche, and how cellular parameters, such as cell size and shape contribute to shoot morphogenesis. Importantly, in the context of an ERC synergy grant, we have established a new line of research aimed

at decoding context dependent genetic networks using the *Arabidopsis* root tip as a model. Using a combination of biochemical and structural methods, we have deciphered the complex DNA binding behavior of WUSCHEL (WUS), a conserved homeodomain transcription factor, essential for the maintenance of stem cells in the shoot apical meristem of many plants. WUS has been reported to bind to diverse DNA motifs and to act as transcriptional activator and repressor. However, the mechanisms underlying this remarkable behavior had remained unclear. In the reporting period, we quantitatively delineated WUS binding to three divergent DNA motifs and resolved the relevant structural underpinnings by x-ray crystallography. We were able to show that WUS exhibits a strong binding preference for TGAA repeat sequences, while retaining the ability to weakly bind to TAAT elements. We found this behavior to be attributable the formation of dimers through interactions of specific residues in the homeodomain that stabilize WUS DNA interaction. Our results therefore provide a first mechanistic basis for dissecting WUS dependent regulatory networks in plant stem cell control (Sloan et al. Nature Communications 2020).

Another long-standing interest of our lab is to decipher the regulatory programs that allow stem cells to communicate with their niche. In the apical stem cell system of plants, local accumulation of the small, highly mobile phytohormone auxin triggers differentiation while at the same time, pluripotent stem cells are maintained throughout the entire life-cycle. We now found that stem cells are resistant to auxin mediated differentiation, but require low levels of signaling for their maintenance. We were able to demonstrate that the WUS transcription factor confers this behavior by rheostatically controlling the auxin signaling and response pathway via direct promoter interaction of a large array of target genes. Finally, we could show that the rheostatic behavior is mechanistically rooted in the ability

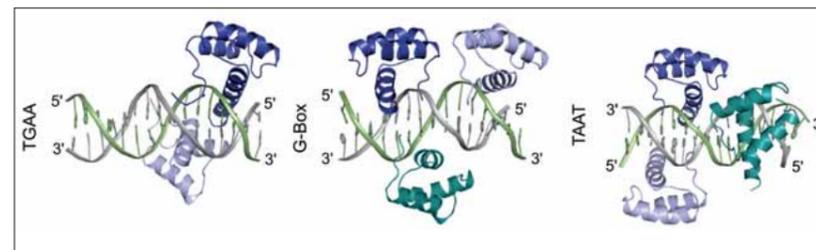


Figure 2
Crystal structure of the WUS homeodomain bound to three divergent DNA motifs. Left panel: TGAA repeat. Central panel: G-Box. Right panel: TAAT containing probe. Taken from Sloan et al. Nature Communications 2020.

of WUS to act via regulation of histone acetylation at target loci, including those with functions in the auxin pathway (Ma et al. Nature Communications 2019). In another study into this direction, we used an integrated approach of inducible genetics, live cell imaging and mathematical modeling to elucidate the role of HECATE (HEC) genes in regulating developmental trajectories of shoot stem cells. Our work revealed that HEC function stabilizes cell fate in distinct zones of the shoot meristem thereby controlling the spatio-temporal dynamics of stem cell differentiation. Importantly, this activity is concomitant with the local modulation of cellular responses to cytokinin and auxin and demonstrated for the first time that the temporal progression of cell fates is regulated independently from positional identity (Gaillochet et al. eLife 2017). Importantly, we were able to follow up on this work by identifying a highly complex network of interacting transcription factors for HEC1, which sketched out a conceptual framework how context dependent transcriptional activities could be realized in the shoot (Gaillochet et al. Plant Journal 2018).

An important new direction for our lab is represented by the interdisciplinary DECODE ERC synergy project, which is aimed at elucidating the genetic architecture of dynamic tissues with single cell resolution. The team consists of two computational groups, Wolfgang Huber (EMBL) and Oliver Stegle (DKFZ), as well as two groups with focus on experimentation, Michael Boutros Uni-HD and DKFZ) and myself. The aim of the project is to reconstruct genetic networks with single cell resolution through computational approaches based on data generated by inducible genetic perturbations with phenotyping by single cell RNA-seq and time-resolved live cell imaging. By working on two model systems, namely *Drosophila*

and *Arabidopsis*, we can leverage experimental strengths and are in a position to carry out comparative analyses. We were able to transfer some of our foundational work in establishing scRNA-seq in *Arabidopsis* roots to rice and this collaboration with the lab of Xiaofeng Gu (CAS, Beijing) resulted in the publication of the first single cell expression atlas in this important crop species (Liu et al. Molecular Plant 2021).

Future directions

Building on our recent findings and leveraging technological developments we aim to develop our research along the following lines:

1. Mechanisms of stem cell induction and maintenance

We are working on translating our knowledge on WUS structure and DNA binding into biological understanding. To this end we have created *Arabidopsis* lines to test the functional potential of WUS variants *in vivo*. In addition to phenotypic and cellular analyses, we will record *in vivo* chromatin binding to derive a mechanistic understanding of WUS regulatory potential. Complementing these approaches, we have developed tools to interrogate single cells of the shoot for their specific response to WUS, both in loss- and gain-of-function settings. These experiments will reveal the cellular heterogeneity in response to stem cell induction and give insights into the complexity of WUS-cofactor combinations. We have systematically screened for WUS interactors, which together with single cell expression data, will allow us to reconstruct biochemically instructed regulatory networks. Finally, we have started to investigate the possibility that WUS acts as an RNA binding protein and will expand this line of investigation in the future.

2. Integration of hormonal, transcriptional and environmental signals for meristem function and morphogenesis

We are currently following three projects in this field: The first one centers on DOF transcription factors, which have been shown to be essential cell type specification switches downstream of auxin. We have found that the expression of a number of these factors is controlled by auxin and WUS and that two of them interact with WUS. We will test if these DOFs encode the long sought-after determinants of spatial specificity between the main meristem and floral meristems. In the second project, we are investigating the interplay of WUS induced stem cell fate with jasmonic acid signaling, one of the major signals in environmental sensing. We have found that jasmonic acid exerts a potent influence on stem cells and in addition that key repressors of the pathway interact with WUS. The third project focuses on a new mutation with massively enlarged meristematic cells (*bce*) that provides an ideal model to study the interaction of cell fate specification pathways with basic cellular parameters for shoot morphogenesis.

3. Decoding context dependent genetic networks in vivo

We have successfully established infrastructure and core technologies required for the project and will now start to interrogate the function of single genes by inducible CAS9 mediated genetic mosaics and scRNA-seq.

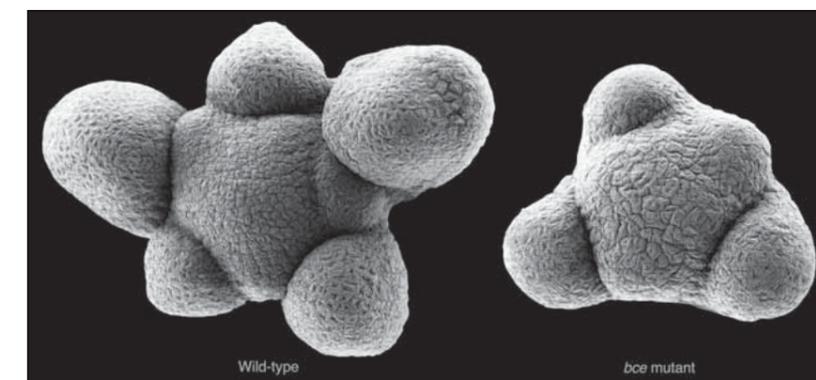


Figure 3
Scanning electron micrographs of wild-type (left) and *bce* mutant (right) shoot meristems. Note the enlarged and misshaped cells in the *bce* background.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 23, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 10, Number of citations 2017-2021: 1591

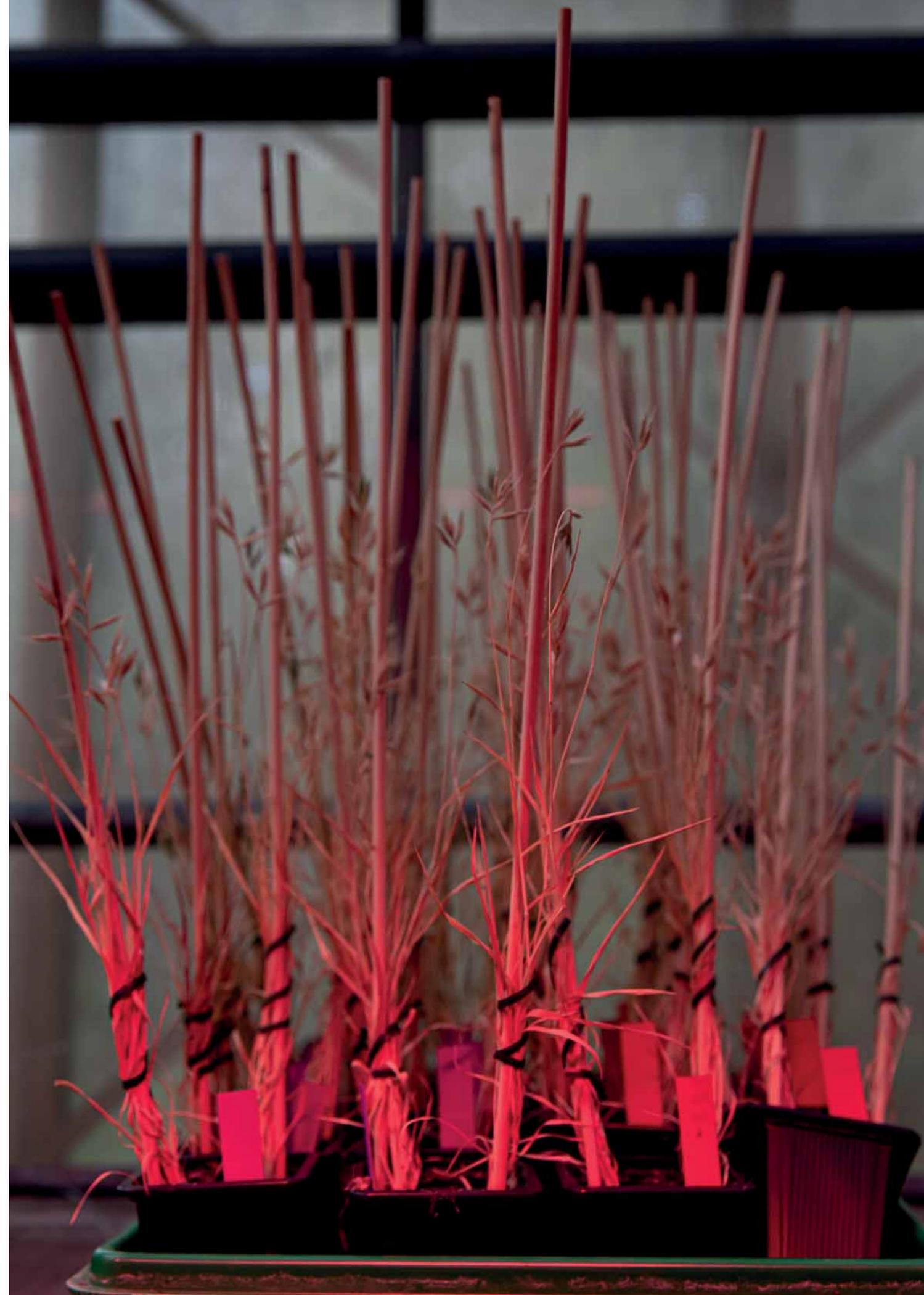
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2.19 PROF. DR. ALEXIS MAIZEL DEVELOPMENTAL PLASTICITY OF PLANTS

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Fields of Interest

Development, cell,
plant, root,
imaging,
quantitative analysis



Summary and outlook

Plants constantly form new organs throughout their life, a major driver of their plasticity and resilience to adverse conditions. An example of this post-embryonic organogenesis is the formation of lateral roots which enhance the plant's capacity to forage its environment for nutrients and stabilize its anchoring. In the model plant *Arabidopsis thaliana*, lateral roots are generated by founder cells located deep in the primary root (Figure 1). The process debuts with the asymmetric expansion of two abutting founder cells that share a common interface. The founder cells expand more along this interface and this prefigures the future dome-shaped lateral root primordium that grows out of the parental root. Accompanying the asymmetric growth, both nuclei migrate toward the common cell wall and the cells divide asymmetrically. Failure to execute these steps leads to aberrantly shaped lateral root or complete arrest. The laboratory is combining precise manipulation of cell properties at cell and tissue scale with live imaging and quantitative analysis to understand the mechanisms responsible for the proper execution of the lateral root initiation program. We have in the last four years made key discoveries about the role of the cell wall and the

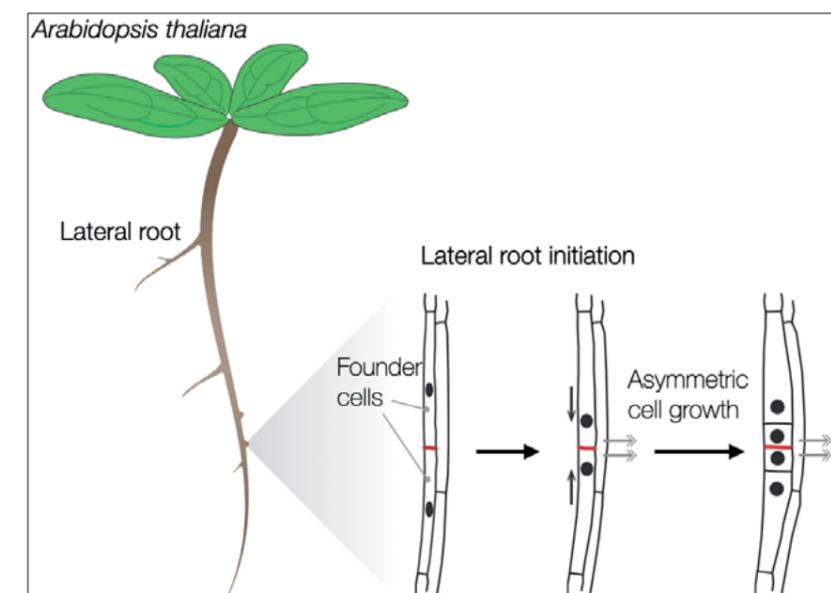


Figure 1

Formation of lateral roots in *Arabidopsis*. Two founder cells expand radially and divide asymmetrically to form a young primordium that keeps dividing and will emerge from the main root.

cytoskeleton in this process. We have also co-developed innovative methods to analyse shape of cells. Our focus in the future will be on understanding how cell polarity, growth and division articulate to specify distinct identities in the developing lateral root.

Research Highlights since 2017

The formation of a new lateral root organ is a self-organizing process during which founder cells invariably undergo a first asymmetric anticlinal division. In the resulting lateral root initiation site, small daughter cells are positioned next to each other in the center and are flanked by larger ones (Figures 1 and 2). We previously showed that radial expansion of

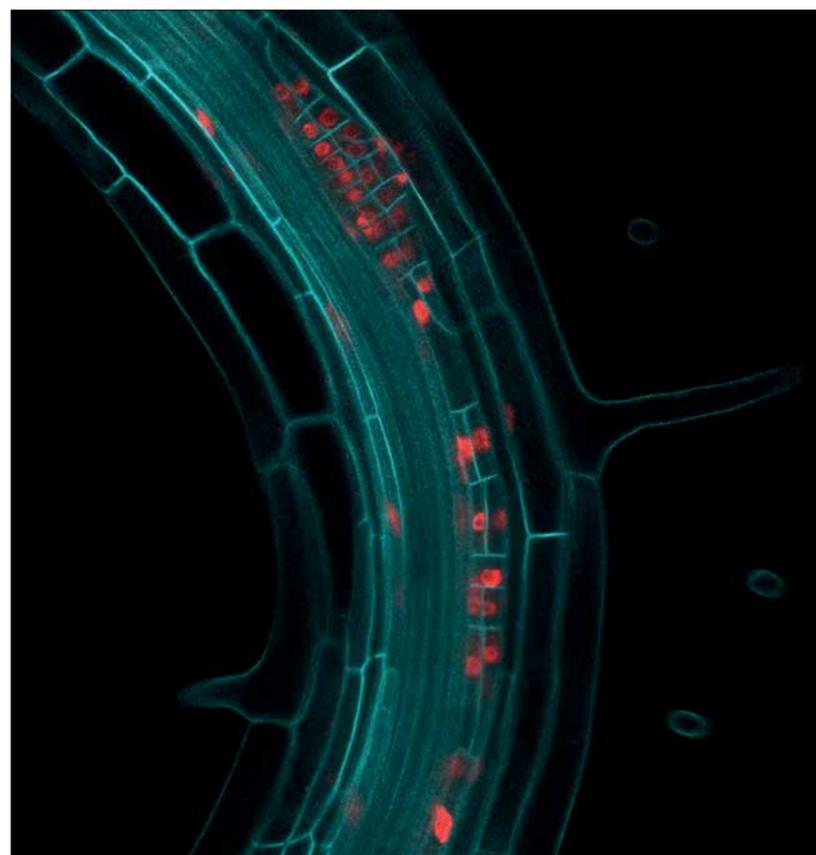


Figure 2
Lateral root primordium visualised by confocal microscopy. Cell contours are visible in blue, nuclei in red.

the founder cells and spatial accommodation by the overlying endodermis are essential for the formation of a lateral root and the first asymmetric cell divisions to occur. In the last four years we made important discoveries on how founder cells tightly control their growth to ensure proper initiation of the new lateral root.

> Cell wall remodelling and control of founder cells asymmetric divisions.

We have discovered that tight control of the cell width is necessary to coordinate asymmetric cell divisions in cells that give rise to a new lateral root organ.

We identify the cell wall remodelling protein EXPA1 as an early marker of pericycle founder cell radial expansion. Based on detailed analyses of expansin a1 mutants, we show that EXPA1 is required for the proper radial expansion which licenses the correct positioning of first anticlinal divisions. This suggests that a specific pericycle width is necessary to trigger asymmetric pericycle cell divisions during lateral root initiation. Although biomechanical processes have been shown to play a role in plant organogenesis, including lateral root formation, our data give mechanistic insights into cell size control during lateral root initiation.

Associated publication: Ramakrishna et al. 2019

> Cytoskeleton remodelling licence asymmetric expansion of founder cells.
We analyzed the microtubule and actin cytoskeleton during the asymmetric radial expansion of lateral root founder cells. We developed new genetic tools for targeted cell specific perturbation of microtubules or actin network. These tools allowed us to demonstrate the existence of an auxin-dependent, microtubule-mediated feedback mechanism that constrains the expansion of the founder cells. We also showed that cell polarization and asymmetric cell division require F-actin network. Thus the reorganization of the cytoskeleton is necessary for the asymmetric expansion and intertwines with auxin signaling to grant a basic polarity to the founder cells.

Associated publication: Vilches Barro et al. 2019

> Quantitative analysis of cell growth.

Quantitative analysis of morphogenesis requires accurate segmentation of individual cells in volumetric images of growing organs. In tight collaboration with teams of the Heidelberg center for scientific computing and EMBL, we employed a convolutional neural network to predict cell boundaries and graph partitioning algorithms to segment cells based on the neural network predictions. This pipeline, called PlantSeg delivers accurate results and generalizes well across different tissues, scales, acquisition settings even on non plant samples. We used PlantSeg to generate a four-dimensional reconstruction of the first stages of lateral root formation and analyze the geometric features of its first divisions (Figure 3). We show that differences exist between the mother cells of anticlinal and periclinal divisions that the periclinal divisions marking the transition to stage II are themselves asymmetric. Consecutive rounds of anticlinal, although of seemingly different 2D geometry, have similar volume and that periclinal divisions are preceded by intense cell growth. Our results suggest that cell growth and division are integrated by the LRP cells which precisely partition volume upon division.

Associated publications: Wolny et al. 2020, Schütz et al. 2021

Future directions

In the coming years, we will concentrate on understanding how cell polarity, growth and division articulate to specify distinct identities in the developing lateral root. Specifically, we will address the following questions.

How do abutting lateral root founder cells coordinately polarize?

The common cell interface between founder cell act as organizing center that polarize each founder cell and organizes lateral root morphogenesis. We will test the importance of this interface by performing ablation of one of the two lateral root founder cells and observe the impact of suppressing this common interface on initiation. Conversely, we will use optogenetics to create new interface between series of pericycle cells and observe the consequences on lateral root initiation.

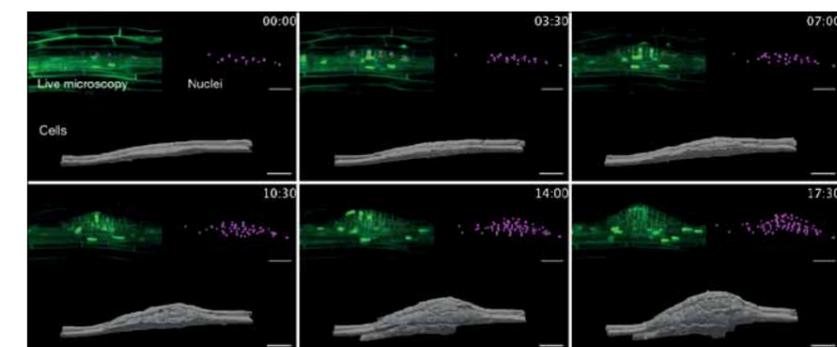


Figure 3
Digital reconstruction of lateral root primordium development. Microscopy images were segmented using PlantSeg to extract nuclei and cell contours.

Several proteins have been shown to localize to this polarizing domain but despite its importance, its molecular composition remains poorly known. Additionally, there is mounting evidence linking membrane nanoclusters to auxin signaling. We will investigate whether signalling lipids and regulators of cytoskeleton dynamics contribute to the polarisation of founder cells. We will also use a proteomic-based approach built on proximity labeling and affinity purification to identify components localizing to this polarizing interface. This axis will uncover new aspects of cell signaling associated with directional cell growth in plants.

How cell growth and division contribute to emergence of cell identities?
The transition from a single layered lateral root primordia to a two layered one is an essential symmetry breaking event and requires the shift of the division plane from anticlinal to periclinal, which, we showed, requires specific cell volume and growth. The PLETHORA transcription factors PLT3,5,7 play a crucial role in orchestrating this transition. We will identify the molecular targets of PLT3,5,7 by RNA-seq. Additionally, we will use single cell RNA-seq to reveal how the lack of the absence of such symmetry breaking event changes the developmental trajectories of cells in LRP. These data will new insights on the molecular mechanisms underlying the process of symmetry breaking and how it impacts cell identity specification.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 13, Number of peer-reviewed articles as first or last/corresponding 2017-2021: 6, Number of citations 2017-2021: 1715

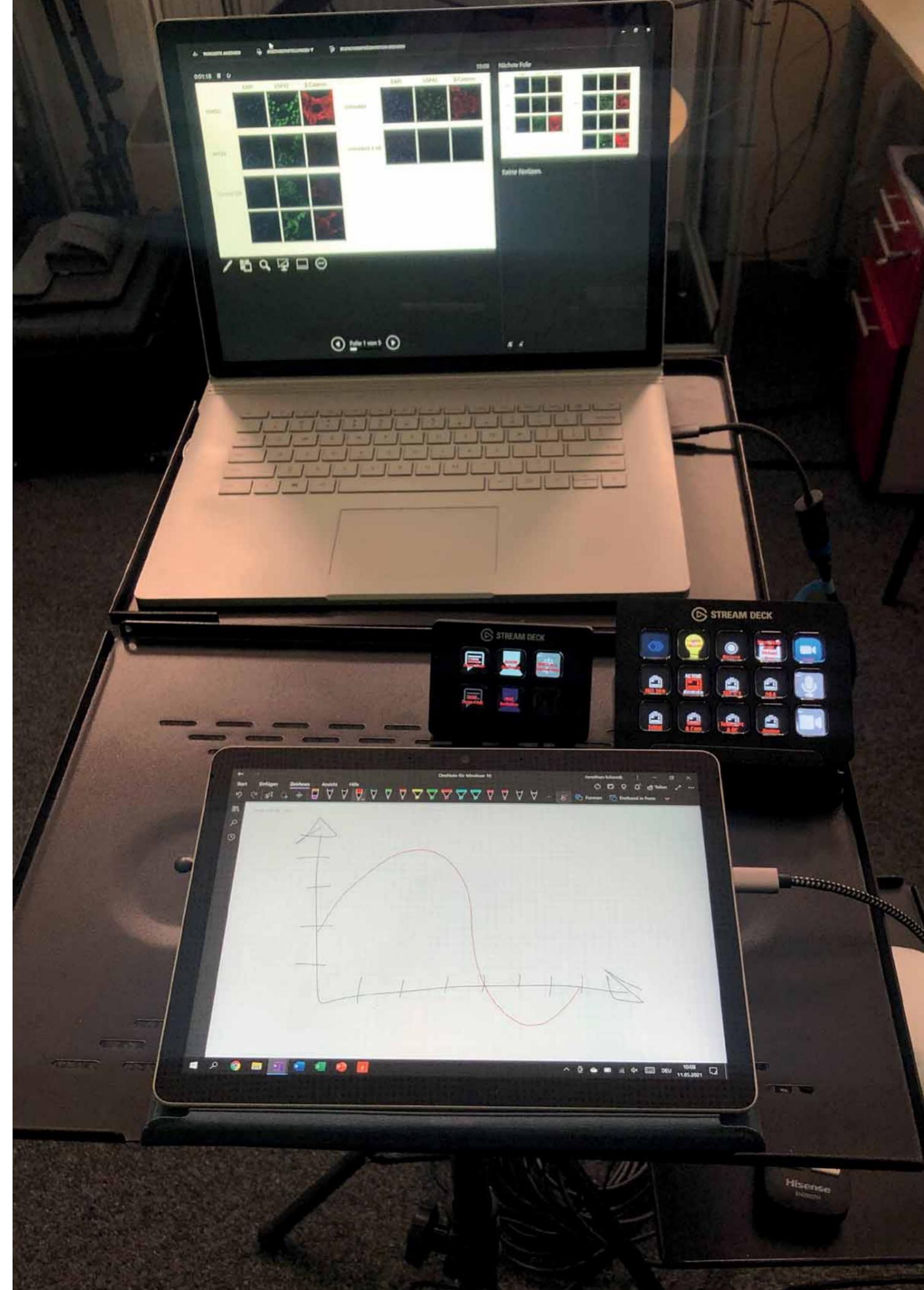
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2.20 PROF. DR. GISLENE PEREIRA CYTOSKELETON, CELL DIVISION AND SIGNALLING

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Fields of Interest

Cell cycle regulation, asymmetric cell division,
mitotic checkpoints, centrosome biology, ciliogenesis,
stem cells, organoids



Summary and outlook

Microtubule organising centres (MTOCs) are key for spatial and temporal organisation of microtubules involved in mitotic spindle formation and spindle alignment in polarised cells, including stem cells. In addition, specialised sub-structures of the mammalian MTOCs (named centrioles) are directly involved in the formation of the primary cilium (Figure 1), an organelle involved in the modulation of signalling pathways relevant for embryonic development and tissue homeostasis. Therefore, it is not surprising that defects that lead to spindle or cilia dysfunction are linked to a variety of human diseases such as polycystic kidney disease, retinal degeneration, obesity, diabetes and cancer.

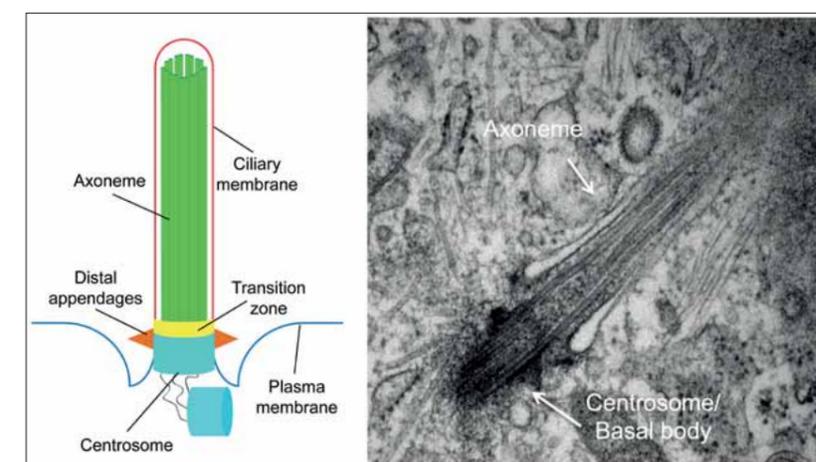


Figure 1
Schematic representation and electron micrograph of sub-domains of the primary cilium of retina epithelial cells.

Our group is particularly interested in understanding how changes in microtubule organisation influence cell behaviour and growth of stem or stem-like cells, how primary cilia are formed, how cilia regulate signalling and how de-regulated cilia biogenesis is linked to disease development or tissue degeneration.

Our past work laid the ground for the discovery and elucidation of a key surveillance mechanism, the spindle position checkpoint that contributes to error-free chromosome segregation during mitosis. Furthermore, we uncovered a mechanism that regulates centrosome

asymmetry during mitosis and established novel players that contribute to initial steps of cilia formation and cilia length control.

Research Highlights since 2017

Control of genome integrity and cell division:

The mitotic spindle apparatus, a complex assembly of microtubules, centrosomes and associated proteins, plays essential roles in chromosome partitioning in mitosis as well as cell fate determination in asymmetrically dividing cells. Considering the essential role of the mitotic spindle, cells developed an elaborated system to deal with damaged spindles, as those structures cannot be simply eliminated. Our past work helped to identify the mitotic spindle position checkpoint (SPOC) in the asymmetrically dividing model system budding yeast. The SPOC is a surveillance mechanism that stops mitotic exit and cell division when mitotic spindle damage leads to spindle misorientation or spindle collapse. In cells with damaged spindles, SPOC deficiency causes chromosome missegregation, which ultimately leads to cell death. The SPOC discovered in yeast inspired the identification of the Centrosome Orientation Checkpoint in *Drosophila* germline stem cells. Similar to SPOC, this checkpoint prevents cell division in response to spindle misorientation.

The SPOC detects spindle orientation errors and transmits a hold-signal to the cell cycle machinery to stop cell cycle progression until defects are corrected. Based on our past work, we now understand relatively well the most downstream events leading to cell cycle arrest. We could show that upon spindle misorientation, the protein kinase Kin4 is key to inhibit the mitotic exit network (MEN), the signalling pathway that promotes progression from mitosis into G1 and cytokinesis. In the past five years, we extended this view and demonstrated that mitotic-cyclin-dependent kinase (Cdk1) activity positively regulates the SPOC and inhibits cell division independently of Kin4 (Caydasi et al., Nat Comm. 2017; Meitinger and Pereira, MBoC 2017).

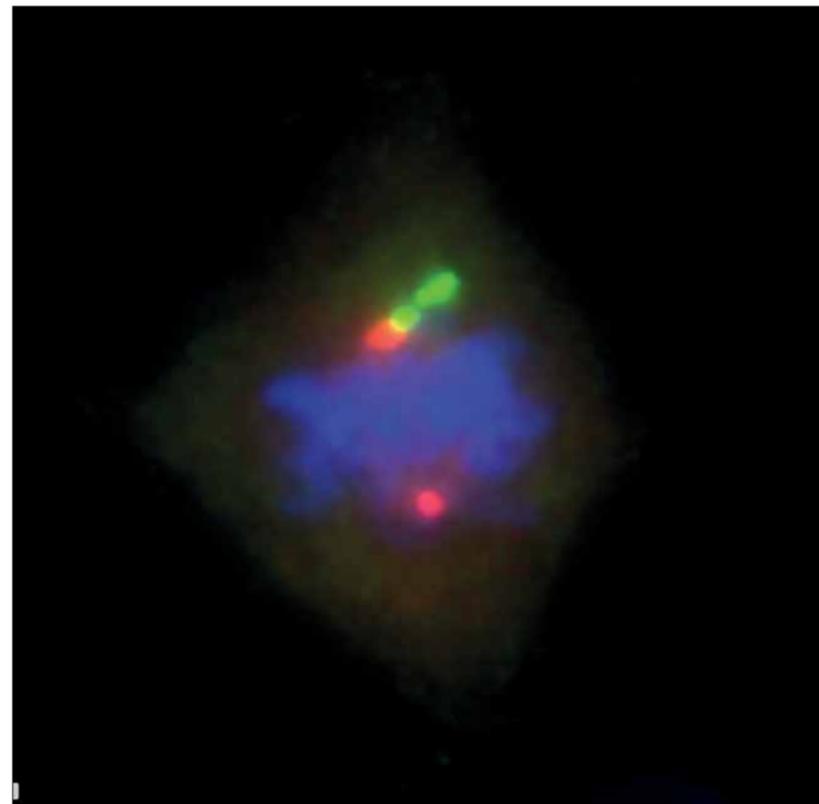


Figure 2
Nek2 knockout (KO) cells retain a primary cilium during mitosis. The immunofluorescence image shows a mitotic Nek2 KO retina epithelial cell carrying a cilium (stained in green) at one of the two centrosomes (stained in red). The DNA is stained in blue.

Using high-throughput approaches, we uncovered more than 20 novel SPOC genes and showed that they are key for the regulation of distinct aspects of SPOC signalling. Our analysis led us to propose that the SPOC involves an immediate Kin4-dependent response to microtubule disturbance. However, this per se is not sufficient. Cells require the activity of a subset of genes, including components of the chromatin-remodelling complex SWR1-C, to maintain a robust SPOC arrest. Our work suggests that SWR1-C promotes SPOC via transcriptional regulation and opens the question of how nuclear and cytoplasmic events are coordinated during checkpoint activation (Caydasi et al, BioRxiv, under revision).

Centrosome asymmetry and cilia formation in higher eukaryotes:

Our laboratory is also interested in centrosome and cilia biology in mammalian cells, particularly on the regulation of appendage proteins and their function in ciliogenesis. Appendages are macromolecular assemblies that are formed at the distal and sub-distal end of the mother centriole. Recently, we established the protein LRRC45 as part of appendage complexes that are present in differentiated and stem cells. Functional analysis defined LRRC45 as a key component required for cilia formation, chromosome fidelity, and organization of cytoplasmic macromolecular assemblies of centriolar proteins (known as centriolar satellites). This work is of general importance because it shows that, although appendage proteins form a common complex, individual components have specific and defined functions. It is the cooperative nature of appendage components that allow the initiation of cilia biogenesis at centrosomes (Kurtulmus et al., JCS 2018).

Appendage proteins are released from the mother centriole in mitosis by a mechanism that is still undefined. Our work identified the conserved kinase Nek2 as a regulator of appendage proteins. We showed that Nek2 reduces the association of a subset of appendages prior to mitosis. In the absence of Nek2, appendages are retained at the mother centriole and contribute to retention of a ciliary remnant (Figure 2). Live-cell imaging showed that the daughter cell that inherits the ciliary remnant was able to reform a cilium and activate the sonic hedgehog (SHH) signalling pathway almost immediately after cell division. We propose that Nek2 removes appendages from the mother centriole at the onset of mitosis to prevent asymmetric inheritance of a ciliary remnant in mitotic cells (Viol et al., JCB 2020). This analysis contributed to the understanding of appendage regulation and function of Nek2 kinases. We believe that our findings are relevant to stem cell and cancer biology. Neural stem cells were reported to retain a ciliary remnant in mitosis during asymmetric cell divisions. This capacity is lost during differentiation, implying a regulation at the level of centrosomes. In addition, Nek2 is overexpressed in a variety of cancer cell types that also lost the ability to form a cilium. We propose that appendage regulation by Nek2 might be an underlying mechanism that contributes to asymmetric cell division of certain stem cells or cilia loss in tumours overexpressing Nek2.

Future directions

How the SPOC senses spindle defects on a molecular level and how cells silence the SPOC after error correction are important open questions. Although we have now identified a large number of new SPOC components, the molecular function of the majority of them remains unclear. Also important is the fact that daughter cell-confined components, including the conserved GTPase Cdc42 and at least three Cdc42 effector proteins, play a fundamental role in the timely control of mitotic exit by means that are not fully understood. We envisage that compartmentalisation of signalling molecules is key for the timely control of mitotic exit in respect to spindle orientation. How cells establish mitotic promoting and inhibitory zones on a molecular level will be the focus of our future research using yeast as a model system. Building on our work in yeast, we plan to investigate how mammalian cells coordinate spindle/centrosome orientation with cell cycle progression using organoids or *Drosophila* germline stem cells, which have an established centrosome orientation checkpoint mechanism. Here, we will focus on the conserved cell cycle regulators that we found in our screens in budding yeast cells.

We will continue to dissect the molecular basis for centrosome asymmetry and cilia formation and function. Here, we will focus on the identification of Nek2 substrates, characterization of appendage protein complex composition and the link between signaling pathways, including Wnt and SHH, and cilia initiation. We are also interested in understanding how cilia length is controlled, as hyper-elongated cilia are linked to disease development but the mechanisms by which cells control cilia length are not well understood. To address these questions, we will employ a combination of in vitro and in vivo studies using human and murine cells in 2D and 3D-cultures. Part of these studies will be performed in collaboration with colleagues of Heidelberg or Karlsruhe as part of collaborative programs, including the SFB873 ("Maintenance and differentiation of stem cells in development and disease"), SFB1324 ("Mechanisms and function of Wnt signalling"), Heidelberg/Karlsruhe Excellence Cluster 3DMM20 and University of Heidelberg FoF1/HMLS explorer programme.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 10, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 6, Number of citations 2017-2021: 1705

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2.21 PROF. DR. G. ELISABETH POLLERBERG POLLERBERG DEVELOPMENTAL NEUROBIOLOGY

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Fields of Interest

Molecules involved in the growth and path finding of nerve cell fibers (axons) during development and regeneration, in particular cell adhesion molecules and their interaction partners (ALCAM, ERMs, Ephrins)



Summary and outlook

The molecules underlying axon growth during development and regeneration of nerve fibers are not yet all known. We focus on the immunoglobulin-superfamily cell adhesion molecule (IgSF-CAM) ALCAM/CD166 and its interaction partners. We could identify three novel interaction partners, the intracellular linker protein ERM (Ezrin, Radixin, Moesin), the repulsive guidance protein Ephrin B3, and the Alarmin HMGB1 (High Mobility Group). For each of these interactions we could demonstrate a role for the growth and/or recovery of retinal ganglion cell axons.

The publication of these results was not yet possible due to too high daily work load, lack of a long-time scientific assistant, and postdocs/ PhD students leaving academia, losing the motivation to publish. The hope is that this can be accomplished now. Moreover, axon growth in hydrogels will be analysed in collaboration (Cellendes, Technology Park Reutlingen).

Research Highlights since 2017

Starting with a working hypothesis based on data originating from nanopatterned ALCAM, we could indeed show its interaction with the ERM linker protein, anchoring ALCAM to the cortical cytoskeleton beneath the axonal plasma membrane. Producing a variety of mutated ERM forms (constitutively active /passive) and using inhibitors, we could show that the ALCAM-ERM interaction reduces ALCAM endocytosis, depending on ERM phosphorylation and ALCAM ubiquitination. By stimulation of ALCAM we could achieve a recovery of ERM-inhibitor-induced collapsed axons. Together the findings reveal that ALCAM/ERM interactions are pivotal for the multifunctional role of this CAM in axon elongation and regeneration.

Another ALCAM interaction partner which we could identify is EphrinB3, a protein in the axonal plasma membrane leading to axon retraction when activated. Activating Ephrin B3 (by fusion protein hFc-EphB3) and at the same time also activating ALCAM (homophilically by ALCAM), we found that this interaction fine-tunes the velocity of growing axons at the optic nerve head where retinal ganglion cell axons have to perform delicate steering reactions to successfully leave the eye and reach the brain.



We also identified HMGB1 as an ALCAM interaction partner and showed that ALCAM stimulation enhances the expression levels of HMGB1 in retinal neurons. We found that HMGB1 is able to stimulate axonal growth and to guide axons (in vitro). As HMGB1 is released in wound sites by necrotic and immune cells, this points to an important role of the interaction for injured axons.

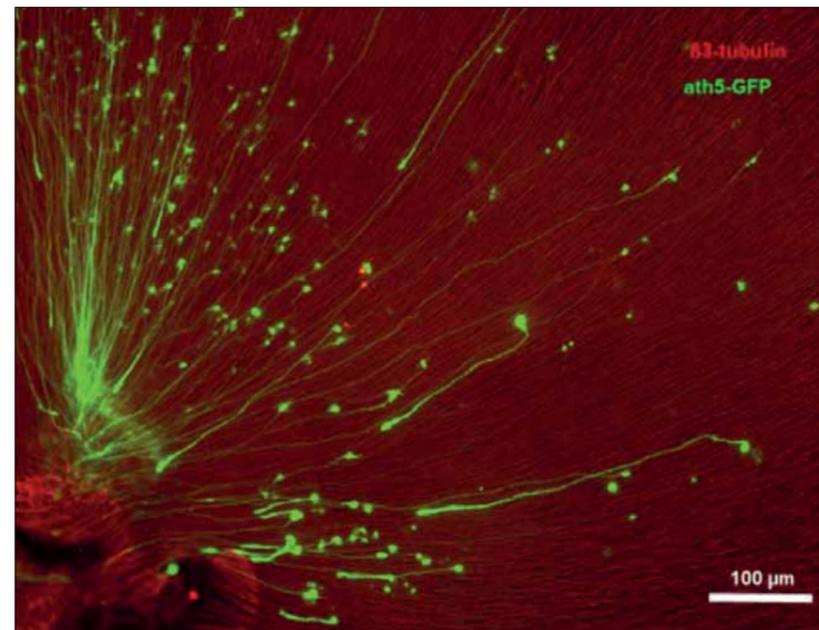


Figure 1

Axons of retinal ganglion cells (green) extending in the embryonic retina on pre-existing axons (red) to the central retina (lower left corner) where they slow down and perform steering reactions to leave the retina and head to the developing brain.





2.22 DR. MICHAEL RAISSIG INDEPENDENT RESEARCH GROUP

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Fields of Interest

Stomatal development, grass stomata, cell identity, cell polarity, cell differentiation and morphogenesis, transcription factor biology, transcriptomics, stomatal physiology, gas exchange kinetics, form-function relationship, evolution of stomatal form, *Brachypodium distachyon*, *Kalanchoë laxiflora*



Summary and outlook

Stomata are breathing pores on plant leaves that exchange gases between the plant and the environment. Stomata in grasses like *Brachypodium distachyon* are particularly fast and efficient due to their innovative form, where two lateral subsidiary cells (SCs) flank the central, dumbbell-shaped guard cells (GCs; Fig. 1A). The developing GC and SC lineages are strictly organized in a base-to-tip developmental gradient and their development is tightly coordinated (Fig. 1B). We have previously shown that *BdMUTE* is the key SC-identity factor and that SC recruitment is abolished in *bdmute* almost reverting the graminoid morphology to an ancestral, two-celled, yet functional stomatal form (Fig. 1B). Comparative transcriptomics of the developing leaf with (wild type) and without (*bdmute*) SCs revealed *BdJIXING* (*BdJIX*), which is important for asymmetric SMC divisions. Unlike previously discovered SMC polarity factors like PANGLOSS1 (*PAN1*), which localize at the GMC/SMC interface, YFP-*BdJIX* shows a complementary, opposite polarization (Fig. 1B). Careful dissection of the *bdpan1* and the *bdjix* phenotypes suggested distinct roles of these players in SMC division. Comparative transcriptomics of mature leaf zones with and without SCs identified the type III secreted peroxidase *BdPOX*. *BdPOX* restricts GC elongation and optimizes stomatal morphology for water-use efficient gas exchange. In future, we will further decipher SC formation in *Brachypodium* using proximity-labelling and single-cell transcriptomics. In addition, we will study the distinct ontogenetic origin of SCs in the CAM model *Kalanchoë laxiflora*. Finally, we will investigate the morphogenetic processes shaping the dumbbell-shaped GCs in grass stomata using imaging, genetics and transcriptomics.

Research Highlights since 2017

How biological form is generated through coordination of cell division patterns and acquisition of distinct cell fates and how a specific form affects its function are some of the most fundamental questions in biology. Plant stomata—small, adjustable cellular valves at the plant-environment interface that balance photosynthetic carbon dioxide uptake with water vapour release—offer an outstanding developmental, evolutionary and physiological model system to link development, morphology and physiological function. Unlike most other plants, grasses form morphologically innovative stomatal complexes, which feature dumbbell-shaped rather than kidney-shaped guard cells (GCs) that are flanked by two lateral subsidiary cells (SCs; Fig. 1). This derived form accelerates opening and closing kinetics and enhances water-use efficiency, which might contribute to the grass family's evolutionary success and to the fact that domesticated grasses like rice, maize and wheat provide ~2/3 of human caloric intake.

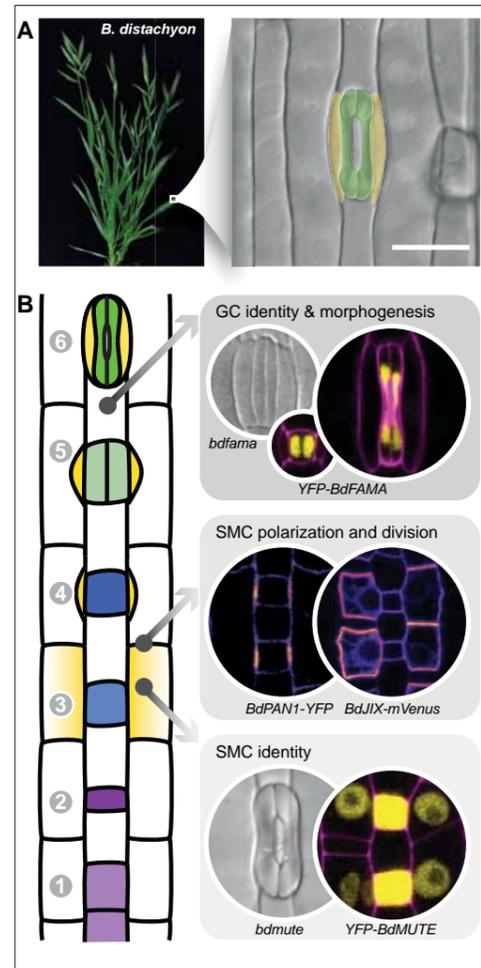


Figure 1
Grass stomatal development. (A) The wild grass *Brachypodium distachyon* forms graminoid stomata, where two lateral SC (yellow) flank the central, dumbbell-shaped GCs (green). (B) The three key processes involved in making graminoid stomata; *BdMUTE*-regulated SMC establishment (bottom panel), SMC polarization and division (middle panel) and GC morphogenesis (top panel).

Our research aims to decode the developmental blueprint of grass stomata and link stomatal form to stomatal function. The Stomatal Biology group—established in May 2018 as an Emmy Noether research group—combines genetics, (single-cell) transcriptomics and microscopy with gas exchange physiology mostly in the grass model *Brachypodium distachyon*. Our main research question are (1) how lateral SCs form, (2) how GCs acquire their unique shape, and (3) how the “graminoid” stomatal morphology improves stomatal physiology.

To approach how SCs are formed, we took advantage of the SC-less *bdmute* mutant and comparatively assessed the transcriptome of developing wild-type and SC-less *Brachypodium* leaves. One of the downregulated genes was *BdJIXING* (*BdJIX*) coding for a small protein of unknown function, which we mutated using CRISPR/Cas9. Mutant plants showed defects in the asymmetric divisions of SMCs resulting in skewed transversal rather than longitudinal divisions leading to missing SCs in the mature zone (Fig. 2A). This phenotype was reminiscent of classical polarity mutants discovered in the maize SC lineage like the receptor-like kinase *ZmPANGLOSS1* (*ZmPAN1*). A mutant in *BdPAN1* showed the identical phenotype to *bdjix* suggesting that *BdJIX* played a role in SMC polarization. Indeed, the double mutant showed strong synergistic defects suggesting independent roles in guiding SMC polarization and asymmetric division. While *BdPAN1-YFP* was strongly polarized towards the GMC/SMC interface, *BdJIX-YFP* was excluded from this domain and found in the apical, basal and distal wall of SMC (Fig. 2C-D). Expression of *BdJIX* but not

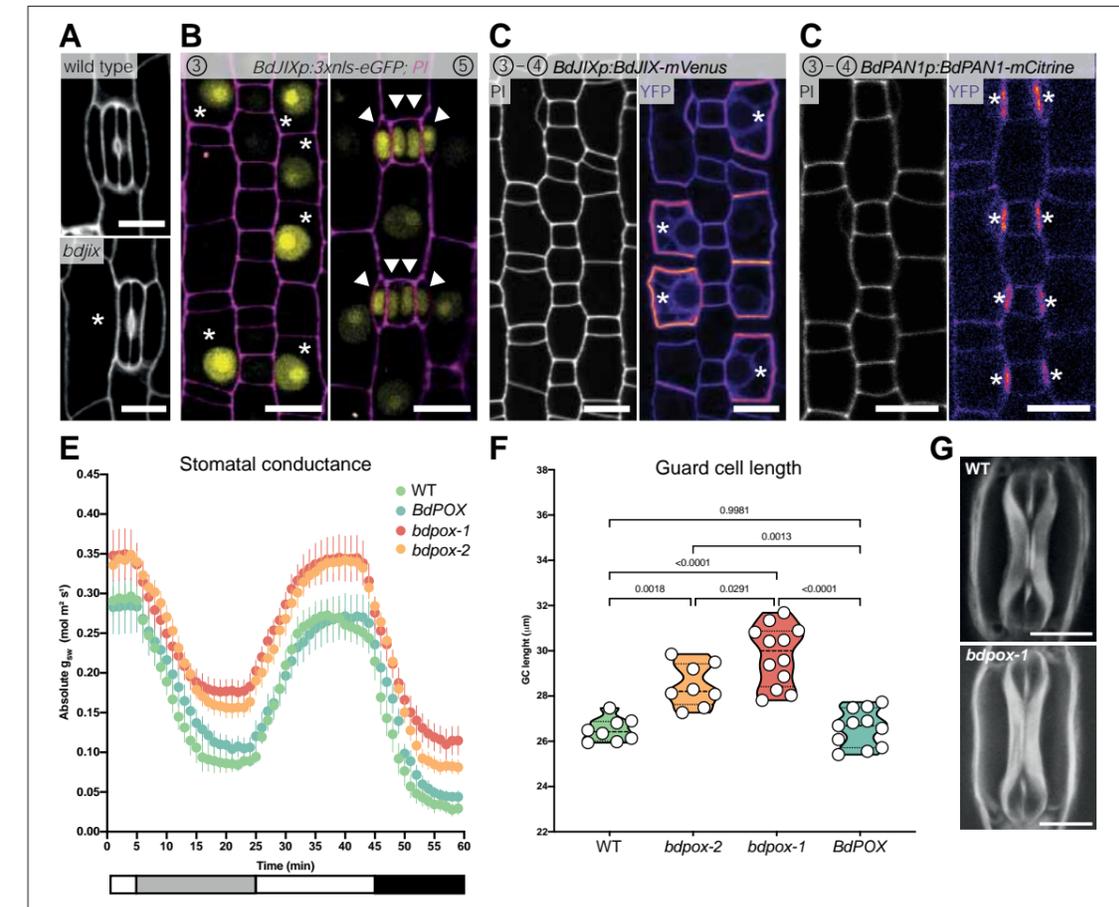


Figure 2
Identification of novel players in grass stomatal development. (A) *BdJIX* is involved in SC formation. (B-D) *BdJIX* is primarily expressed in SMC (B), and shows an opposite polarization (C) compared to other polarity regulators in the SMCs like *BdPAN1* (D). (E-G) The secreted peroxidase *BdPOX* is required to reduce stomatal conductance (E) by restricting GC length (F, G).

BdPAN1 was *BdMUTE*-dependent and *BdPAN1* was required for polarized localization of *BdJIX*. Finally, quantitative analysis of nuclear migration toward the division site in SMC and frequency of failed SMC divisions suggested that *BdPAN1* has a more prominent role in nuclear migration whereas *BdJIX* might be involved in orienting the SMC division plane (Zhang et al. in prep).

To investigate how SCs functionally contribute to graminoid stomatal gas exchange, we also performed comparative transcriptomics of the mature *B. distachyon* leaf with and without SCs. We prioritized the downregulated candidate genes ($n=179$) according to their expression pattern, their functional annotation and the availability of existing mutations. For this, we took advantage of a set of 2200 chemically mutagenized families that were whole-genome sequenced to map all of the 1.5 Mio mutations in this population. We then developed an assay using leaf-level gas exchange measurements to assess stomatal opening and closing kinetics of over 50 candidate mutants. During this screen over the course of almost two years we assessed light response kinetics of 120 *Bd21-3* wild-type individuals in an environmentally fluctuating greenhouse. In addition, we microscopically determined the seasonal variability of stomatal morphology in a subset of these plants. We observed systemic environmental effects on gas exchange measurements and remarkable intraspecific plasticity of stomatal anatomical traits (Nunes et al. in prep - A). The reverse genetics screen yielded four mutant candidates with defects in steady-state gas exchange and/or stomatal kinetics. Two independent mutant alleles in the type III secreted peroxidase *BdPOX*, for example, showed consistently higher gas exchange than wild type (Fig. 2E). While stomatal density was unaffected in *bdpox*, the mutants displayed 20% longer GCs

(Fig. 2F-G). Calculation of stomatal gas exchange capacity based on anatomical parameters (anatomical g_s max) reliably predicted physiologically measured g_s max. This suggested that indeed the anatomical defect caused higher gas exchange rates in *bdpox*. Finally, confocal imaging and signal quantification of UV autofluorescence indicated that there are differences in the polyphenolic compounds in GC cell walls during GC elongation and morphogenesis. This suggested a role for *BdPOX* in crosslinking polyphenolic compounds like lignin to restrict GC elongation in *B. distachyon* for water-use efficient gas exchange (Nunes et al. in prep - B).

Future directions

Our future aims are to understand (1) how SCs develop in grasses in more detail, (2) how ontogenetically distinct SCs are formed in non-grass models, and (3) how grass GCs acquire their unique form.

The identified genetic modules involved in SC formation (Fig. 1) will be leveraged to gain an exhaustive understanding of grass SC recruitment. We will further investigate *BdMUTE*'s role in establishing SC fate through single-cell transcriptomics of developing stomatal cells, study *BdMUTE*'s cell-autonomous function in the GC lineage through non-mobile rescue constructs, and *BdMUTE*'s grass-specific neofunctionalization through domain swaps. Proximity labelling approaches of oppositely polarized *BdPAN1* and *BdJIX* will discover novel players in SMC polarization and the development of time-lapse imaging protocols will unravel aspects of the extremely polarized division generating SCs. Finally, novel mutants affecting SC formation that we found in a forward genetic screen using the genome-indexed NaN population will identify novel players of SC development.

SCs are only loosely defined and beyond grasses it is merely assumed that they play a relevant role in stomatal function. We, therefore, started to work with the succulent and CAM photosynthesis model *Kalanchoë laxiflora*, which shows anisocytic stomata with radially arranged SCs that (unlike in grasses) stem from amplifying GC precursor divisions (Fig. 3). We will gene-edit and fluorescently tag genes homologous to stomatal fate and polarity factors in *Arabidopsis* and *Brachypodium* to analyze how the stomatal gene regulatory module was rewired to generate anisocytic stomata. Ideally, we will recover mutants without SCs and can test if and how these cells contribute to stomatal function in *K. laxiflora*.

Finally, the Poaceae-specific morphogenesis of dumbbell-shaped GCs remains enigmatic. To identify factors downstream of *BdFAMA*, we will employ comparative transcriptomics of *bdfama* (Fig. 1) and wild-type leaves. Time-lapse imaging of cytoskeletal dynamics during

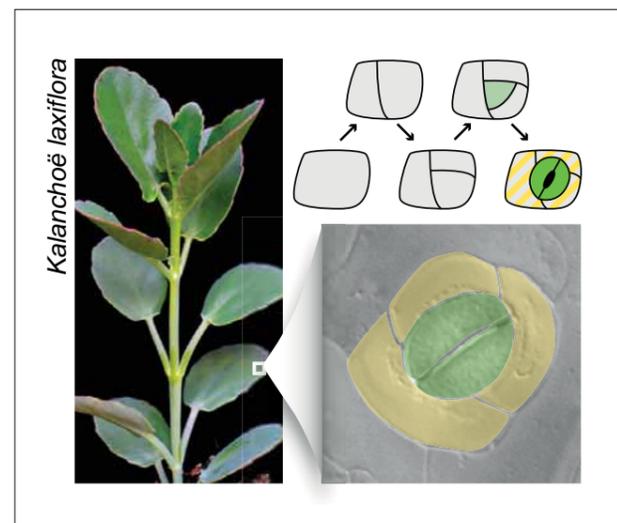


Figure 3
Kalanchoë laxiflora as a model for distinct SC ontogeny. *K. laxiflora* features anisocytic stomata, where three radially arranged SCs (yellow) surround kidney-shaped GCs (green; bottom panel). SCs derive from circular asymmetric divisions of the GC precursor (top panel).

GC morphogenesis in wild-type and morphogenesis mutants will enable mechanistic insight into this process. Novel mutants affecting GC development that we identified using mutant screens (e.g. *tlp* affecting GC differentiation) or reverse genetics (i.e. *bdpox* with longer GCs) identified novel players and will allow us to functionally test how morphological aspects contribute to stomatal physiology.

Stomata are cellular gatekeepers at the interface between a plant individual and the global climate. Understanding how diverse stomatal morphologies form and how specific forms affect stomatal function and water use efficiency will help to counter the devastating effects of climate change on global agriculture and help to feed the world by improving crop performance and resilience.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 5, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 4, Number of citations 2017-2021: 515

Spiegelhalder RP, Raissig, M.T. (2021). Morphology made for movement: formation of diverse stomatal guard cells. *Curr Opin Plant Biol* 63:102090. doi:10.1016/j.pbi.2021.102090

Haas, A.S. and Raissig, M.T. (2020). Seed Sterilization and Seedling Growth on Plates in the Model Grass *Brachypodium distachyon*. *Bio-protocol*: e3700–e3700.

Nunes, T.D.G., Zhang, D., and Raissig, M.T. (2020). Form, development and function of grass stomata. *Plant J.* 101: 780–799.

Raissig, M.T., Matos, J.L., Anleu Gil, M.X., Kornfeld, A., Bettadapur, A., Abrash, E., Allison, H.R., Badgley, G., Vogel, J.P., Berry, J.A., and Bergmann, D.C. (2017). Mobile MUTE specifies subsidiary cells to build physiologically improved grass stomata. *Science* 355: 1215–1218.



2.23 PROF. DR. THOMAS RAUSCH PLANT MOLECULAR PHYSIOLOGY

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Fields of Interest

Molecular stress physiology in crop plants, transcriptional regulation of metabolic pathways (fructans, lignin, secondary metabolites), ligno-cellulosic feedstock for bioeconomy



Summary and outlook

Our research has explored i) the role of transcriptional regulation of fructan metabolism in chicory (a key source for industrial inulin production) under cold stress, ii) the transcriptional regulation of lignin biosynthesis in *Miscanthus sinensis*, an important ligno-cellulosic lifestock for bioeconomy, and iii) the impact of different temporal UV-B stress patterns on the accumulation of flavonols in the model plant *Arabidopsis*. For chicory, novel insight was provided for the involvement of R2R3MYB-, DREB-, and NAC-transcription factors (TFs) in the regulation of its fructan exohydrolase genes. For *Miscanthus*, the role of NAC- and R2R3MYB-TFs (activators and repressors) for the lignification process during plant development was elucidated. For *Arabidopsis*, the comparison of pulsed versus continuous UV-B exposure revealed the importance of interspersed recovery periods.

Research Highlights since 2017

In our chicory project, we could demonstrate the differential transcriptional regulation of the three fructan exohydrolase isoform genes involving several TF classes, thereby providing new insight into their respective functions during taproot development, stress exposure and post-harvest storage. A particular highlight has been the demonstration of synergistic effects between certain R2R3MYB-TF and DREB-TF in the promoter activation of a fructan exohydrolase gene.

In the *Miscanthus* project, while largely confirming transcriptional regulation patterns of genes involved in lignin biosynthesis in other monocots like maize or sorghum, our research has demonstrated how different activating R2R3MYB-TFs from *Miscanthus* cause differential effects on lignin composition when ectopically expressed in tobacco. This indicates that by engineering specific activating R2R3MYB-TFs a distinct manipulation of lignin composition is possible.

For the *Arabidopsis* project addressing the role of specific temporal UV-B exposure patterns, we could observe a substantial increase in flavonol accumulation in the pulsed exposure mode as compared to continuous exposure. Here, the short intervening recovery periods did not only partially reduce the effects of internal feedback loops but also caused less growth inhibition. This model study may provide valuable insight for application in horticulture: By applying specific stress patterns, the accumulation of health-promoting secondary metabolites can be boosted without severe loss in plant productivity.

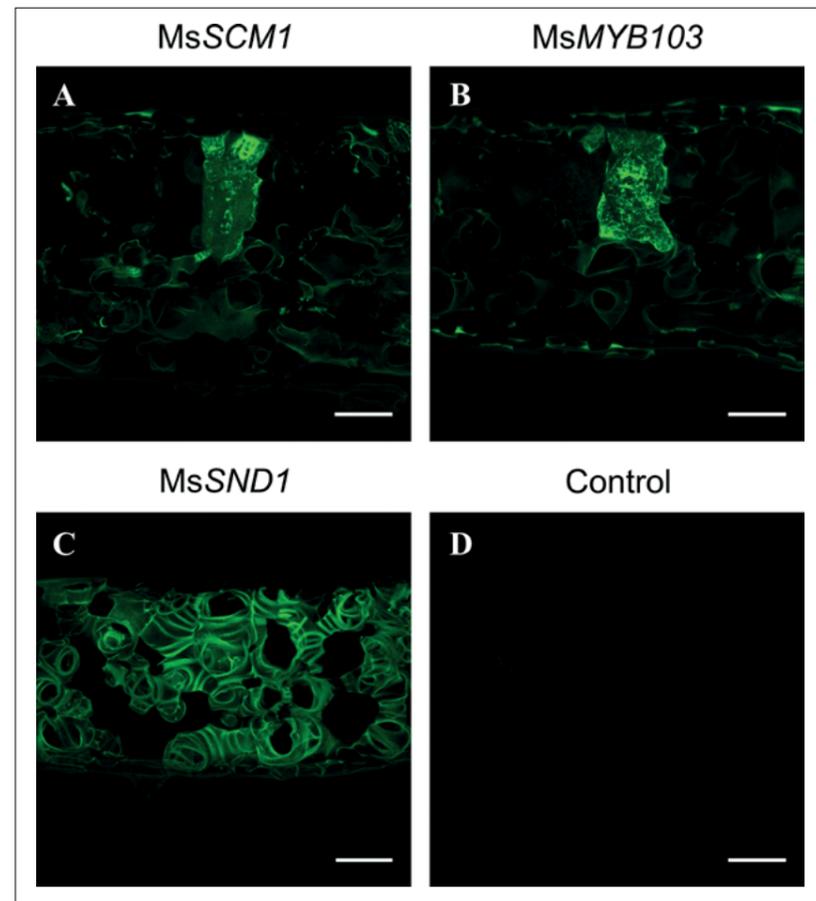


Figure 1
Lignin formation induced by ectopic expression of different *Miscanthus sinensis* transcription factors (MsSND1, MsSCM1, and MsMYB103) in *N. benthamiana* leaves

After 5 days of ectopic expression, leaf cross-sections were stained with Basic Fuchsin to observe fluorescence under a confocal microscope. An empty vector control served as control. Scale bar 50 μm . *Golfier et al. (2021)*, submitted.

Future directions

After my retirement in 2019, I am continuing my research on the regulation of fructan metabolism in chicory in a collaborative project with the Südzucker company. To further advance our insight into the functionalities of different fructan exohydrolase isoform genes and the transcription factor network regulating their differential expression and cold stress or post-harvest storage, we are currently using Crispr-Cas technology (for more details see Steffen Greiner's report). In 2021, I have been elected as Managing Director of the Heidelberg Centre for the Environment (HCE), an interdisciplinary research incubator of the University's Excellence Strategy. The topic of bioeconomy is one of the HCE's focus areas, which I hope to strengthen in the future.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 15, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 4

Huang, X., Luo, W., Wu, S., Long, Y., Li, R., Zheng, F., Greiner, S., **Rausch, T.**, Zhao, H. (2020) Apoplastic maize fructan exohydrolase Zm-6-FEH displays substrate specificity for levan and is induced by exposure to levan-producing bacteria. *Journal of Biological Macromolecules*, <https://doi.org/10.1016/j.jbiomac.2020.06.254>

Yang, Y., Lenherr, E.D., Gromes, R., Wang, S., Wirtz, M., Hell, R., Peskan-Berghöfer, T., Scheffzek, K., **Rausch, T.** (2019) Plant glutathione biosynthesis revisited: redoxmediated activation of glutamylcysteine ligase does not require homo-dimerization. *Biochemical Journal* 476: 1191-1203

Höll, J., Lindner, S., Walter, H., Joshi, D., Poschet, G., Pflieger, S., Ziegler, T., Hell, R., Bogs, J., **Rausch, T.** (2019) Impact of pulsed UV-B stress exposure on plant performance: How recovery periods stimulate secondary metabolism while reducing adaptive growth attenuation. *Plant Cell and Environment* 42(3): 801-814. doi: 10.1111/pce.13409. Epub 2018 Aug 13

Wei, H., Zhao, H., Su, T., Bausewein, A., Greiner, S., Harms, K., **Rausch, T.** (2017) Chicory R2R3-MYB transcription factors CiMYB5 and CiMYB3 regulate 1 fructan 1-exohydrolase 2 expression in response to abiotic stress and hormonal cues. *J Exp Bot*, doi:10.1093/jxb/erx210

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



2.24 PROF. DR. KARIN SCHUMACHER CELL BIOLOGY

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Fields of Interest

Cell biology, membrane trafficking,
ion homeostasis, vacuole biogenesis,
cell growth



Summary and outlook

The endomembrane system of higher plants has evolved several unique features that serve the needs imposed by their sessile lifestyle and reflect the plasticity of growth and development at the cellular level. The identity of the individual eukaryotic endomembrane compartments is not only characterized by their respective protein ensembles but also by their luminal pH. Luminal acidification is driven by the V-ATPase, a rotary nano-engine that energizes secondary active transport and is essential for diverse pH-dependent trafficking events in the secretory and endocytic pathways. Due to this dual function in transport and trafficking the V-ATPase is of pivotal importance for cellular homeostasis and over the past years, we have made substantial progress in understanding the structure, function and regulation of the V-ATPase in the model plant *Arabidopsis*. The trans-Golgi network/early endosome (TGN/EE), the central sorting hub for protein trafficking and the vacuole are the two compartments that are characterized by the presence of specific V-ATPase isoforms (Figure 1) and we have made several major contributions to understanding their molecular identity and functional dynamics. We have implemented the use of genetically-encoded sensors for pH and Ca^{2+} and are making use of these tools in dissecting how the V-ATPase

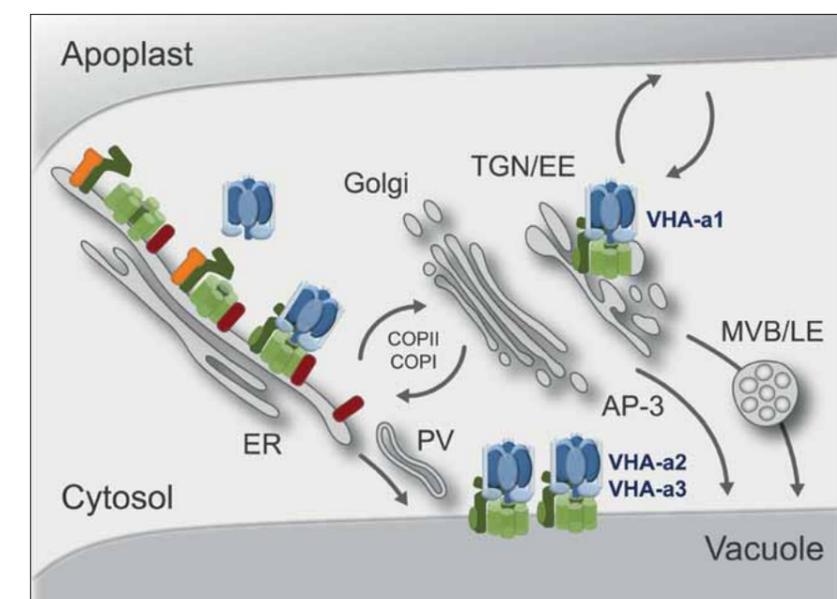


Figure 1
V-ATPase assembly and localisation in *Arabidopsis thaliana*

Assembly of the V-ATPase Vo domain (green) takes place in the endoplasmic reticulum (ER) and requires the assembly factor AtVMA21 (red). The information necessary for targeting V-ATPases to different subcellular sites is contained in subunit VHA-a that exists as three different isoforms in *Arabidopsis*. V-ATPase complexes containing VHA-a1 are targeted via COPII mediated ER export to the trans-Golgi network/early endosome (TGN/EE), whereas holoenzymes containing VHA-a2 or VHA-a3 are targeted in a COPII-independent manner to the vacuolar membrane.

is integrated in diverse cellular and metabolic networks. Moreover, we are interested in the evolution of V-ATPase isoforms in the plant kingdom and pursue a systematic approach addressing the role of the endomembrane system during male gametophyte development.

Research Highlights since 2017

pH-homeostasis in the endomembrane system

Adaptation of the cellular protein-repertoire according to the developmental program and in response to environmental changes requires a highly specific targeting and trafficking machinery. In plants, the trans-Golgi network/early endosome (TGN/EE) is the central dynamic hub in which exo- and endocytic trafficking pathways converge and thus specificity of cargo routing needs to be achieved. The identity of the TGN/EE is determined by the presence and activity of the V-ATPase and luminal pH is a key determinant for protein trafficking (Dragwidge et al., 2019). We have identified the V-ATPase targeting domain in VHA-a1 and have shown that it is conserved among seed plants (Figure 2). Despite its ubiquitous expression and highly specific localisation VHA-a1 is only essential during male gametophyte development and acts redundantly with the tonoplast-localised isoforms VHA-a2 and VHA-a3 in all vegetative cells (Lupanga et al., 2020). Moreover, we have shown that TGN/EE-localization of the V-ATPase is dependent on the presence of two members of the ClC-family of anion transporters pointing to a crucial yet mechanistically unresolved connection between ion homeostasis and protein trafficking (Schöll et al. 2021). Together with the group of Ursula Kummer we have established a mathematical model that allows us to test predictions which players along with the V-ATPases are critical for pH-homeostasis (Holzheu et al., 2021).

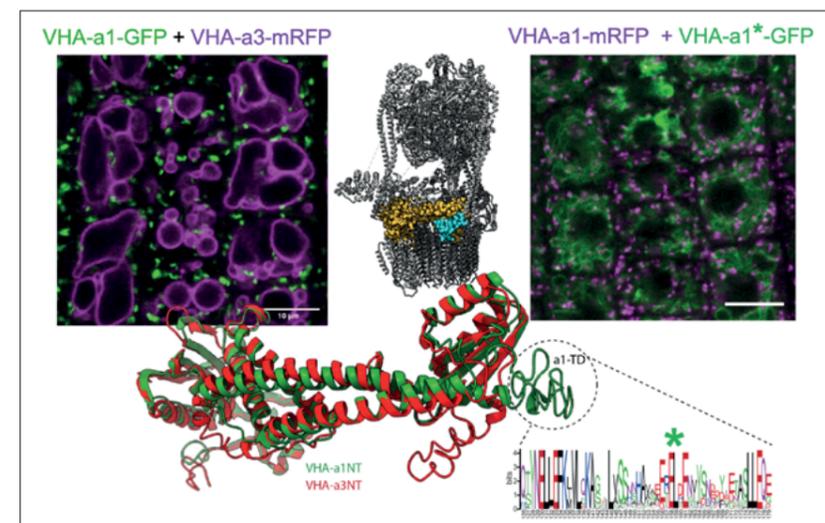


Figure 2
Targeting of VHA-a1 is mediated by a region in the cytosolic N-terminus that is necessary and sufficient for COPII-mediated ER-export and retention of the V-ATPase in the TGN/EE. Exchange of two highly conserved acidic residues as well as a leucine residue (*) within the exposed targeting domain (a1-TD) leads to rerouting of VHA-a1 to the tonoplast.

Vacuole biogenesis

The success of land plants is closely linked to the evolution of a large central vacuole that not only enables plants to buffer changes in the availability of essential nutrients and to detoxify the cytosol when challenged by harmful molecules but most importantly allows cells to fill large volumes at low metabolic cost. By taking up most of the cell volume vacuoles act as a hydrostatic skeleton that in combination with the cytosol and the cell wall provides turgor pressure, the driving force underlying cell growth and reversible changes in cell volume. Despite their manifold and essential function of vacuoles, the molecular mechanisms underlying their biogenesis have not been determined. We have shown that the ER plays a major role in vacuole biogenesis and have since established tools that allow us to visualize and manipulate the formation of ER-derived provacuoles (Figure 3). In this context, we contributed to demonstrating that the homotypic fusion and vacuolar protein sort-

ing (HOPS) tethering complex interacts with phosphoinositides in the regulation of vacuole fusion and fragmentation (Brillada et al., 2018) and that HOPS and CORVET (Class C core vacuole/endosome tethering) mediate distinct vacuolar trafficking pathways in coordination with different sets of SNARE proteins and RAB GTPases (Takemoto et al., 2018).

Genetically encoded indicators

Over the past decade, one line of research in our lab has been dedicated to developing and employing improved genetically encoded fluorescent indicators (GECIs). With regard to pH-imaging, we established transgenic lines expressing pH-GECIs in all compartments of the secretory system that allowed us to determine pH in the ER, the different domains of Golgi-stacks, the TGN/EE as well as in the apoplast. Using these tools in different mutant backgrounds, we have been able to show that pH in the TGN/EE is a major determinant of protein trafficking (Dragwidge et al. 2019) and that pathogen-induced pH changes in the apoplast are involved in regulating the growth-defense balance (Kesten et al., 2019). Regarding calcium imaging, we demonstrated that R-GECO1 exhibits a significantly increased signal change compared with ratiometric NES-YC3.6 and could show that R-GECO1 due to its superior sensitivity is able to report stimulus-induced Ca^{2+} -signals on a cellular scale (Keinath et al., 2015). By converting the intensimetric R-GECO1 into a ratiometric indicator, we were eventually able to apply in vivo calibration protocols that allow us to determine absolute calcium concentrations based on in vivo imaging (Waadt et al., 2017). As pH and Ca^{2+} dynamics are interdependent, we developed 2-in-1-GEFIs that enable us to monitor both concentrations simultaneously and could show that in response to several treatments, increases of cellular Ca^{2+} concentrations overlapped in time and space with decreases of pH (Waadt et al., 2020).

Future directions

pH-homeostasis in the endomembrane system

Despite being a highly conserved protein complex present in all eukaryotes, the Arabidopsis V-ATPase has several unique features including S-acylation of VHA-a2 and VHA-a3. Determining its atomic structure via cryo-EM would not only allow us to address the structural consequences of S-acylation but would also provide important insights into the evolution of subunits that are present in the plant and mammalian complex but lacking in the yeast V-ATPase. Using proximity labelling approaches, we aim to determine the mechanistic base for differential ER-export and TGN/EE-retention of VHA-a isoforms and if the V-ATPase at the TGN/EE acts as a pH-sensor that directly interacts with the trafficking machinery. The liverwort *Marchantia* that has a single VHA-a isoform provides us with a unique opportunity to study the evolution of the TGN/EE-targeting motif and we will investigate if and how the five VHA-a isoforms of the moss *Physcomitrium* are differentially localized. Homozygous *vha-a1* mutants appear wildtype-like during vegetative development, however as they can not be stably maintained, we have so far not been able to quantitatively assess their phenotype in sub-optimal growth conditions or in stress situations in which the TGN/EE has been implicated. Using lines carrying a rescue construct in which VHA-a1 expression is driven by a pollen-specific promoter will allow us to determine if neo-functionalization of VHA-a1 has resulted in specific features related to stress tolerance or if differential localization is indeed only essential during pollen development.

Vacuole biogenesis

Determining the molecular mechanisms underlying ER-derived provacuole formation requires tools that allow us to monitor and manipulate delivery of individual cargo proteins. We will establish tools for chemical and optogenetic protein dissociation in combination with proximity labelling to follow VHA-a3 from the ER to the vacuole and identify proteins involved in the formation of provacuoles. Several mutants identified in a screen for aberrant vacuole biogenesis remain to be characterised (Figure 3) and a chemical genetics screen will be employed to circumvent the lethality associated with strong defects in vacuole biogenesis. Last but not least, candidate proteins for the interaction between vacuoles and the cytoskeleton as well as for ER-vacuole contact sites will be characterized.

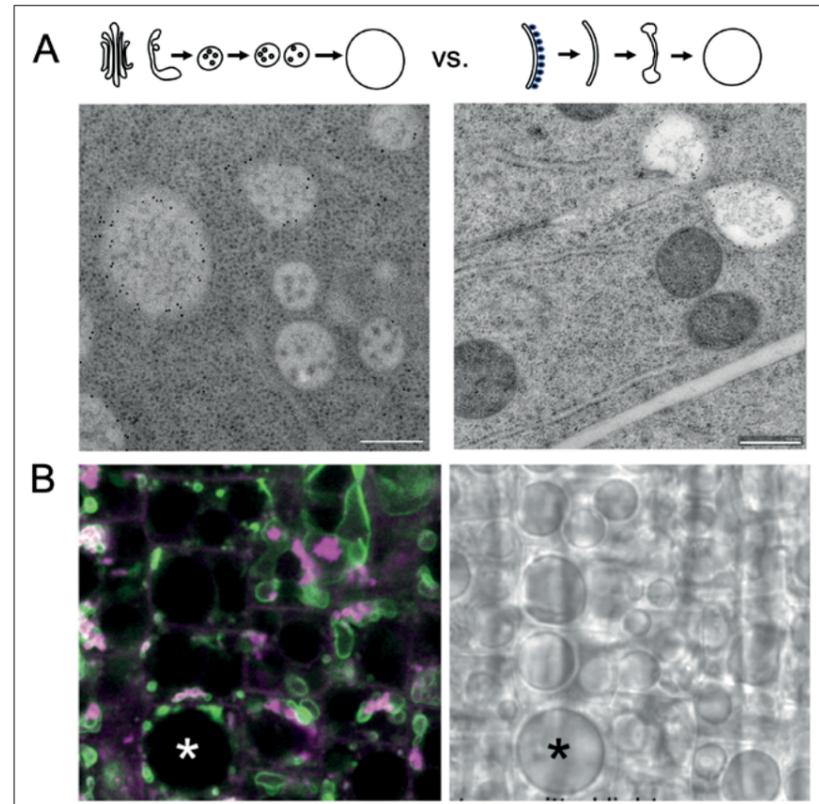


Figure 3A

Immunogold-labelling of the tonoplast H⁺-pyrophosphatase indicates that homotypic fusion of MVBs is not sufficient for vacuole biogenesis as important tonoplast markers that are absent from MVBs are abundant in ER-vacuole intermediate structures known as provacuoles.

Figure 3B

The *bgo1* (big gloomy orb) mutant contains large vacuole-like structures devoid of VHA-a3-GFP

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 14, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 6, Number of citations 2017-2021: 3072

Scholl, S., Hilmer, S., Krebs, M., and **Schumacher, K.** (2021). CLCd and CLCf act redundantly at the TGN/EE and prevent acidification of the Golgi stack. *JCS*.

Holzheu, P., Krebs, M., Larasati, C., **Schumacher, K.**, and Kummer, U. (2021). An integrative view on vacuolar pH homeostasis in *Arabidopsis thaliana*: Combining mathematical modeling and experimentation. *Plant J*.

Waadt, R., Köster, P., Andrés, Z., Waadt, C., Bradamante, G., Lampou, K., Kudla, J., and **Schumacher, K.** (2020). Dual-Reporting Transcriptionally Linked Genetically Encoded Fluorescent Indicators Resolve the Spatiotemporal Coordination of Cytosolic Abscisic Acid and Second Messenger Dynamics in *Arabidopsis*. *Plant Cell* 32, 2582–2601.

Lupanga, U., Röhrich, R., Askani, J., Hilmer, S., Kiefer, C., Krebs, M., Kanazawa, T., Ueda, T., and **Schumacher, K.** (2020). The *Arabidopsis* V-ATPase is localized to the TGN/EE via a seed plant-specific motif. *Elife* 9. 43-44

Waadt, R., Krebs, M., Kudla, J., and **Schumacher, K.** (2017). Multiparameter imaging of calcium and abscisic acid and high-resolution quantitative calcium measurements using R-GECO1-mTurquoise in *Arabidopsis*. *New Phytol.* 216, 303–320.

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Fields of Interest

Fluorescent indicators, live imaging,
Ca²⁺ signaling, cellular pH homeostasis,
nitrate homeostasis, membrane transport,
proton pumps, vacuoles



Summary and outlook

Understanding the molecular mechanisms behind plant-environmental sensing and the associated response reactions is key to develop strategies to engineer crop plants with improved tolerance towards biotic and abiotic stress conditions. To investigate physiological adaptations on a molecular level, fluorescence-based indicators are powerful tools that enable non-invasive measurements of various biochemical and biophysical cellular parameters. The combined power of fluorescence-indicator based techniques and classical biochemistry, genetics and more recently computational modelling, allowed us to gain new insight into regulation of vacuolar pH and into apoplasmic pH signalling in response to biotic stress. Furthermore, our work has contributed to understand the regulation of tip-focused Ca²⁺ gradients during polar cell growth and we were able to develop advanced routines for Ca²⁺ imaging in plant cells.

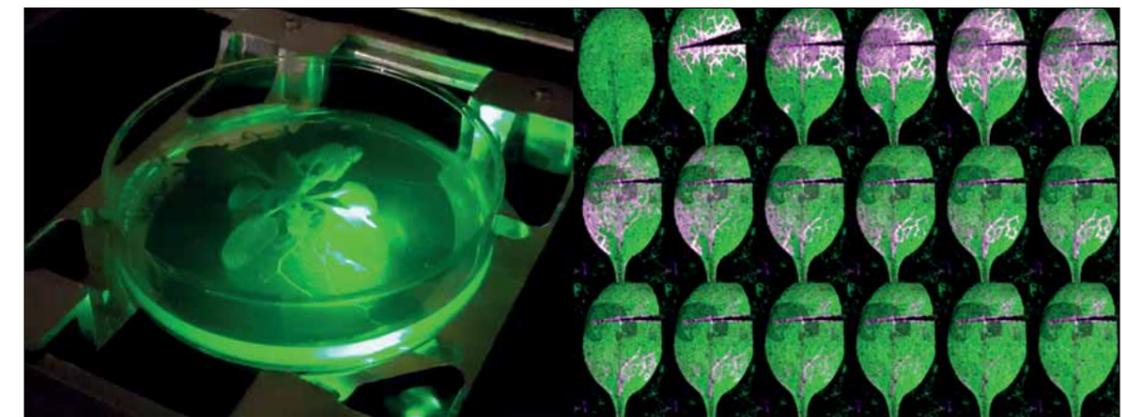


Figure 1

Wound-triggered long-distance Ca²⁺ signals in *Arabidopsis* leaves visualised with genetically-encoded indicator R-GECO1-mTurquoise (Waadt et al. 2017)

Research Highlights since 2017

Fluctuating environmental conditions trigger diverse physiological and developmental adaptations in plants. Our efforts aim to understand how plants sense and integrate such environmental information. To investigate physiological adaptations and signalling events on a molecular level, we use state-of-the-art fluorescent indicator tools to non-invasively assess biological processes in real-time and on a cellular scale. To be able to study the complex mechanism of vacuolar acidification in *Arabidopsis* more efficiently and with high-

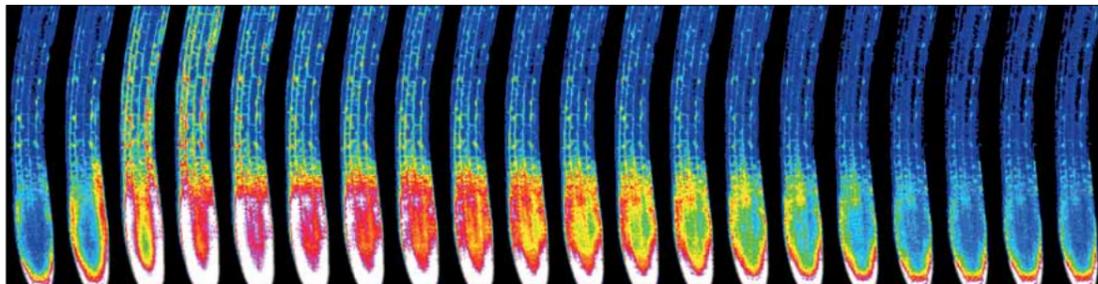


Figure 2
ATP-induced increase of $[Ca^{2+}]_{cyt}$ in *Arabidopsis thaliana* roots visualized with R-GECO1 (Waadt et al. 2017).

er throughput compared to previously established microscopic methods, we developed a plate-reader based platform to measure vacuolar pH using the pH sensitive fluorescent dye 5-CFDA. Experimentally obtained data of vacuolar pH were then incorporated into a mathematical model that provided an integrative view on vacuolar pH regulation in plants. (Holzheu et al. 2021). Besides the investigation of vacuolar pH we have been developing genetically-encoded pH indicators that allow us to target specific cellular domains or compartments such as the apoplastic space of plant cells or the cytosolic domain beneath the plasma membrane. Based on these tools we contributed in elucidating the infection mechanism of the pathogenic fungus *Fusarium oxysporum* which induces rapid apoplastic acidification due to proton pump activation at the plasma membrane (Kesten et al. 2019). Physiological adaptations in plant cells not only involve changes in cellular pH but are often governed by transient concentration changes of the second messenger calcium. Based on our previous work in which we established the red-emitting intensimetric Ca^{2+} indicator R-GECO1 in *Arabidopsis*, we contributed to highlight the role of cyclic nucleotide-gated Ca^{2+} channels (CNGCs; CNGC6, CNGC9 and CNGC14) for coordinating the tip-focused Ca^{2+} gradient in polarly growing root hairs cells of *Arabidopsis* (Brost et al. 2019). To overcome limitations inherent to intensimetric indicator methods, we developed a series of ratiometric Ca^{2+} indicators that combine the high dynamic range of single-fluorescent protein Ca^{2+} indicators with the advantages of ratiometric imaging (Waadt et al. 2017). To further evolve Ca^{2+} imaging in plants we established protocols that enable calibration of Ca^{2+} indicators in intact plant tissues which is a prerequisite to correlate transient $[Ca^{2+}]_{cyt}$ changes with the biochemical activity of the respective Ca^{2+} decoding proteins (Waadt et al. 2017).

Future directions

To get a better understanding of the mechanisms that drive the extraordinary flexibility of plants on a metabolic and physiological level we will continue to further improve existing imaging-based platforms for Ca^{2+} and pH and would like to extend the repertoire of genetically-encoded indicators for the important nutrient and signalling molecule nitrate. Besides, future work will be directed towards vacuolar pH regulation in which our previous work suggested a new and so-far unrecognized mechanisms for vacuolar acidification that relies on vacuolar stored Ca^{2+} acting as battery to fuel vacuolar H^+ uptake via reverse transport activity of vacuolar Ca^{2+}/H^+ exchangers. We aim to proof this exciting hypothesis to find out whether such an alternative mechanism acts in the background of the established proton pump-mediated vacuolar acidification machinery. New fluorescent indicator applications amenable to monitor high concentrations of Ca^{2+} in the acidic environment of the vacuolar lumen will have to be established aside.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 9, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 1, Number of citations 2017-2021: 92

Holzheu, P., **Krebs, M.**, Larasati, C., Schumacher, K., and Kummer, U. (2021). An integrative view on vacuolar pH homeostasis in *Arabidopsis thaliana*: Combining mathematical modeling and experimentation. *Plant J.* 8, 1–16.

Lupanga, U., Röhrich, R., Askani, J., Hilmer, S., Kiefer, C., **Krebs, M.**, Kanazawa, T., Ueda, T., and Schumacher, K. (2020). The *Arabidopsis* V-ATPase is localized to the TGN/EE via a seed plant-specific motif. *Elife* 9, 1–40.

Kesten, C., Gámez-Arjona, F.M., Menna, A., Scholl, S., Dora, S., Huerta, A.I., Huang, H., Tintor, N., Kinoshita, T., Rep, M., et al. (2019). Pathogen-induced pH changes regulate the growth-defense balance in plants. *EMBO J.* 38, e101822.

Brost, C., Studtrucker, T., Reimann, R., Denninger, P., Czekalla, J., **Krebs, M.**, Fabry, B., Schumacher, K., Grossmann, G., and Dietrich, P. (2019). Multiple cyclic nucleotide-gated channels coordinate calcium oscillations and polar growth of root hairs. *Plant J.* 99, 1–14.

Waadt, R., **Krebs, M.**, Kudla, J., and Schumacher, K. (2017). Multiparameter imaging of calcium and abscisic acid and high-resolution quantitative calcium measurements using R-GECO1-mTurquoise in *Arabidopsis*. *New Phytol.* 216, 303–320.



2.25 PROF. DR. SABINE STRAHL GLYCOBIOLOGY

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Fields of Interest

Glycobiology; glycosyltransferases
and functions of glycans in yeast
and mammals; congenital disorders
of glycosylation



Summary and outlook

Glycosylation is the most abundant, complex and diverse protein modification known. Among the different types of glycosylation, N-glycosylation and O-mannosylation are essential in fungi and animals, and underlie the pathophysiology of severe congenital disorders in humans. Using yeast and mammalian model systems our group is studying the molecular mechanism of the initial steps of protein O-mannosylation in the endoplasmic reticulum (ER) and the roles of O-mannosyl glycans, which can be dynamically adapted to changes in development or the environment.

In addition to their important roles for canonical target proteins, O-mannosyl glycans function in ER protein quality control. Glycoproteomics and high throughput screenings in the eukaryotic model baker's yeast gave us new insights into the different functions of O-mannosylation. In continuation, we now aim to define general principles of O-mannosyl glycan functions and dynamics with respect to key factors of ER protein quality control and to explore operation and regulatory mechanisms of the protein O-mannosyltransferases especially in terms of stress conditions.

Work from our and other groups suggests a multi-layered interconnection between O-mannosylation and N-glycosylation in yeast and humans. However, mechanistic details are just emerging. Using a combination of omics, biochemistry and cell biology, we showed that defects in O-mannosylation induce cellular processes that result in aberrant cell-cell adhesion and inter alia impact on the N-glycosylation machinery in human cell models. Based on our findings, we will further study mammalian O-mannosylation and its relation to N-glycosylation at the operating, regulatory and functional levels.

Research Highlights since 2017

In eukaryotes, around one third of all proteins enter the secretory pathway and become glycosylated. Protein N-glycosylation and O-mannosylation are initiated at the ER, where the target polypeptides and the donor saccharides are synthesized and eventually covalently linked. Only if properly glycosylated and folded can proteins leave the ER and travel through the Golgi apparatus to their final destinations. On their way, N-linked and O-mannosyl glycans can be further trimmed and/or elongated in orchestrated reactions. This results in immensely diverse and complex glycans that allow dynamic adaptations to developmental and environmental changes (Figure 1).

In baker's yeast O-mannosylation is the only type of O-glycosylation, making *Saccharomyces cerevisiae* an ideal eukaryotic model to study the molecular mechanism and functions of this essential posttranslational protein modification (reviewed in Neubert and Strahl, 2016).

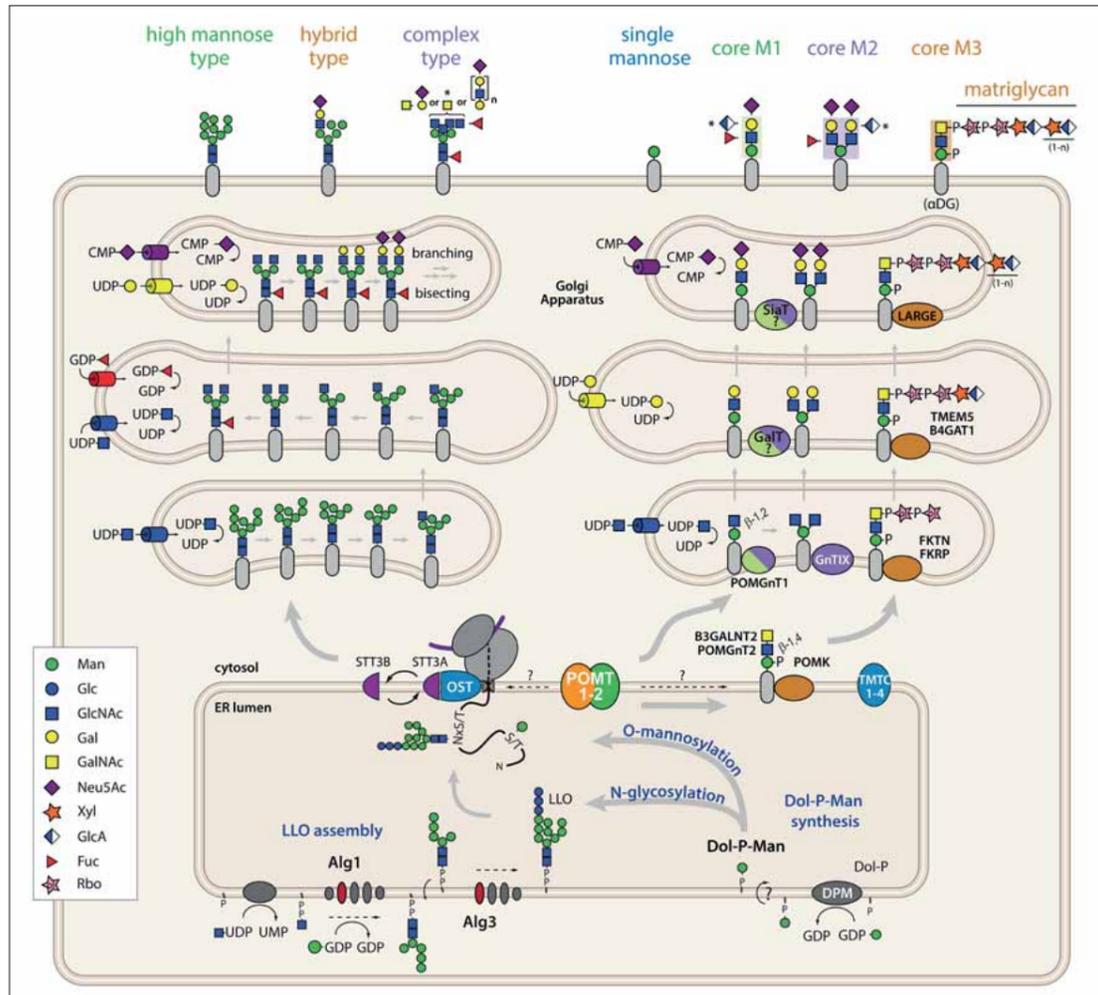


Figure 1
N-glycosylation and O-mannosylation in mammals. ER: assembly of the lipid-linked glycan donors. In N-glycosylation, the LLO glycan is transferred by OST to acceptor proteins. In O-mannosylation, POMTs (PMTs in yeast) transfer mannose from Dol-P-Man to acceptor proteins. Golgi: protein-linked glycans may be extended. N-glycosylation: left panel; O-mannosylation: right panel.

A dual role of O-mannosylation has been shown in yeast. In addition their important functions for canonical target proteins, O-mannosyl glycans may exclude unfavourable folding intermediates from futile folding attempts and mark them for degradation (unfolded protein O-mannosylation, UPOM) (Figure 2). In an important step towards the full understanding of the multiple functions of protein O-mannosylation we recently established the first yeast O-mannose glycoproteome (Halim et al., 2015; Neubert et al., 2016). Among the PMT target proteins, we identified key factors of ER protein quality control and stress signaling pathways, opening exciting opportunities to explore roles of O-mannosyl glycans. In frame of SFB1036, we now took advantage of a tandem fluorescent timer reporter to monitor different aspects of protein dynamics and identified more than 30 proteins affected in abundance and/or stability when O-mannosylation is compromised (Castells-Ballester et al., 2018). Among those are stress sensors as well as other crucial components of protein folding and quality control machineries suggesting an important role for O-mannosyl glycans for ER protein homeostasis. O-mannosylation and protein folding must balance each other to ensure functionality of canonical target proteins and UPOM. Exactly adjusting PMT activity to ER protein load most likely enables O-mannosylation of highly diverse protein substrates without unintentionally interfering with protein folding. Accordingly, PMT levels are coordinated with ER stress and their abundance is controlled on a transcriptional as well as on a translational level (Castells-Ballester et al., 2019).

Protein O-mannosylation is initiated at the luminal side of the ER membrane, by a conserved family of protein O-mannosyltransferases (PMTs in yeast; POMTs in mammals; Figure 1). The yeast *Pmt1-Pmt2* complex proved to be a central hub for UPOM and the unfolded protein response (UPR) (Zatorska et al., 2017; Castells-Ballester et al., 2019). These integral ER membrane proteins feature 11 transmembrane domains and a large hydrophilic loop domain (MIR) facing the ER lumen. Structural data gained in close collaboration with the groups of I. Sinning (BZH, Heidelberg University) and H. Schwalbe (BMRZ, University of Frankfurt) allowed to propose putative sugar/substrate binding sites which were confirmed to be important for enzymatic activity *in vivo* (Chiapparino et al., 2021).

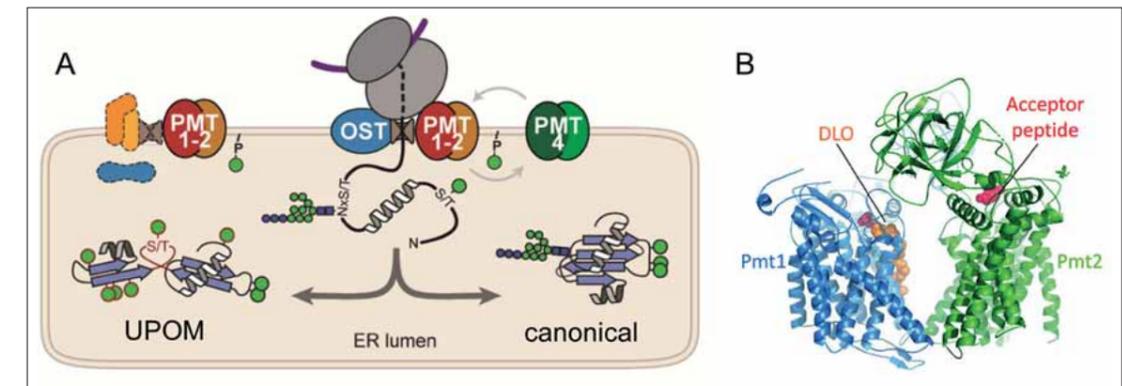
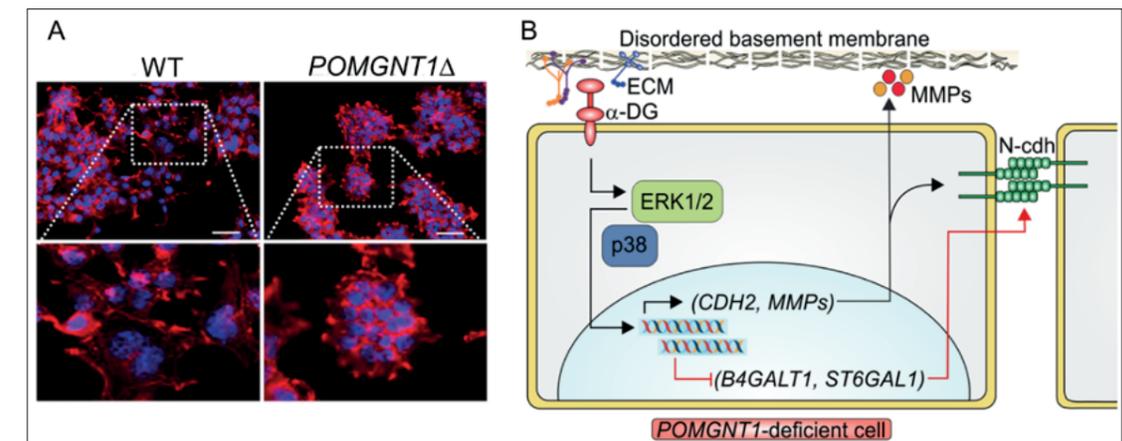


Figure 2
A) The biosynthesis of O-mannosyl glycans in the ER. Canonical target proteins are O-mannosylated by PMT family members and may be N-glycosylated by the OST complex. Some proteins are only O-mannosylated in case they misfold (unfolded protein O-mannosylation; UPOM). B) From Neubert and Strahl (2016) Cryo-EM structure of the *Pmt1-Pmt2* complex at 3.2 Å resolution; from Bai et al. (2019).

In human, defects in protein O-mannosylation results in severe congenital muscular dystrophies with brain and eye malformation such as muscle-eye-brain disease (MEB). Defective O-mannosylation of α -dystroglycan (α DG) has been recognized as a major pathomechanism. Our previous work showed that changes in O-mannosylation also impact on E-cadherin (Cdh) -mediated cell-cell adhesion during murine embryonic development and in human gastric carcinoma (Lommel et al., 2013; Carvalho et al., 2016). However, whether the observed defects only apply to E-Cdh is unclear, as are the underlying molecular mechanisms. In the frame of FOR2509, we took advantage of glyco-engineered HEK 293 cells and MEB patient-derived fibroblasts, and now demonstrate that, in general, aberrant O-mannosylation impacts on cadherin-mediated cell-cell adhesion. Our study suggests a model in which due to defects of O-mannosyl glycans on α -DG, ERK and p38 signaling cascades are activated. As a consequence, epithelial-mesenchymal transition (EMT)-like transcriptional events result *inter alia* in the induction of N-cadherin. In addition, transcriptional modulation of N-glycan modifying enzymes contributes to increased N-Cdh homotypic interactions. In combination, these events result in enhanced cell-cell adhesion and disor-

Figure 3
A) Morphology of *POMGNT1* knock-out cells. B) Hypothetical model: Loss of O-mannose-linked glycan on α DG abolishes interaction with extracellular matrix (ECM) components. As a consequence EMT-like events are triggered resulting *inter alia* in the induction of N-cadherin. Modulation of N-glycan modifying enzymes contribute to increased N-cadherin homotypic interactions. From Noor et al. (2021).



dered basement membranes thereby contributing to the molecular pathogenesis of MEB disease (Figure 3; Noor et al., 2021).

Future directions

To elucidate the multi-layered role of O-mannosylation in ER protein quality control it is imperative to clarify the functions of these modifications for the diverse target proteins (canonical and UPOM) under physiological and stress conditions. Based on our yeast glycoproteomics and high throughput analysis, we now aim to address this issue on the molecular level focusing on selected candidate proteins. Furthermore, knowing how Pmt1-Pmt2 work and which regulatory mechanisms they are subjected to is mandatory to grasp the molecular interconnection between O-mannosylation, UPR and other stress conditions. Building on our recent findings, we wish to unravel different roles of Pmt1 and Pmt2 in the complex and study how stress conditions impact on Pmt1-Pmt2 activity, UPOM and UPR. Our work revealed a multi-layered interconnection between O-mannosylation and N-glycosylation in mammals. However, mechanistic details are just emerging. Thus, in the future we aim to study mammalian O-mannosylation and its relation to N-glycosylation at the operating, regulatory and functional levels and dig even deeper into the pathomechanisms of O-mannosylation defects. A large repertoire of methods and tools as well as MS-directed analyses necessary for this work are established in our lab.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 10, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 5, Number of citations 2017-2021: 1129

Noor, S.I., Hoffmann, M., Rinis, N., Bartels, M.F., Winterhalter, P.R., Hoelscher, C., Hennig, R., Himmelreich, N., Thiel, C., Ruppert, T., Rapp, E., and **Strahl, S.** (2021). Glycosyltransferase POMGNT1 deficiency strengthens N-cadherin-mediated cell-cell adhesion. *J Biol Chem.* 296, 100433.

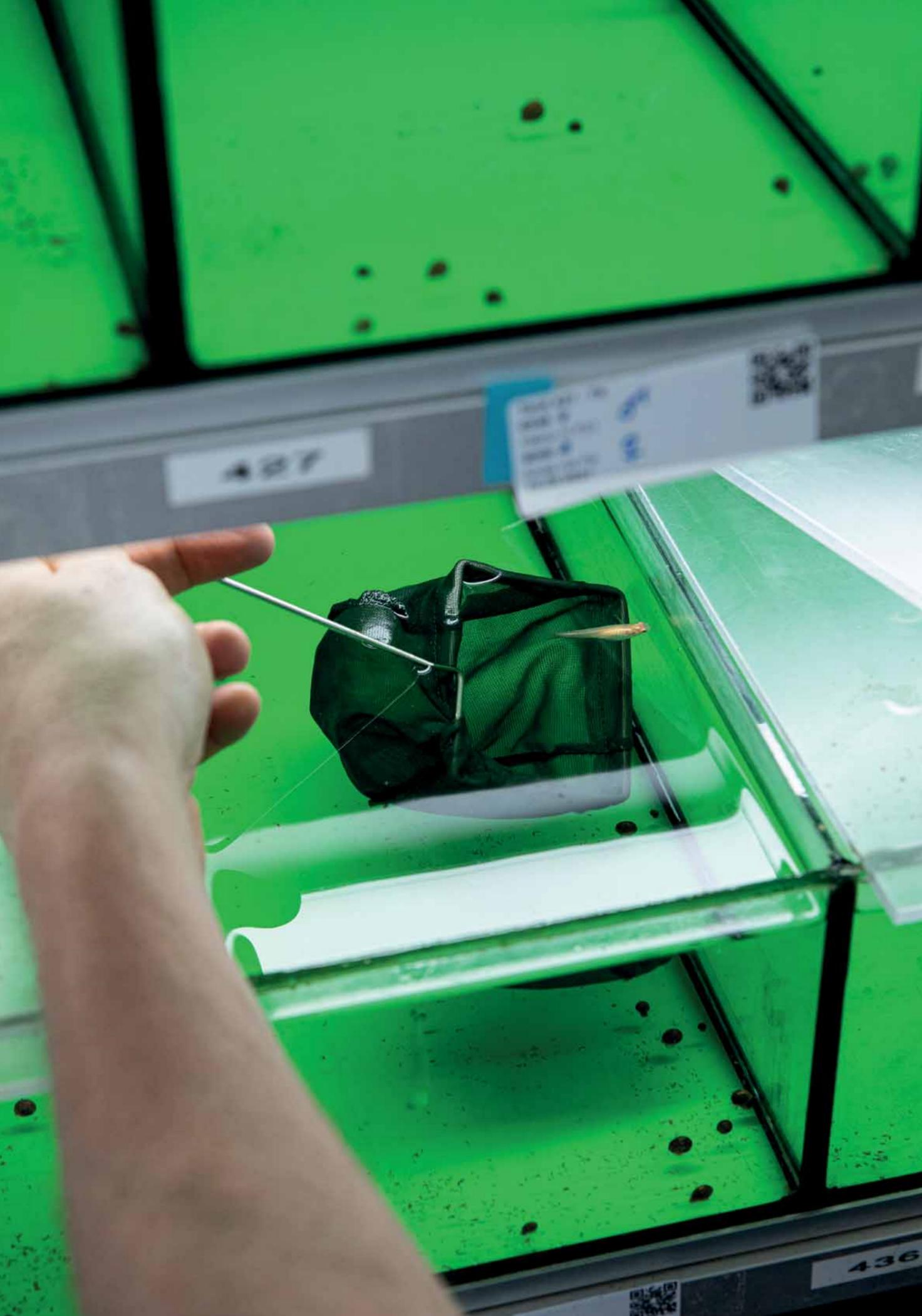
Chiapparino, A., Grbavac, A., Jonker, H.R., Hackmann, Y., Mortensen, S., Zatorska, E., Schott, A., Stier, G., Saxena, K., Wild, K., Schwalbe, H., **Strahl, S.***, and Sinning I.* (2020) Functional implications of MIR domains in protein O-mannosylation. *Elife* 9:e61189.

Castells-Ballester, J., Rinis, N., Kotan, I., Gal, L., Bausewein, D., Kats, I., Zatorska, E., Kramer, G., Bukau, B., Schuldiner, M., and **Strahl, S.** (2019) Translational Regulation of Pmt1 and Pmt2 by Bfr1 Affects Unfolded Protein O-Mannosylation. *Int J Mol Sci.* 20(24):6220.

Castells-Ballester, J., Zatorska, E., Meurer, M., Neubert, P., Metschies, A., Knop, M., and **Strahl, S.** (2018) Monitoring Protein Dynamics in Protein O-Mannosyltransferase Mutants In Vivo by Tandem Fluorescent Protein Timers. *Molecules.* 23(10):2622.

Zatorska, E., Gal, L., Schmitt, J., Bausewein, D., Schuldiner, M., and **Strahl, S.** (2017) Cellular Consequences of Diminished Protein O-Mannosyltransferase Activity in Baker's Yeast. *Int J Mol Sci.* 18(6). pii: E1226.





2.26 PROF. DR. JOACHIM WITTBRODT ANIMAL PHYSIOLOGY/DEVELOPMENTAL BIOLOGY

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Fields of Interest

Molecular developmental genetics, stem cells in development, growth, regeneration (eye, heart, brain, intestine; medaka: *Oryzias latipes*, zebrafish: *Danio rerio*), genetics of individuality in morphogenesis, regeneration, physiology and behaviour. Vertebrate (fish, mammalian) organoids. Technology development: Crispr/Cas, imaging, organoids



Summary and outlook

The main focus over the past years was the genetics underlying the balance of proliferation and differentiation in fish stem cells. A particular focus was on the role of retinal stem and progenitor cells and their role in establishing and maintaining the perfect shape of the eye which is fundamental for its functional homeostasis. We have shown that the neuroretina is creating shape out of itself via a programmed behavior of neuroretinal stem cells as predicted by computational models and validated *in vivo*.

We have expanded our scope towards the genetics of individuality and genome/environment interactions. For that we have established an inbreeding panel representing a natural population of medaka (MIKK panel) that we are now using to address the multifactorial contribution to genome environment interactions in the context of regeneration, heart function and behaviour to name a few.

Our ongoing progress in Crispr/Cas and base editing technology was instrumental for the establishment of defined genetic conditions that now allow addressing the acute loss of key players in development and disease. Complementing the analyses in the context of the genetics of individuality we have in particular focussed on the heart as well as on particular aspects of the protein glycosylation machinery. In both settings we introduced human specific mutations to the respective fish orthologues, validating the human candidate genes and establishing experimental models at the same time. With the fish (retinal) organoids recently established by the lab we can exploit all established tools in a new, synthetic context.

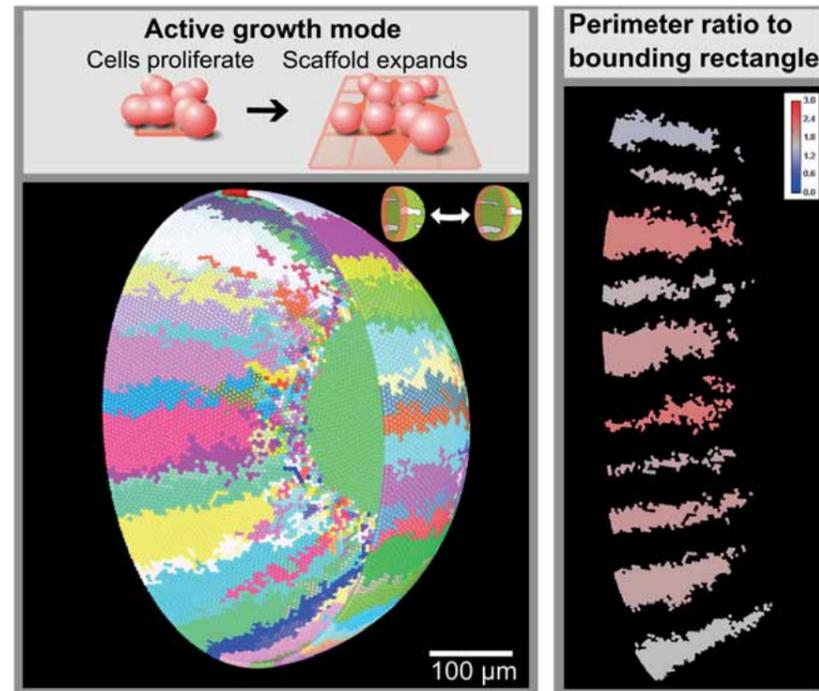


Figure 1
Modeling of retinal growth and function indicates crucial driver role for stem and progenitor cells of the neural retina.

Research Highlights since 2017

In live-long growing organs like the teleost eye, a functional homeostasis has evolved. Combining clonal analysis with a computational agent based model, we have unraveled how tissue-specific stem cells for the neural retina (NR) and retinal pigmented epithelium (RPE) of medaka (*Oryzias latipes*) shape the eye by coordinating their growth rates. Precise NR cell division timing indicates an upstream role in driving growth. RPE cells divide with greater variability, consistent with a downstream, responding role. We have shown that NR cells orient division axes to regulate organ shape and retinal topology. We highlight a mechanism for growth coordination, where one tissue integrates cues to synchronize growth of nearby tissues. This strategy facilitates the modulation cell proliferation parameters in one tissue to adapt whole-organ morphogenesis in a complex vertebrate organ as addressed in a combination of modelling and experimental validation.

Extending these findings we propose an integrator for eye growth governed on the level of progenitor cells of the neural retina. How body and organs balance their relative growth is of key importance for coordinating size and function in continuously growing species (like fish). We demonstrate that a central growth regulator, Igf1 receptor (Igf1r), is necessary and sufficient for proliferation control in the postembryonic retinal stem cell niche: the ciliary marginal zone (CMZ). Targeted activation of Igf1r signaling in the CMZ uncouples neuroretinal growth from body size control. Strikingly, Igf1r operates on the level of progenitor cells, stimulating their proliferation and increasing retinal size while preserving its structural integrity. We have revealed a modular organization in which progenitor differentiation and neurogenesis are self-organized and highly regulated. We position Igf signaling as a key module for controlling retinal size and composition, with important evolutionary implications.

Regeneration responses in animals are widespread across phyla. We identified a marked difference in the regenerative capacity between medaka and zebrafish (*Danio rerio*). In contrast to zebrafish, medaka Müller glia cells (OIMGCs) act as progenitors and exhibit a restricted regenerative capacity. After injury, they proliferate but fail to self-renew and

ultimately only restore photoreceptors. In contrast to zebrafish, proliferating OIMGCs do not maintain *sox2* expression. When experimentally sustained in medaka its retina regenerates similar to zebrafish via OIMGCs. A single, cell-autonomous factor reprograms OIMGCs and establishes a regeneration-like mode. This positions medaka to delineate key regeneration factors, an aspect that we are actively pursuing in the context of the MIKK population genomic resource.

The establishment of simplified retinal structures for high throughput analysis of compounds facilitating regenerative properties on the one hand, and self growing and preparing light sensor on the other is one of the central aims in the cluster 3DMM20. Here we initially focussed on retinal organoids derived from pluripotent mammalian stem cell. However they are limited by their long developmental time and unpredictable success rates. We overcame these limitations by deriving organoids from rapidly developing teleosts. We established conditions to quantitatively derive retinæ from teleost primary embryonic pluripotent cells. Within four days, aggregates quantitatively execute key steps of eye development: retinal specification, morphogenesis, and differentiation. The unprecedented efficiency and rapid development of fish-derived organoids in combination with advanced genome editing techniques immediately allowed to systematically probe the impact of the physical environment.

Individual variation is a critical parameter in the interplay of intrinsic and extrinsic factors that produce states of health and disease, and must be understood to translate findings from models to the human context. A significant barrier in achieving this is the fact that

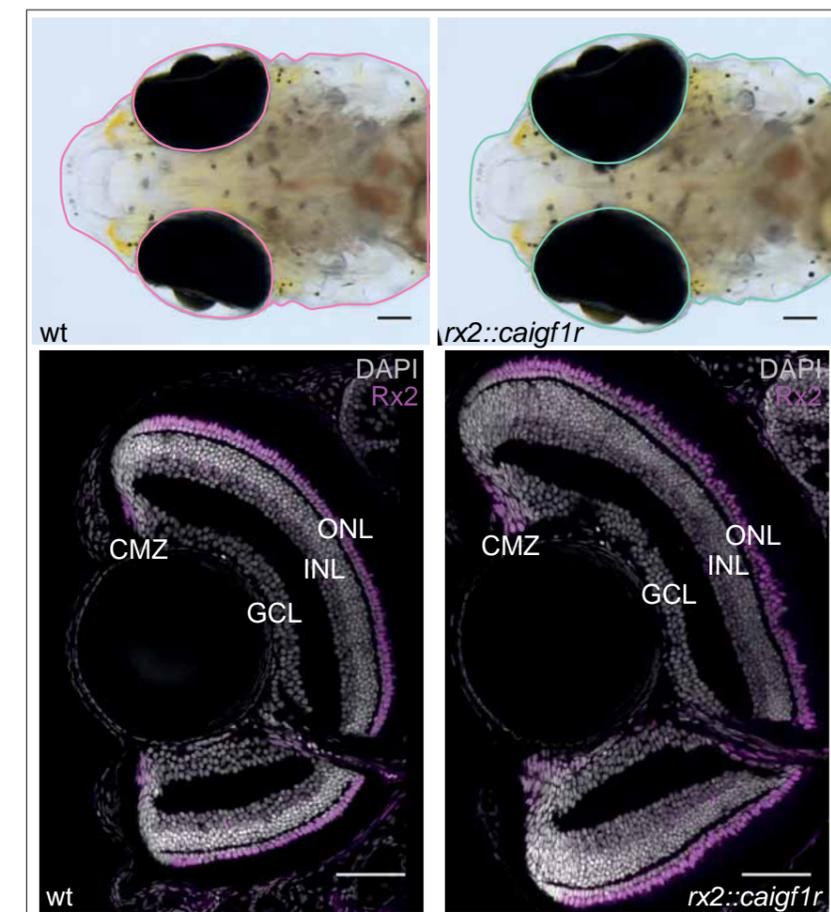


Figure 2
Retinal progenitor cells (but not stem cells) coordinate organ size via IGF signaling

current genetic models of complex vertebrates were deliberately bred to reduce variation as a source of noise and ensure experimental reproducibility. In the ERC Syncergy project IndiGene we tackle these issues using the unique properties of medaka, which can be fully inbred from the wild. We have inbred and performed whole-genome sequencing of a panel of 111 diverse medaka lines originating from a single natural population (MIKK panel) and are now performing in-depth phenotyping of these fish at scales ranging from organismal to molecular phenotypes.

CRISPR/Cas9 efficiently induces targeted mutations via non-homologous-end-joining but for genome editing, precise, homology-directed repair (HDR) of endogenous DNA stretches is a prerequisite. To favour HDR, many approaches interfere with the repair machinery or manipulate Cas9 itself. Using Medaka and mouse (in collaboration with Marc Freichels group) we show that the modification of 5' ends of long dsDNA donors strongly enhances HDR, favours efficient single-copy integration facilitating successful gene replacement or tagging.

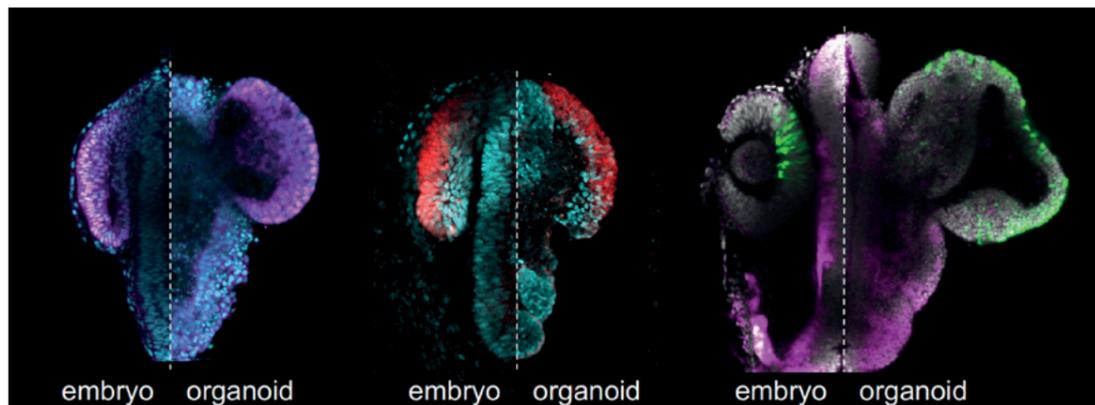
Future directions

In the coming years the focus of the lab will be on the genetics of individuality and the establishment of a hybrid synthetic retina. We will continue our efforts on elucidating the action of the retinal stem cell niche, in particular addressing the role of the immune system in shaping the niche. All of those activities crucially depend of the continuous method development for precise and targeted genome editing or advanced imaging of whole organisms. Long term collaborations with Ewan Birney (EBI), the cluster 3DMM20 and Tilo Baumbach (KIT) provide the complementary expertise as basis for shifting the frontiers into the uncharted territories.

We have developed quantitative high throughput approaches to detect polygenic traits contributing the the function of the heart. To do so we have been screening all lines of the MIKK panel for cardiac function in a genome/environment (temperature) analysis. We have established F2 offspring of the extreme phenotypes and will sequence 1200 individuals to correlate genotype and phenotype. Identified loci will be subjected to a CRISPR based targeted mutagenesis to ultimately assess the relative contribution, additive and synergistic effects of the linked loci.

This analysis is performed for a range of quantitative assays, heart function, regeneration, behaviour, impact of environmental toxins, brain and body morphology to name a few. All data linking genome to phenome will be made available to ultimately allow a link between species from fish to men and back. We expect to establish complex, tractable models for development and disease.

Figure 3
Fish retinal organoids recapitulate organismal development.



Based on the identification of a so far unknown immunological surveillance of the retinal stem cell niche we are extending our scope to other stem cell niches and aim to understand the implications for evolution and stem cell related diseases. We particularly focus on the interaction of stem cells with macrophages and the underlying signaling activities. The establishment of fish retinal organoids allows to address the relative contribution of stem cell regulation in the presence or absence of a pruning surveillance system. We will employ organoid systems to complement the in vivo analysis and the most immediate question addressed will be the origin of stem cells in the ciliary marginal zone. This is of relevance for basic research as much as for applied approaches such as the establishment of a synthetic retina in 3D organotypic systems. To ensure progress in all fields we will continue developing the technology fostering our disruptive approaches.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 32, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 12, Number of citations 2017-2021: 12737 (according to Google Scholar)

Zilova, L., Weinhardt, V., Tavhelidse, T., Schlagheck, C., Thumberger, T., and **Wittbrodt, J.** (2021). Fish primary embryonic pluripotent cells assemble into retinal tissue mirroring in vivo early eye development. *eLife* 10, e66998.

Becker, C., Lust, K., and **Wittbrodt, J.** (2021). Igf signaling couples retina growth with body growth by modulating progenitor cell division. *Development* 148, dev.199133.

Tsingos, E., Höckendorf, B., Sütterlin, T., Kirchmaier, S., Grabe, N., Centanin, L., and **Wittbrodt, J.** (2019). Retinal stem cells modulate proliferative parameters to coordinate post-embryonic morphogenesis in the eye of fish. *eLife* 8, 3470.

Gutierrez-Triana, J.A., Tavhelidse, T., Thumberger, T., Thomas, I., Wittbrodt, B., Kellner, T., Anlas, K., Tsingos, E., and **Wittbrodt, J.** (2018). Efficient single-copy HDR by 5' modified long dsDNA donors. *eLife* 7, 142.

Lust, K., and **Wittbrodt, J.** (2018). Activating the regenerative potential of Müller glia cells in a regeneration-deficient retina. *eLife* 7, 7028.

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Fields of Interest

Aquatic ecology and toxicology;
fish biology; embryo toxicity;
teratogenicity; genotoxicity; neurotoxicity;
endocrine disruption; pharmaceuticals;
microplastics; nanoplastics; histopathology;
cytopathology; river and lake restoration



Summary and outlook

Tremendous efforts have been undertaken to reduce environmental contamination and to improve the quality of aquatic ecosystems. A huge body of regulations has been installed, and most environmental compartments are regularly controlled. However, despite improvements in water quality, fish populations still show deficits. The reasons being manifold, specifically acting anthropogenic trace contaminants such as endocrine disruptors or pharmaceuticals are considered as candidate reasons. Thus, there is an urgent need to develop procedures to identify trace contaminants. Given the trend towards alternative test methods, fish cell culture and embryo toxicity testing systems are developed by the COS

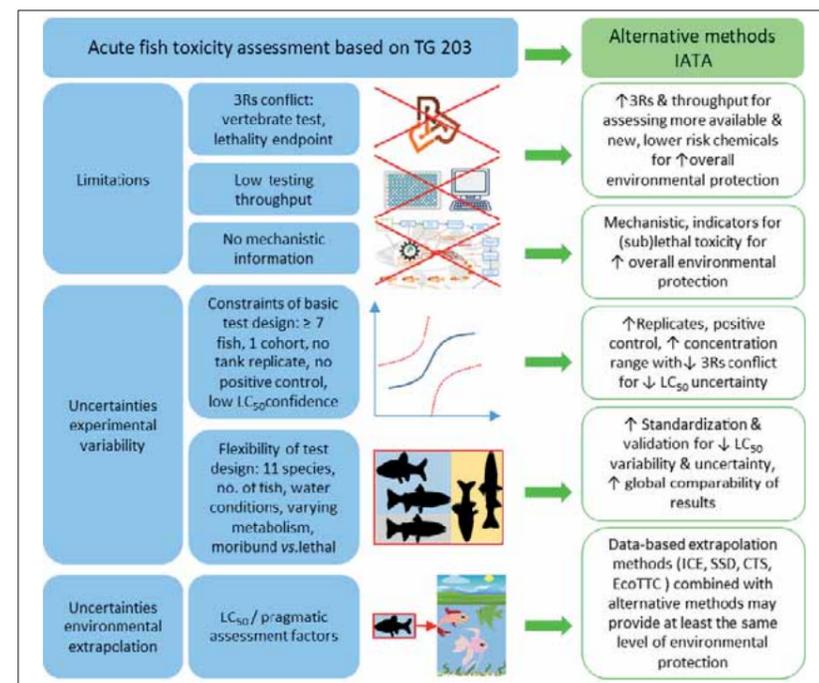


Figure 1
Limitations and uncertainties for the use of the OECD TG 203 acute fish toxicity test versus alternative methods based IATAs: Acute fish toxicity assessment based on TG 203 contains limitations in terms of significant practical disadvantages as well as uncertainties in experimental variability, both of which may be reduced by the use of alternative methods combined within IATAs.

Aquatic Ecology and Toxicology Group as components of a test battery for sensitive and reliable detection of a variety of specific endpoints.

Research Highlights since 2017

Since the 2017 COS report, major activities of the Aquatic Ecology and Toxicology Group at the COS have focused on the following issues:

General toxicity testing: Behavior-based endpoints in (zebra)fish embryos to complement the list of endpoints for the identification of neurotoxicity (Zindler et al. 2020). For various cytochrome P450 isoforms, the biotransformation capacity of various developmental stages of (zebra)fish has been identified (Loerracher et al. 2021). A major review article on fish embryo biotransformation capacities has been issued. Together with the members of an OECD expert group, major contributions have been made to the discussion about the suitability of the fish embryo toxicity test as an alternative for acute toxicity testing with adult fish (Paparella et al. 2021).

Teratogenicity testing: Within the Horizon 2020 project EU-ToxRisk, attempts have been to establish the zebrafish embryo as an alternative to mammalian teratogenicity testing (Brotzmann et al. 2021). Atlases have been developed to systemize and categorize morphopathological alterations in zebrafish development (von Helffeld et al. 2020).

Adverse effects of pharmaceuticals: Within the scope of a cooperation with Karlsruhe and Tübingen Universities, a battery of bioassays has been established to characterize adverse effects of neuroactive pharmaceuticals (Zindler et al. 2020) as well as pharmaceuticals used to control diabetes. In parallel, effects of artificial sweeteners in fish have been studied.

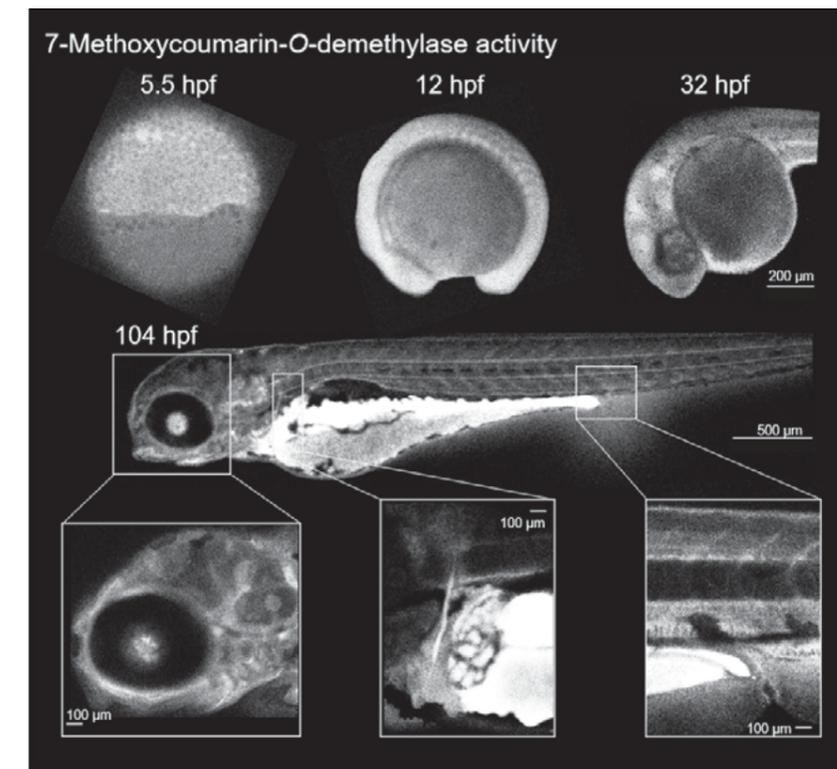


Figure 2
Developmental pattern of 7-methoxycoumarin-O-demethylase (MCO) activity in zebrafish (*Danio rerio*) embryos after 3 h exposure to 1 mM 7-methoxycoumarin (Loerracher and Braunbeck, 2021).

Micro- and nanoplastic particles: Methods have been developed to track micro- and nanoplastic particles and associated organic contaminants from the water phase via the gut to organs of various developmental stages of zebrafish (Batel et al. 2018, 2020). Together with the MiWa consortium, a major review paper on the occurrence and fate of microplastic particles has been compiled (Triebkorn et al. 2019).

Endocrine disruption: Together with Dr. Lisa Baumann and a larger consortium within EU-funded programs, endpoints for thyroid-related endocrine disruption are implemented in existing or novel OECD guidelines as well as adverse outcome pathways (AOPs; see research profile Dr. Lisa Baumann).

Future 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 cover more specific modes of bioactivation, teratogenicity, neurotoxicity and immunotoxicity. Experiments into fate and effects of microplastic particles will be extended to elucidate effects by nanoplastic particles. With respect to thyroid-related endocrine disruption, manifold gaps of knowledge will have to be closed in upcoming years.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 61, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 33, Number of citations 2017-2021: 677

Brotzmann, K., Wolterbeek, A., Kroese, D., and **Braunbeck, T.** (2021). Neurotoxic effects in zebrafish embryos by valproic acid and nine of its analogues: the fish-mouse connection? *Arch. Toxicol.* 95, 641–657.

Loerracher, A.K., and **Braunbeck, T.** (2021). Cytochrome P450-dependent biotransformation capacities in embryonic, juvenile and adult stages of zebrafish (*Danio rerio*) – a state-of-the-art review. *Arch. Toxicol.* 95, 2299-2334.

Paparella, M., Scholz, S., Belanger, S., Bichere, P., **Braunbeck, T.**, Connors, K., Faßbender, C., Halder, M., Lillicrap, A., Liska, R., Schirmer, K., Stoddart, G., Thomas, P., and Walter-Rohde, S. (2020). Limitations and uncertainties of acute fish toxicity assessments are reducible by alternatives. *ALTEX* 38, 20-32.

von Hellfeld, R., Brotzmann, K., Baumann, L., Strecker, R., and **Braunbeck, T.** (2020). Adverse effects in the fish embryo acute toxicity (FET) test: a catalogue of unspecific morphological changes versus more specific effects in zebrafish (*Danio rerio*) embryos. *Environ. Sci. Eur.* 32:122

Zindler, F., Tisler, S., Loerracher, A.K., Zwiener, C. and **Braunbeck, T.** (2020). Norfluoxetine is the only metabolite of fluoxetine in zebrafish (*Danio rerio*) embryos that accumulates at environmentally relevant exposure scenarios. *Environ. Sci. Technol.* 54, 4200-4209

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Fields of Interest

The focus of my work is the endocrine system of zebrafish (*Danio rerio*), which I use as research model to understand the development of different organs and how environmental pollutants can affect these systems, leading to adverse effects in wildlife.



Summary and outlook

Endocrine disrupting chemicals (EDCs) derive from various, mostly man-made sources such as pesticides, pharmaceuticals and cosmetics. EDCs induce adverse effects by disrupting the hormone system, and they are associated with altered reproductive function in humans and wildlife, increased incidence of cancer, disrupted growth and (neuro-) developmental delays in offspring, as well as changes in immune function. To investigate these issues, my work with zebrafish combines basic as well as applied research aspects and, thus, creates a unique interdisciplinary combination of developmental biology, endocrinology and ecotoxicology. My current and future work is focused on the interaction of the thyroid hormone system with sensory organ development; e.g., we could demonstrate that different thyroid-disrupting chemicals induce morphological and functional changes in early development of zebrafish eyes (Fig. 1).

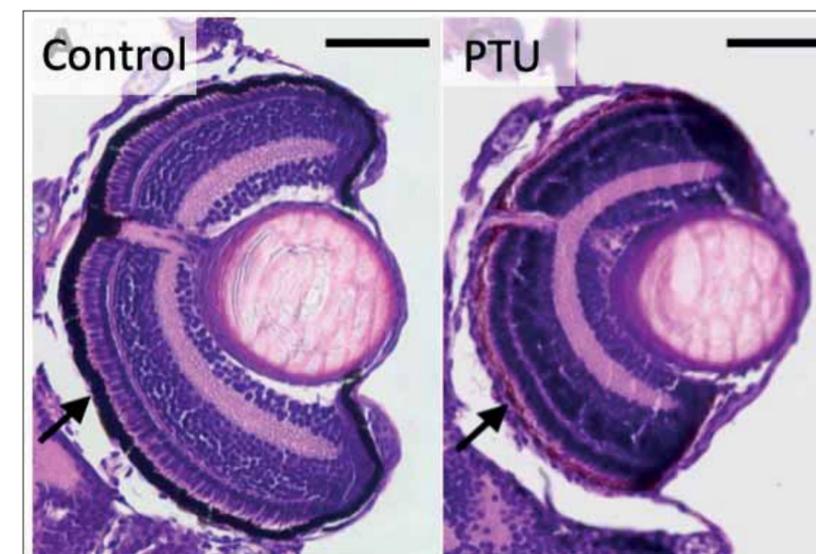


Figure 1
Histological section of zebrafish eyes at 5 days post fertilization. Left: control fish with normal retinal structures and retinal pigment epithelium (arrow); right: fish exposed to propylthiouracil (PTU) from 0-5 days post fertilization with reduced eye size, disrupted retinal structures and reduced pigmentation of the retinal pigment epithelium (arrow).

Research Highlights since 2017

Over the last years, I have focused on the thyroid hormone (TH) system, which is highly conserved across vertebrates for regulation of different developmental processes. This makes the TH system an extremely interesting model to understand fundamental mechanisms of vertebrate development and to investigate the impact of environmental EDCs on early development. In fish, especially the development of the eyes is crucial for survival of developing larvae. Our data demonstrate that different modes of EDCs result in uniform apical responses of disrupted eye development in zebrafish, leading to cellular changes in the retina (altered photoreceptor structure and patterning) and impaired visual capacities of the larvae (altered optokinetic response and photomotor behavior). Moreover, with a transcriptomic analysis of the eyes of exposed zebrafish, we identified different TH-regulated pathways of eye development and function, which were differentially expressed following the treatments with different EDCs. These results provide first insight into the transcriptional consequences of thyroid disruption in developing fish, and, at the same time, advance our knowledge on the role of THs in the regulation of eye development in fish. Based on our research data and available literature, we have developed an AOP (adverse outcome pathway) for thyroid disruption of fish eye development (Fig. 2), which has been submitted to the AOP Wiki (<https://aopwiki.org/aops/363>). AOPs represent an important conceptual framework to support ecotoxicology research and risk assessment by allowing to link a molecular initiating event to an adverse outcome at a population-relevant level of biological organization. Our AOP demonstrates how environmental EDCs can have severe impact on survival of fish by disrupting proper development of their visual system.

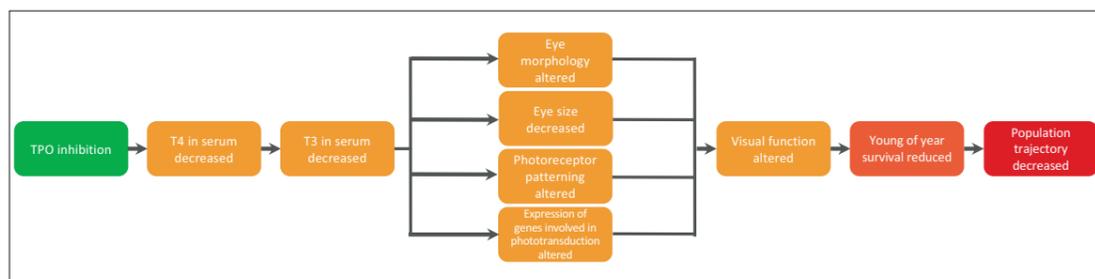


Figure 2
AOP (adverse outcome pathway)
for thyroid disruption of fish eye
development

Future directions

While our knowledge on the interaction of THs with eye development is increasing, there remain big gaps regarding the impact of TDCs on the development of the nervous system and associated sensory organs of fish. In addition to the optic sense, fish also rely on a highly evolved olfactory sense, as well as mechanosensation, i.e. orientation and “hearing” with their lateral line, a system unique to fish and amphibians. Research on the TH regulation of these two ecologically highly relevant senses is scarce, but recent studies indicate that THs are indeed important regulators of their development. We will address the still existing knowledge gaps on TH-regulated development of fish eyes, lateral line and olfactory epithelium, and set them into an ecological context by investigating the impact of EDCs on these very important sensory systems. We will assess the influence of THs on development of these organs at the molecular, morphological, physiological and behavioral level to identify critical endpoints for new AOPs of thyroid disruption in fish.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 12, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 4, Number of citations 2017-2021: 236

Holbech, H., Matthiessen, P., Hansen, M., Schüürmann, G., Knapen, D., Reuver, M., Flamant, F., Sachs, L., Kloas, W., Hilscherova, K., Leonard, M., Arning, J., Strauss, V., Iguchi, T., **Baumann, L.** (2020). ERGO: Breaking down the wall between human health and environmental testing of endocrine disruptors. *International Journal of Molecular Sciences*, DOI: 10.3390/ijms21082954

Batel, A., **Baumann, L.**, Carteny, C., Cormier, B., Keiter, S., Braunbeck, T. (2020). No effects in histological, enzymatic and chemical analyses upon long-term ingestion of differently sized microplastic particles in zebrafish (*Danio rerio*). *Marine Pollution Bulletin*, DOI: 10.1016/j.marpolbul.2020.111022

Baumann, L., Holbech, H., Weltje, L., Moissl, A., Hennies, M., Schmidt-Posthaus, H., Segner, H., Braunbeck, T. (2019). Does hepatotoxicity interfere with endocrine activity in zebrafish (*Danio rerio*)? *Chemosphere*, DOI: 10.1016/j.chemosphere.2019.124589

Baumann, L., Segner, H., Ros, A., Knapen, D., Vergauwen, L. (2019). Thyroid disruptors interfere with molecular pathways of eye development and function in zebrafish. *International Journal of Molecular Sciences*, DOI: 10.3390/ijms20071543

Rehberger, K., Werner, I., Hitzfeld, B., Segner, H., **Baumann, L.** (2017). 20 years of fish immunotoxicology – what we know and where we are. *Critical Reviews in Toxicology*, DOI: 10.1080/10408444.2017.1288024

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Fields of Interest

Targeted genome editing,
developmental biology,
stem cells,
congenital disorders of glycosylation



Summary and outlook

Patients suffering from the rare Congenital Disorders of Glycosylation (CDG) show a variety of multisystemic phenotypes (with late onset) mainly affecting skeletomuscular and neuronal structures. We still lack understanding how such diverse, yet specific phenotypes come about. To investigate pathways affected by hypoglycosylation, we use state-of-the-art targeted genome editing to establish mutant medaka fish lines based on the genetics of CDG-patients. These mutants strikingly recapitulate the multisystemic phenotypes described in patients. To molecularly dissect the onset and progression of these phenotypes, we integrate genetics, transcriptomics, phenotyping and imaging as well as lipidomics, glycomics and (glyco-) proteomics to identify phenotype causing target candidates of *N*-glycosylation, *O*- and *C*-mannosylation. In the organismal context, we finally want to understand how systemic hypoglycosylation differentially impacts on different cell types.

Research Highlights since 2017

In close collaboration with members of the Wittbrodt lab, I developed the online CRISPR and base editor prediction tools CCTop (Stemmer, et al. 2015) and ACEofBASEs (Cornean, et al. 2021) as well as a method for precise integration of single copy large donor fragments via homology directed repair (Gutierrez, et al. 2018) as well as rapid phenotyping and genotyping pipelines (Hammouda, et al. 2019). In the last years, I generated precisely edited medaka fish also within the first funding round of the Research Unit FOR 2509 "The concert of dolichol-based glycosylation: from molecules to disease models". Being involved in the project P10 ("The Role of ER-Resident Glycosylation in Vertebrate Neural Development") led by Joachim Wittbrodt, we used precision genome editing to establish defined allelic variants based on the genetics of patients suffering from the rare genetic diseases addressed as Congenital Disorders of Glycosylation (CDG). In these patients, glycosylation - the post-translational protein modification - is affected. ER-resident glycosylation mainly comes in three flavors: *N*-glycosylation, *C*- and *O*-mannosylation. Applying targeted genome editing to core players of these different pathways resulted in specific, yet different phenotypes.

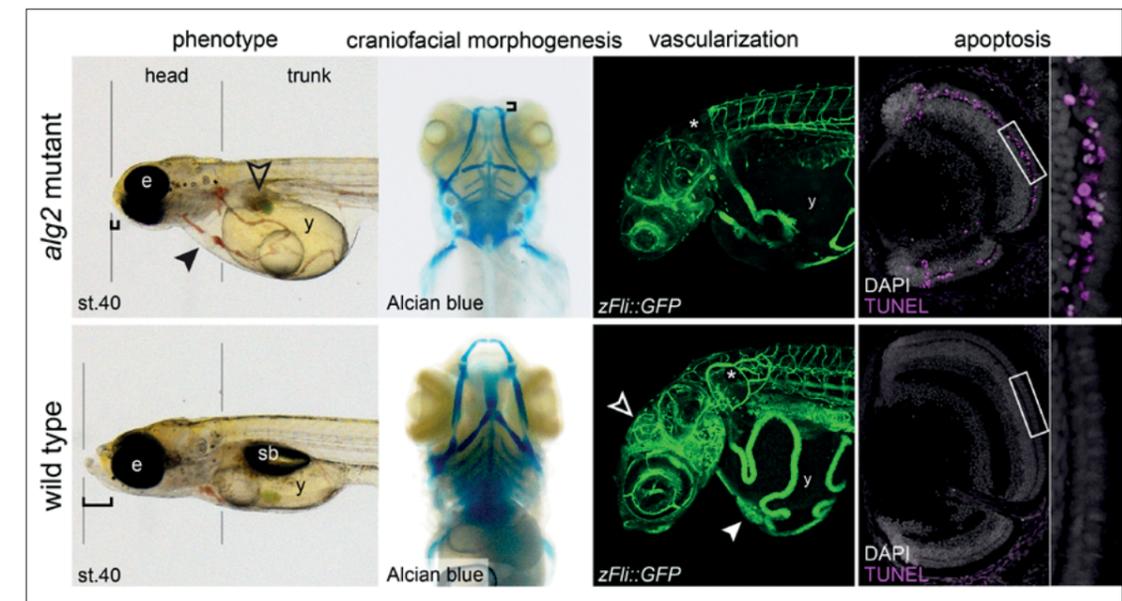
N-glycosylation

A translational model for the alpha-1,3/1,6-mannosyltransferase (Alg2) in medaka not only recapitulated the multisystemic phenotypes described in ALG2-CDG patients (e.g. craniofacial dysmorphisms and affected vasculature), but interestingly revealed that a systemic hypo-*N*-glycosylation status differentially impacts on different cell types and tissues during late embryogenesis (Gücüm, et al. 2021). In collaboration with groups of the Research Unit FOR 2509, we confirmed that the mutant fish embryos exhibited a hy-

po-*N*-glycosylation like the ALG2-CDG index patient. Strikingly, proteomics of *alg2* mutant embryos revealed two modes of action: a compensatory yet insufficient effort depicted by the upregulation of glycosylation-relevant factors, and secondly, a particular reduction of rod-photoreceptor-specific proteins reflected by the progressive loss of rod-photoreceptors, a condition known as retinitis pigmentosa.

C-Mannosylation

Besides *N*-glycosylation, we investigated the impact of aberrant *C*-mannosylation upon targeted mutation of *dpy19l1* and *dpy19l3* in medaka (Cirksena, et al. 2021). *C*-mannosylation of thrombospondin type 1 repeats containing proteins is necessary for their folding, secretion and function. In cell culture, our collaboration partners found ADAMTS16 to be reduced in a secretome analysis. The *dpy19l3* mutant embryos partially developed coloboma, i.e. a failure in optic fissure closure, a process driven by ADAMTS16.



Future directions

In the second funding round of FOR 2509, I am acting as project leader of project P10 and will build on the success of my previous work to establish and investigate further CDG-patient-based mutant alleles in medaka, namely PMM2- and Alg3-CDG for *N*-glycosylation, Pomt1- and Pomt2-CDG for *O*-Mannosylation and DPY19L1-4-CDG for *C*-mannosylation. I will apply our established collaborative pipeline to unravel target proteins of aberrant glycosylation to investigate necessary glycosylation sites on these candidates for potentially causing the investigated phenotypes.

I will further exploit the unique opportunity of the medaka *alg2* mutant model being fully rescuable by injection of full length *alg2* mRNA for linear structure-function analysis. Further, the proteins downregulated in the *alg2* mutant eyes will be analyzed for potential *N*-glycosylation sites and causality to induce retinitis pigmentosa when mutated. As a member of the Wittbrodt lab I will continue developing advanced CRISPR tools for biomedical and basic research.

Figure 2

The systemic hypo-*N*-glycosylation state in homozygous *alg2*-mutant medaka embryos results in a multisystemic phenotype with late onset. Craniofacial cartilages and vascularization are dramatically underdeveloped. Although *N*-glycosylation is globally reduced, exclusively the rod-photoreceptor cells in the retina undergo apoptosis, a condition known as retinitis pigmentosa.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 17, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 3, Number of citations 2017-2021: 1270

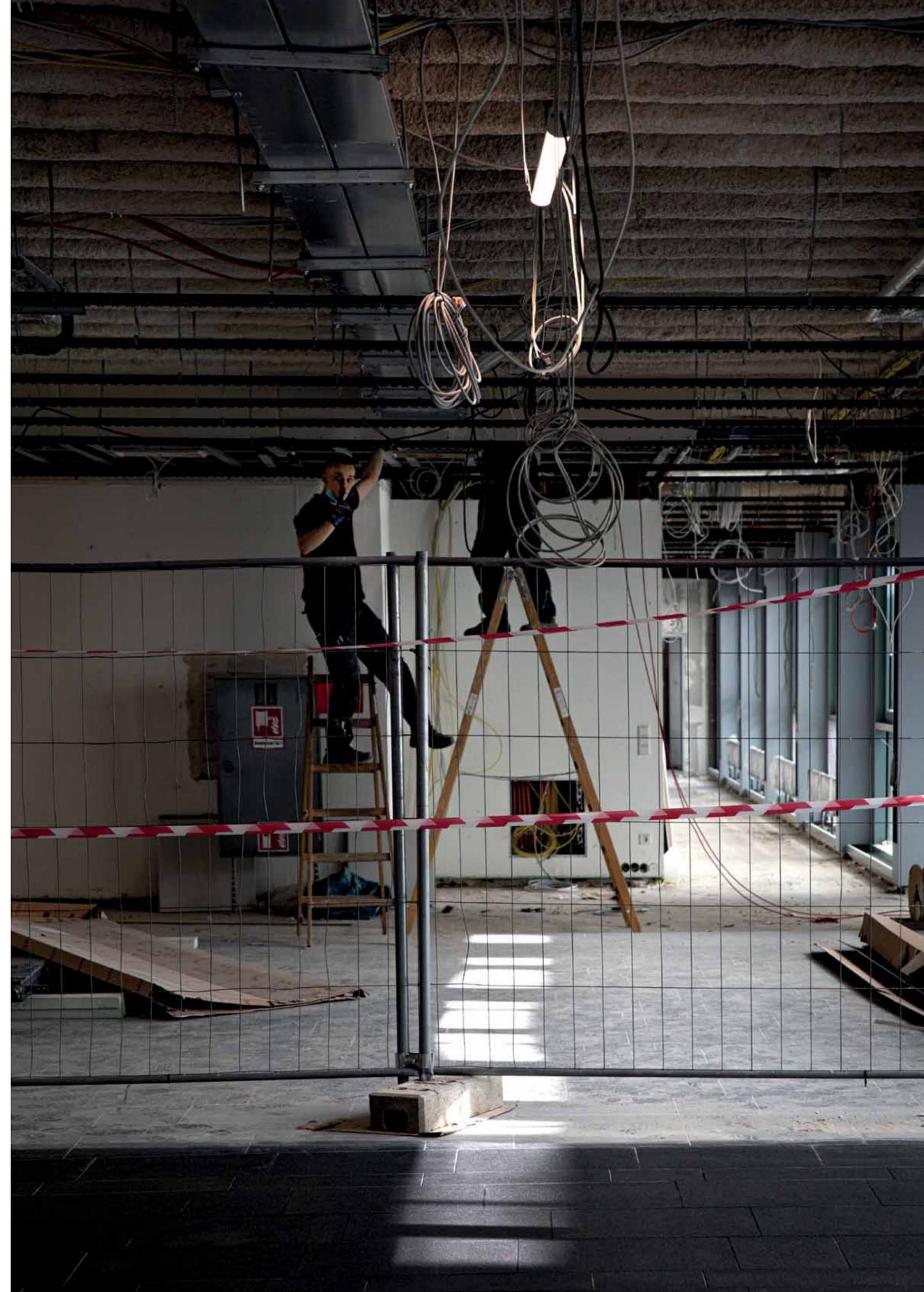
Cornean, A., Gierten, J., Welz, B., Mateo, J., **Thumberger, T.** and Wittbrodt, J. (2021). Precise *in vivo* functional analysis of DNA variants with base editing using ACEofBASEs target prediction. bioRxiv

Gücüm, S., Sakson, R., Hoffmann, M., Grote, V., Becker, C., Pakari, K., Beedgen, L., Thiel, C., Rapp, E., Ruppert, T., **Thumberger, T.** (2021). A patient-based medaka *alg2* mutant as a model for hypo-N-glycosylation. *Development* 148, 11, dev199385.

Cirkseña, K., Hütte, H. J., Shcherbakova, A., **Thumberger, T.**, Sakson, R., Weiss, S., Jensen, L. R., Friedrich, A., Todt, D., Kuss, A. W., et al. (2021). The C-Mannosylome of Human Induced Pluripotent Stem Cells Implies a Role for ADAMTS16 C-Mannosylation in Eye Development. *Mol Cell Proteomics* 20, 100092.

Hammouda, O. T., Böttger, F., Wittbrodt, J. and **Thumberger, T.** (2019). Swift Large-scale Examination of Directed Genome Editing. *Plos One* 14, e0213317.

Gutierrez-Triana, J. A., Tavhelidse, T., **Thumberger, T.**, Thomas, I., Wittbrodt, B., Kellner, T., Anlas, K., Tsingos, E. and Wittbrodt, J. (2018). Efficient single-copy HDR by 5' modified long dsDNA donors. *Elife* 7, e39468.



2.27 DR. SEBASTIAN WOLF INDEPENDENT RESEARCH GROUP

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Fields of Interest

Cell wall, signalling, development,
vascular patterning, receptors

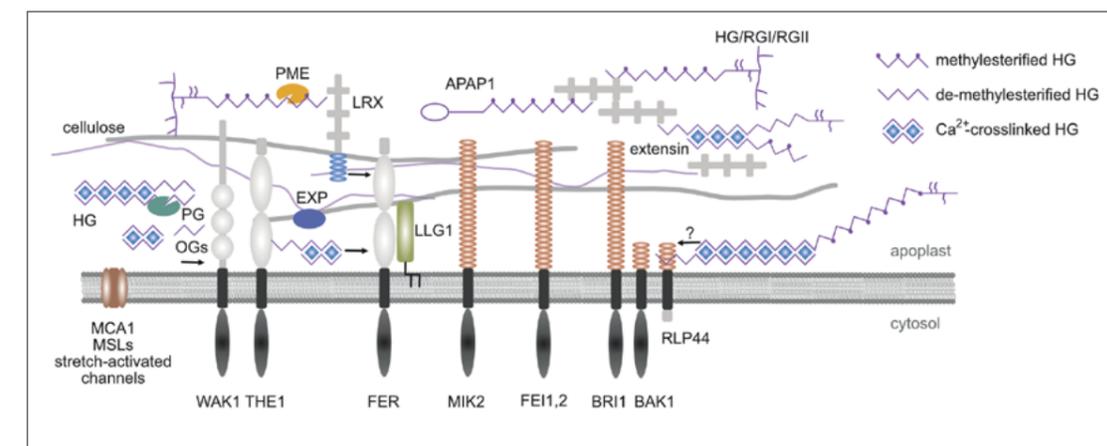


Summary and outlook

Our research aims to decipher how signals from the plant's extracellular matrix, the cell wall, are generated and perceived, and how this cell wall-mediated signalling intersects with the regulatory mechanisms of plant physiology and development. Cell walls encase each plant cell and tie neighbouring cells to each other, eliminating cell migration as a means for shape generation. Instead, cell walls control morphogenesis through selective and coordinated restriction of cell expansion. To control growth, but also to respond to environmental cues, the physical and chemical properties of the cell wall are assumed to be under constant surveillance (Figure 1). We have discovered that information about the state of pectin, the most complex and dynamic cell wall component, is conveyed to intracellular signalling pathways that regulate growth and stress responses, but also the maintenance of cell identity. Focusing on cell fate patterning in meristems, we study how cell wall signalling affects cellular decision making. A particular emphasis of our work rests on the plasma membrane and its multitude of cell surface receptors as the interface between cell wall and the cell interior. We mainly use the reference plant *Arabidopsis thaliana* as a model system, but are also interested in cell wall biology in plants of the genus *Miscanthus*, widely regarded as one of the most promising bioenergy crops, to improve biomass valorisation. We have identified a battery of cell wall biosynthesis-driving *Miscanthus* transcription factors and aim to unravel the transcriptional networks containing potential targets for cell wall optimization.

Figure 1

Overview over cell wall receptor candidates with simplified cell wall components. Receptors of the WAK (WAK1), CrRLK1L (THE1, FER), and LRR-RLK/RLP (MIK2, RLP44) classes are indicated. HG = homogalacturonan, RGI = rhamnogalacturonan I, RGI2 = rhamnogalacturonan II, PME = pectin methyltransferase, PG = polygalacturonase, EXP = expansin. LRX = leucine-rich repeat extensin proteins.



Research Highlights since 2017

Our work has identified a pathway linking surveillance of the major cell wall component pectin with brassinosteroid (BR) signalling, a plant hormone pathway critically involved in growth control. This feedback from the wall is integrated with BR signalling at the plasma membrane, through RECEPTOR-LIKE PROTEIN 44 (RLP44), which upon triggers from the cell wall is required and sufficient to elicit BR signalling activation by interacting with the BR receptor complex (Figure 1). Our data also demonstrate that in the vasculature of the root, the BR receptor complex is required to activate RLP44, which in turn promotes procambial cell identity by activating the receptor complex for the peptide hormone phytosulfokine (PSK). Interestingly, the BR and PSK receptor complexes compete for RLP44. These results are in line with the emerging theme of dynamic, promiscuous, and flexible interactions among plasma membrane receptor networks integrating signalling information in order to tune cellular responses to external cues. In light of the plethora of possible receptor interactions at the plasma membrane, an important question is how specific responses can be achieved. We could show that routing of RLP44 into PSK or BR signalling is encrypted in its phosphorylation state, which is responsive to the cell wall state. Currently, we investigate whether the integration of cell wall and BR/PSK signalling is controlled by membrane sub-compartmentalization and cell wall interaction. Importantly, our work described above also revealed a novel function for PSK receptor-mediated signalling, depending on the interaction with RLP44: the maintenance of cell identity. Loss of PSK signalling results in a cell fate switch of procambial cells towards xylem identity. Thus, cell wall signalling is integrated with intracellular regulatory networks balancing differentiation, cell fate maintenance, and proliferation. The finding that cell wall signalling can affect cell fate, although initially surprising, is reminiscent of the role of the animal extracellular matrix in stem cell maintenance and the control of cell differentiation, and prompted us to investigate cell identity control by the cell wall in a dedicated ongoing project. To facilitate cell wall manipulation in a temporally and spatially controlled manner, we devised, in collaboration with the group of Thomas Greb, a toolkit for inducible, cell type-specific expression that covers many cell types in the three main meristems, the root apical meristem, the shoot apical meristem, and the vascular cambium (Figure 2). Since publication of our toolkit, many scientists have requested seeds and plasmids from our lab or from public repositories, indicating that our resource caters to a widespread need. For our own research, this expression system has been instrumental in modifying cell wall properties in individual meristem domains, resulting in the finding that specific cell wall properties are required to maintain stem cell identity. In addition, we could reveal that cell wall-triggered BR signalling exerts cell autonomous and non-cell autonomous effects that differ between cell types. Moreover, in collaboration with Sigal Savaldi-Golstein (Technion, Haifa, Israel),

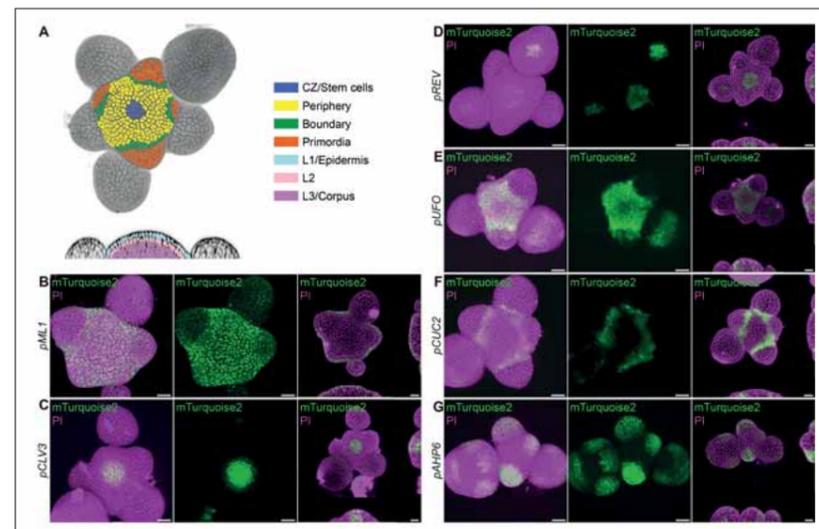


Figure 2
Cell type-specific expression resource for trans-activation in SAM domains. A) overview over SAM architecture. B-G) Reporter (ER-localized mTurquoise2) trans-activation by SAM domain-specific driver lines.

we used the expression system to reveal that optimal levels of BR signalling are required for normal cell division plane orientation in the root meristem. Currently, in collaboration with Holger Puchta (KIT, Karlsruhe), we use a modified, more efficient version of CRISPR/Cas9 in combination with our set of trans-activation lines to eliminate individual genes from a cell type or whole cell types from an organ in an inducible manner. The ultimate goal of this collaboration (funded by the “Heidelberg Karlsruhe Strategic Partnership”) is to increase the phenotypic range of relevant traits through selective elimination of cell types and silencing of individual meristems.

Species of the genus *Miscanthus* are considered the most promising group of bioenergy crops, as they combine high biomass production with low input requirements. However, how secondary cell wall biosynthesis, the main driver of terrestrial biomass accumulation, is regulated in *Miscanthus* is largely unknown. In close collaboration with the group of Thomas Rausch, we have identified key genes involved in secondary cell wall biosynthesis from *Miscanthus* and we could delineate a transcription factor hierarchy consisting of NAC and MYB proteins regulating this process. Using RNA-seq analysis in combination with cell wall profiling, we found that individual members of the small MYB transcription factor family we isolated, differ in their target spectrum of regulated genes, which is reflected in biotechnologically interesting differences in cell wall, particularly in lignin, quality. In addition, we could identify specific motifs in the regulatory sequences of the target genes of individual TFs, which will enable us to predict TF targets in the *Miscanthus* genome.

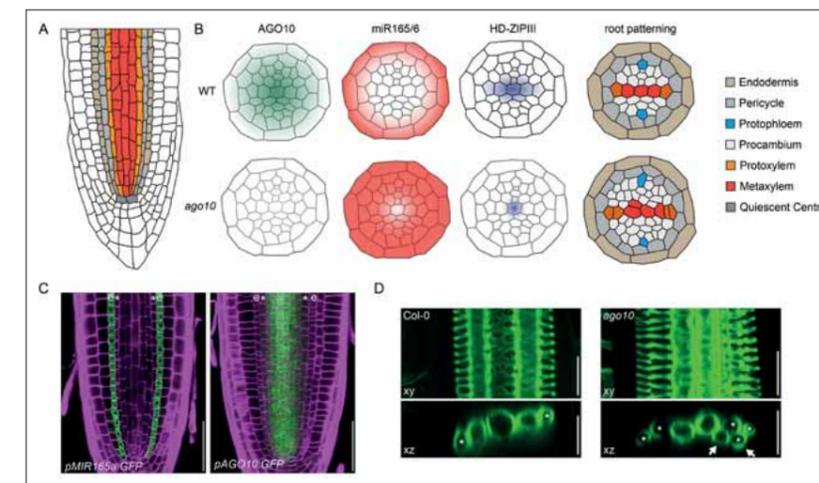


Figure 3
AGO10 is required for cell fate specification and control of formative cell division in the root. A, Schematic representation of the root meristem. B, Cross sections through the meristem 150 µm above the quiescent centre. C, Endodermal expression of a pMIR165a reporter and graded expression of the pAGO10::GFP reporter. D, Ectopic xylem patterning in ago10 due to precursor proliferation.

Future directions

The main goal of my research is to decipher how cell wall-mediated signalling intersects with the regulatory mechanisms of plant development. To this end, we will leverage our knowledge acquired over the course of the last years as well as our unpublished data to decipher integration of cell wall state and LRR-RLK mediated signalling at the plasma membrane. We will test the hypothesis that pectin state is a chemical signal that modulates growth regulation and development through. This would constitute a fully resolved cell wall signalling pathway, i.e. connect a distinct cell wall molecule with cellular signalling outcomes relevant for development. Furthermore, building on exciting preliminary data, and the generation of a suite of genetic tools, we will establish of the shoot apical meristem as a system to study the role of cell wall signalling on cell identity and patterning. The project is based on our observation that cell wall properties not only have a profound impact on the architecture of the shoot apical meristem, which harbours the stem cell niche responsible for most above-ground plant organs, but also directly affects cell identity, reminiscent of the role of extracellular matrix signalling in animal systems. This novel line of research should provide unprecedented insight into the role of feedback from the cell wall

on patterning mechanism and the control of cell identity. In addition, we hope to identify new cell wall signalling components underlying the communication between the cell intra- and the extracellular space, a fundamental theme for all organisms. Related to the regulation of cell fate acquisition in the vascular tissue, we have recently revealed that a ARGONAUTE10 is required for regulating a conserved suite of transcription factors involved in xylem specification and tailoring their activity to the environmental conditions (Figure 3). In the future, we want to unravel how xylem patterning and proliferation of xylem precursor cells in response to the environment is integrated with growth and development.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 10, Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 8, Number of citations 2017-2021: 1384

El Arbi, N., Schürholz, A.-K., Schiffner, A., Prados, I.H., Böhme, F., Wenzl, C., Zhao, X., Zeng, J., Lohmann, J.U., and **Wolf, S.** (2021). ARGONAUTE10 is required for cell fate specification and the control of formative cell divisions in the Arabidopsis root meristem. bioRxiv 10.1101/2021.02.05.429893

Holzwardt, E., Wanke, F., Glöckner, N., Höfte, H., Harter, K., and **Wolf, S.** (2020). A Mutant Allele Uncouples the Brassinosteroid-Dependent and Independent Functions of BRASSINOSTEROID INSENSITIVE 1. *Plant Physiol.* 182, 669–678.

Holzwardt, E., Huerta, A.I., Glöckner, N., Garnelo Gómez, B., Wanke, F., Augustin, S., Askani, J.C., Schürholz, A.-K., Harter, K., and **Wolf, S.** (2018). BRI1 controls vascular cell fate in the Arabidopsis root through RLP44 and phytosulfokine signaling. *Proc. Natl. Acad. Sci.* 115, 11838–11843.

Schürholz, A.-K., López-Salmerón, V., Li, Z., Forner, J., Wenzl, C., Gaillochet, C., Augustin, S., Barro, A.V., Fuchs, M., Gebert, M., et al. (2018). A Comprehensive Toolkit for Inducible, Cell Type-Specific Gene Expression in *Arabidopsis*. *Plant Physiol.* 178, 40–53.

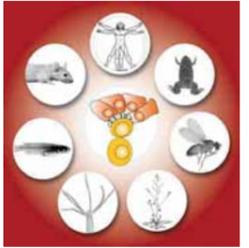
Golfier, P., Volkert, C., He, F., Rausch, T., and **Wolf, S.** (2017). Regulation of secondary cell wall biosynthesis by a NAC transcription factor from *Miscanthus*. *Plant Direct* 1, e00024.



RESEARCH CONSORTIA

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MAINTENANCE AND DIFFERENTIATION OF STEM CELLS IN DEVELOPMENT AND DISEASE



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Prof. Dr. Jan Lohmann

Centre for Organismal Studies, Heidelberg University

www.sfb873.de

The basic principles controlling stem cell self-renewal and differentiation are strikingly conserved during evolution, while at the same time regulatory pathways can differ between various stem cell systems in the same organism and between homologous stem cell niches in different organisms. The CRC873 consortium takes full advantage of a diverse set of model systems to illuminate the cellular and molecular mechanisms governing stem cell function. Thus, members of the CRC873 are in a unique position to derive important insights into the evolution of stem cells and the underlying regulatory modules, in addition to advancing our understanding of essential stem cell control mechanisms.

The overarching goal of the CRC873 is to define the regulatory principles underlying the balance between maintenance, expansion and differentiation of stem cells in diverse systems on a mechanistic level.

This question is tackled by studying intrinsic and extrinsic control of stem cell behavior in various tissues, such as blood, the nervous-system, gut, or germline in a wide range of model systems including *Arabidopsis*, *Hydra*, *Drosophila*, medaka, *Xenopus*, as well as mouse and human. In addition to analyses of normal stem cell function, we focus on diseases such as cancer or infection, since they not only can serve as steppingstones for translational research, but also represent important models for stem cell dysregulation. During the funding periods, the members of the CRC873 developed into a tightly connected community. Consequently, our subprojects are not only productive, but collaborations between research groups working on diverse stem cell systems and model organisms have been established and brought to fruition. Importantly, promising new directions emerged as a direct result of these interactions. In the second funding period, we pioneered the use of quantitative approaches, such as biophysical analyses or genomic approaches with single cell resolution to describe stem cell regulation at the systems level. Another important aspect was the development of innovative tools for *in vivo* lineage tracing and our members made important contributions in this area. These experimental strategies generated large amounts of quantitative data, and therefore standardized data extraction and mining, as well as mathematical modeling have become essential for an increasing number of projects.

Building on these achievements, several exciting new directions have emerged in the reporting period. At the biological level, the topic of plasticity has taken center stage. Stem cell systems dynamically respond to challenges, such as infections, or abiotic stress, and the regulatory machinery has evolved to enable appropriate responses across diverse systems. Consequently, the mechanisms underlying stem cell plasticity, such as the role of immune or stress associated pathways, currently represents a focus of our research. Similarly, the question of the evolutionary origin of stem cells and their regulatory mechanisms attracts more attention, based on the realization that work on diverse systems has converged on a small number of important principles. At the technical and conceptual level, the transition from bulk to single cell approaches has been a major technological innovation, since interrogating individual cells instead of averaging across pools of cells has the potential to redefine our understanding of stem cell function and stem-cell niche interactions. Importantly, single cell assays also make evolutionary studies more meaningful, since they allow direct comparisons of highly defined cell types and thus filter out the noise caused by divergent cell compositions. Reflecting the fundamental biology of stem cells, the CRC873 is structured into two major focus areas:

(A) Mechanisms of stem cell self-renewal: Using suitable model systems we elucidate essential molecular mechanisms of stem cell control and identify conserved and divergent regulatory modules governing the fundamental decision process of self-renewal and differentiation. These results serve as a resource for comparative studies to define the pathways regulating stem cell fate during development and disease.

(B) Cell-cell interactions in the stem cell niche: In addition to cell-intrinsic mechanisms, extrinsic cues mediated by the microenvironment, commonly referred to as the stem cell niche, maintain stem cell fate and control the balance between self-renewal and differentiation. This research area focuses on the nature and function of the cell types comprising the niche and the molecules involved in the bi-directional cross talk between the niche and the corresponding stem cells in normal and disease states. Cross species and cross kingdom comparisons are used to identify the most relevant components that generate the functional stem cell-niche units.

In summary, the CRC873 focuses on two key aspects of stem cell biology, namely control of self-renewal versus differentiation, as well as stem cell-niche interactions in a dynamic environment. Importantly, we approach these features across species and kingdom boundaries *in vitro* and *in vivo*. Our mission is to mechanistically elucidate the components identified so far and to rigorously compare the defined modules between the diverse systems.

Heidelberg University, Centre for Organismal Studies

Jun. Prof. Dr. Lázaro Centanin
Dr. Ulrike Engel
Prof. Dr. Thomas Greb
Prof. Dr. Thomas Holstein
Prof. Dr. Ingrid Lohmann
Prof. Dr. Jan Lohmann
Prof. Dr. Gislene Pereira
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Prof. Dr. Carmen Ruiz de Almodóvar

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Prof. Dr. Henrik Kaessmann

Heidelberg University, Institute of Applied Mathematics

Prof. Dr. Anna Marciniak-Czochra

Heidelberg University, Institute of Physical Chemistry

Prof. Dr. Motomu Tanaka

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Prof. Dr. Sacha Dietrich
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Prof. Dr. Carsten Müller-Tidow

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Prof. Dr. Michael Boutros
Prof. Dr. Gergana Dobreva

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Dr. Marieke Essers
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Dr. Michael Milsom
Prof. Dr. Christof Niehrs
Prof. Dr. Hans-Reimer Rodewald
Prof. Dr. Claudia Scholl
Prof. Dr. Andreas Trumpp



Opening of the CRC873 symposium 2017 in the "Alte Aula", the old central lecture hall of Heidelberg University.

Key publications

Sloan, J., Hakenjos, J., P., Gebert, M., Gumiero, A., Stier, G., Wild, K., Sinning, I. and Lohmann, J. U. (2020). Structural basis for the complex DNA binding behavior of the plant stem cell regulator WUSCHEL. *Nature Communications* 11, 2223.

Stolper, J., Ambrosio, E. M., Danciu, D. P., Buono, L., Elliott, D. A., Naruse, K., Martínez-Morales, J. R., Marciniak-Czochra, A., Centanin, L. (2019). Stem cell topography splits growth and homeostatic functions in the fish gill. *Elife* 8:e43747.

Tsingos, E., Höckendorf, B., Sütterlin, T., Kirchmaier, S., Grabe, N., Centanin, L., Wittbrodt, J. (2018). Retinal stem cells modulate proliferative parameters to coordinate post-embryonic morphogenesis in the eye of fish. *Elife* 8:e42646.

Papagiannouli, F., Tamirisa, S., Rempel, E., Ermakova, O., Trost, N., Zhou, J., Mundorf, J., Brunel, S., Ruhland, N., Boutros, M., Lohmann, J.U., and Lohmann, I. (2018). Decoding the regulatory logic of the *Drosophila* male stem cell system. *Cell Reports* 24, 3072-3086.

Caydasi, A. K., Khmelinskii, A., Duenas-Sanchez, R., Kurtulmus, B., Knop, M., Pereira, G. (2017). Temporal and compartment-specific signals coordinate mitotic exit with spindle position. *Nature Communications* 8, 14129.

DFG CRC1324

MECHANISMS AND FUNCTIONS OF WNT SIGNALING



since 2017

Spokesperson (1st funding period, 2017-2021):
Prof. Dr. Thomas Holstein
 Centre for Organismal Studies, Heidelberg University

Spokesperson (2nd funding period, 2021-2025):
Prof. Dr. Michael Boutros
 BioQuant and Medical Faculty Mannheim, Heidelberg University and DKFZ

<http://sfb1324.de/>

Wnt signaling pathways are of crucial importance for many biological processes and disease in animals and humans. Here, they play a decisive role in early development, cell differentiation and regeneration. Aberrant regulation of Wnt signaling can lead to severe developmental defects and diseases, including tumorigenesis. The overarching goal of the Collaborative Research Center (CRC) 1324 is to gain a mechanistic understanding of Wnt signaling pathways and to investigate its physiological consequences during development and disease in a representative spectrum of model systems. Wnt signaling comprises a complex set of key developmental and disease pathways. Wnt ligands are secreted, lipidated proteins found exclusively in animal systems. Various Wnt ligands can bind to half a dozen different receptor families to activate multiple downstream signaling cascades. These pathways converge from multiple ligand-receptor interactions to a signaling funnel of conserved cytoplasmic factors. During the last years, important progress has been made about components and mechanisms of Wnt signaling. However, a multitude of questions are raised about the specificity and context-dependency of signaling input, signaling mechanisms and the control of signaling output during development and in disease.

In the Heidelberg area we are in the unique situation to have a high density of research groups working on Wnt signaling. Within the CRC 1324, we established strong and productive collaborations between research groups working on Wnt signaling at the COS, the Medical Faculties of Heidelberg University, the Biochemistry Center (BZH), the Institute of Applied Mathematics, the European Molecular Biology Laboratory (EMBL), the German Cancer Research Center (DKFZ), the Karlsruhe Institute of Technology (KIT) and the University Göttingen. Due to the combination of complementary approaches and the integration of quantitative, cutting-edge technologies, the CRC 1324 is in the unique position to address fundamental questions of Wnt signaling. Therefore, we incorporated state-of-the-art technologies including structural biology, quantitative microscopy, genome engineering, and advanced proteomic approaches. By leveraging developmental model systems including Hydra, Xenopus, Drosophila, mouse, as well as human cell models, our research network made important contributions towards the understanding of the context-dependency of Wnt pathways in the first funding period. To mention a few highlights: We uncovered the role of Wnt signaling in genome integrity, we discovered that Wnt secretion factor Evi/Wls is controlled by regulatory ERAD (Endoplasmic-reticulum-associated protein degradation) and we discovered the role of Wnt oscillations in mesoderm segmentation and the function of altered Wnt signaling in aged neural stem cells.

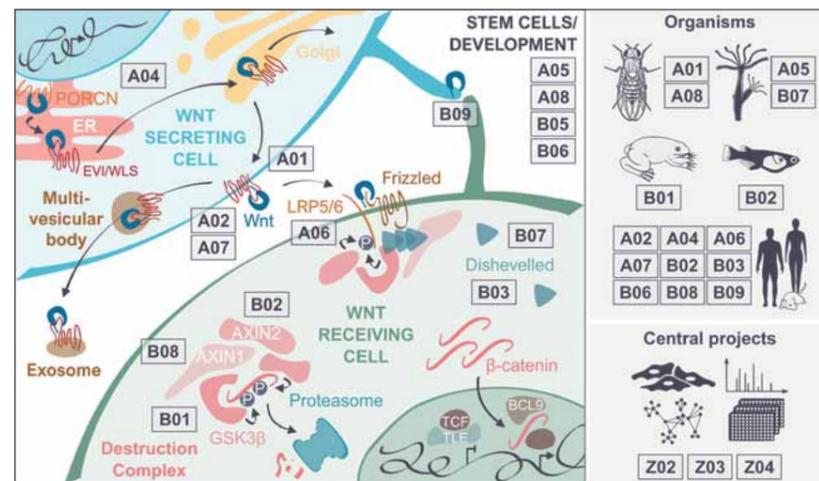
In the second funding period, we now will continue our interdisciplinary approach with a diversity of projects covering structural biology, biochemistry, biophysics, proteomics and genomics, cell biology, developmental biology and tumor biology. While maintaining a strong mechanistic focus, we are now focusing more on fundamental aspects of Wnt signaling at the organismal level. For this reason, we concentrate on two main research areas:

(A) Wnt secretion and receptor-ligand interactions. Here, we investigate how Wnt proteins are produced, modified and secreted into the extracellular space. Furthermore, we analyze Wnt ligand-receptor interactions to understand how they specify the signaling response and induce different signaling cascades.

(B) Wnt coupling to downstream and context-dependent signaling. Here, we address important questions how different Wnt pathways elicit distinct biological responses or how

Wnt signaling is coupled to different downstream factors. In addition, we analyze the spatio-temporal dynamics of Wnt signaling to understand how oscillations and wave patterns are established and regulated.

In summary, the CRC 1324 makes use of an interdisciplinary approach integrating diverse model systems and state-of-the-art technologies to address fundamental questions of the secretion and transmission of Wnt signaling and its spatio-temporal dynamics in a context-dependent manner. Hence, we will contribute towards the understanding of the complexity of Wnt signaling pathways and their role in development, regeneration and tumorigenesis.



Projects and scientific concept of the CRC 1324.

Members of the 1st and 2nd funding period

Heidelberg University

Dr. Sergio Pérez Acebrón (COS)
 Prof. Dr. Hellmut Augustin (Medical Faculty Mannheim)
 Dr. Josephine Bageritz (COS)
 Prof. Dr. Michael Boutros (BioQuant and Medical Faculty Mannheim)
 Prof. Dr. Britta Brügger (BZH)
 Dr. Ulrike Engel (COS)
 Prof. Dr. Thomas W. Holstein (COS)
 Prof. Dr. Jeroen Krijgsveld (Medical Faculty Heidelberg)
 Prof. Dr. Florian Leuschner (Medical Faculty Heidelberg)
 Prof. Dr. Ingrid Lohmann (COS)
 Prof. Dr. Anna Marciniak-Czochra (IWR)
 apl. Prof. Dr. Suat Özbek (COS)
 Prof. Dr. Gislene Pereira (COS)
 Prof. Dr. Matias Simons (Medical Faculty Heidelberg)
 Prof. Dr. Irmgard Sinning (BZH)
 Prof. Dr. Motomu Tanaka (PCI)
 Prof. Dr. Jochen Wittbrodt (COS)
 Dr. Tianzuo Zhan (Medical Faculty Mannheim)

DKFZ

Prof. Dr. Hellmut Augustin
 Prof. Dr. Michael Boutros
 Prof. Dr. Jeroen Krijgsveld
 Prof. Dr. Christof Niehrs
 Prof. Dr. Ana Martin-Villalba

EMBL

Dr. Alexander Aulehla

University of Göttingen (UMC)

Prof. Dr. Holger Bastians
 Prof. Dr. Julia Gross

KIT

Dr. Gary Davidson
 Prof. Dr. G. Ulrich Nienhaus



European Wnt Meeting 2018 hosted by the CRC 1324. (Photo: T.Schwerdt)

Key publications

Ziegler B, Yiallouris I, Trageser B, Kumar S, Mercker M, Kling S, Fath M, Warnken U, Schnölzer M, Holstein TW, Hartl M, Marciniak-Czochra A, Stetefeld J, Stöcker W, Özbek S. (2021). The Wnt-specific astacin proteinase HAS-7 restricts head organizer formation in Hydra. *BMC Biol.* 19(1):120.

Giebel N, de Jaime-Soguero A, García Del Arco A, Landry JJM, Tietje M, Villacorta L, Benes V, Fernández-Sáiz V, Acebrón SP. (2021) USP42 protects ZNRF3/RNF43 from R-spondin-dependent clearance and inhibits Wnt signalling. *EMBO Rep.* 22(5):e51415.

Inverso D, Shi J, Lee KH, Jakab M, Ben-Moshe S, Kulkarni SR, Schneider M, Wang G, Komeili M, Vélez PA, Riedel M, Spegg C, Ruppert T, Schaeffer-Reiss C, Helm D, Singh I, Boutros M, Chintharlapalli S, Heikenwalder M, Itzkovitz S, Augustin HG. (2021). A spatial vascular transcriptomic, proteomic, and phosphoproteomic atlas unveils an angiocrine Tie–Wnt signaling axis in the liver. *Dev Cell.* 56(11):1677-1693.e10.

Lee H, Seidl C, Sun R, Glinka A, Niehrs C. (2020). R-spondins are BMP receptor antagonists in Xenopus early embryonic development. *Nat Commun.* 11(1):5570

Glaeser K, Urban M, Fenech E, Voloshanenko O, Kranz D, Lari F, Christianson JC, Boutros M. (2018). ERAD-dependent control of the Wnt secretory factor Evi. *EMBO J.* 37(4).

Sonnen KF, Lauschke VM, Uraji J, Falk HJ, Petersen Y, Funk MC, Beaupeux M, François P, Merten CA, Aulehla A. (2018). Modulation of Phase Shift between Wnt and Notch Signaling Oscillations Controls Mesoderm Segmentation. *Cell.* 172(5):1079-1090.e12.

DFG RESEARCH UNIT FOR2509

THE CONCERT OF DOLICHOL-BASED GLYCOSYLATION: FROM MOLECULES TO DISEASE MODELS



since 2017

Spokesperson:

Prof. Dr. Sabine Strahl

Centre for Organismal Studies, Heidelberg University

<https://for2509.de/>

The overwhelming success of massive genome sequencing can give the impression that the key to understanding life lies only in our genome. Strikingly, however, there are other layers of complexity; one of the most diverse is the decoration of proteins by combinations of different sugar moieties, which is found in all domains of life. N-glycosylation, O-mannosylation and C-mannosylation are prevalent and highly conserved glycosylation pathways. Their precise interplay is essential for proper protein function, with effects ranging from enabling correct folding and protein localization to establishing precisely shaped gradients of secreted glycosylated morphogens. The glycosylation pathways start in the endoplasmic reticulum and in many cases the acceptor proteins receive more than one type of glycan. The crucial importance of the process is reflected by the wide range of hereditary diseases resulting from disruptions to these pathways (congenital disorders of glycosylation, CDGs) causing devastating, complex multisystemic syndromes.

The extraordinary complexity of glyco-modification has severely hampered systematic approaches aiming at decoding their action across scales. To address this challenge, the Research Unit FOR2509 has gathered experts addressing glycosylation at and across all levels - from the structural to the inter-molecular, cellular, organ and the organismal. They combine genetic, structural and lipid biochemistry, glycomics and (glyco)proteomics, molecular and cell biology, as well as developmental approaches, always with a close focus on the patients' perspective. The interdisciplinary concept of FOR2509 has proved to be very successful. Close collaborations ranging from structural studies to animal models have been established. Numerous joint projects are underway and joint publications have appeared. In December 2020 FOR2509 was extended with a total funding volume of € 1,339,511 per year.

The tight interactions of the participating groups in the first funding period have established new mechanistic insights and novel approaches that shed light on the molecular mechanisms underlying complex glycosylation disorders. Newly established animal models for CDGs provide the basis for the integration of the combined expertise to address the mechanistic implications, starting at the level of altered glycosylation and the resulting consequences for the decoration of the target proteins. Striking evidence underpins a tight interconnection of all three types of glycosylation, highlighting the need to study the complex effects as a team of synergizing experts in order to ultimately resolve structure function relationships and their consequences in the organismal context as a foundation for the development of novel diagnostic tools and new therapeutic approaches.

Members

Heidelberg University

Prof. Dr. Britta Brügger (BZH)

Dr. Thomas Ruppert (ZMBH)

Prof. Dr. Irmgard Sinning (BZH)

Prof. Dr. Sabine Strahl (COS)

Dr. Thomas Thumberger (COS)

Prof. Dr. Joachim Wittbrodt (COS)

University Hospital Heidelberg

PD Dr. Christian Thiel

Goethe-University Frankfurt

Prof. Dr. Harald Schwalbe (BMRZ)

Hannover Medical School

Dr. Hans Bakker
apl. Prof. Dr. Falk Büttner

MPI for Dynamics of Complex Technical Systems, Magdeburg

Dr. Erdmann Rapp



FOR2509 Teaching Workshop
"Protein Glycosylation in Health
and Disease" 2019 in Heidelberg

Key publications

Gücüm S, Sakson R, Hoffmann M, Grote V, Becker C, Pakari K, Beedgen L, Thiel C, Rapp E, Ruppert T, Thumberger T, Wittbrodt J. (2021) A patient-based medaka *alg2* mutant as a model for hypo-N-glycosylation. *J. Development* 148:dev199385. doi: 10.1242/dev.199385. Epub 2021 Jun 9. PMID: 34106226

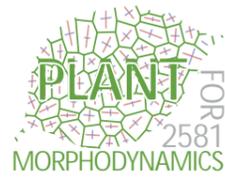
Cirksena K, Hütte HJ, Shcherbakova A, Thumberger T, Sakson R, Weiss S, Jensen LR, Friedrich A, Todt D, Kuss AW, Ruppert T, Wittbrodt J, Bakker H, Büttner FF. (2021) The C-mannosylome of human induced pluripotent stem cells implies a role for ADAMTS16 C-mannosylation in eye development. *Mol Cell Proteomics*. doi: 10.1016/j.mcpro.2021.100092. Online ahead of print. PMID: 33975020

Noor SI, Hoffmann M, Rinis N, Bartels MF, Winterhalter PR, Hoelscher C, Hennig R, Himmelreich N, Thiel C, Ruppert T, Rapp E, Strahl S. (2021) Glycosyltransferase POMGNT1 deficiency strengthens N-cadherin-mediated cell-cell adhesion. *J Biol Chem*. 296:100433. doi: 10.1016/j.jbc.2021.100433. PMID: 33610554

Chiapparino A, Grbavac A, Jonker HR, Hackmann Y, Mortensen S, Zatorska E, Schott A, Stier G, Saxena K, Wild K, Schwalbe H, Strahl S*, Sinning I.* (2020) Functional implications of MIR domains in protein O-mannosylation. *Elife* 9:e61189. doi: 10.7554/elifesciences.61189. PMID: 33357379

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DFG FOR2581 QUANTITATIVE MORPHODYNAMICS OF PLANTS

since 2017

Spokesperson:

Prof. Dr. Alexis Maizel
Centre for Organismal Studies, Heidelberg University

<http://www.for2581.de/>

How do plants shape their tissues and organs? The genetic information encoded in the DNA is an important determinant but how exactly it is used to reproducibly generate the three-dimensional shape of an organism is a long-standing question in biology. Since plants continuously form new tissues and organs, they are ideal models to study how shape is generated. Morphogenesis is a dynamic process that depends on the interplay between pattern formation, cell division, and growth. It is also a multi-scale problem: minor modifications at cell scale can translate into complex structural changes at the tissue or organ scale. As plant cells are strongly mechanically coupled, they are forced to constantly adjust their behaviour relative to each other.

The multidisciplinary research unit (RU) "Quantitative Morphodynamics of Plants" was established in 2017 to tackle the question: How do plants shape themselves? The RU FOR2581 brings together developmental biologists, computer scientists and physicists to quantitatively understand plant morphogenesis. In the last four years, the RU has catalysed remarkable conceptual advances that resulted in several high profile publications and in the development of important new tools allowing the volumetric segmentation of plants cells and advanced quantification of cells in complex tissues. The RU has had a significant impact in the community through training of a dozen young scientists, organising workshops and an international symposium.

Currently in its second phase, the RU capitalises on these successes and the strong interactions between biologists and theorists that were established. Together they formulate and test new hypotheses on how cell size, shape and mechanics contribute to the emergence of complex shapes. They develop and apply novel integrated computational tools for the quantitative analysis plant morphogenesis, from images to predictive models.

The RU is enabling the emergence of a new community of young quantitative plant developmental biologists able to combine traditional cell and molecular biology with computational and physical methods to solve biological problems.

Members

Heidelberg University

Prof. Dr. Thomas Greb (COS)
Prof. Dr. Fred Hamprecht (IWR)
Prof. Dr. Jan Lohmann (COS)
Prof. Dr. Alexis Maizel (COS)

EMBL

Dr. Anna Kreshuk

TU Munich

Prof. Dr. Karen Alim
Prof. Dr. Kay Schneitz

Max Planck Institute for Plant Breeding, Cologne

Dr. Angela Hay
Prof. Dr. Miltos Tsiantis



Participants of the Plant Growth & Form symposium organised by the FOR2581 in September 2019 in Heidelberg.

Key publications

Vijayan, A., Tofanelli, R., Strauss, S., Cerrone, L., Wolny, A., Strohmeier, J., Kreshuk, A., Hamprecht, F., Smith, R. and Schneitz, K., 2021. A digital 3D reference atlas reveals cellular growth patterns shaping the Arabidopsis ovule. *eLife* (2021);10:e63262 DOI: 10.7554/eLife.63262

D. Ramos, J.R., Maizel, A. & Alim, K. Tissue-wide integration of mechanical cues promotes effective auxin patterning. *Eur. Phys. J. Plus* 136, 250 (2021). <https://doi.org/10.1140/epjp/s13360-021-01204-6>

Wolny, A., Cerrone, L., Vijayan, A., Barro, A. V., Louveaux, M., Wenzl, C., Strauss, S., Wilson-Sánchez, W., Lymbouridou, R., Steigleder, S., Pape, et al. (2020). "Accurate And Versatile 3D Segmentation Of Plant Tissues At Cellular Resolution." *Elife* 9. doi: 10.7554/eLife.57613

Kierzkowski, D., Runions, A., Vuolo, F., Strauss, S., Lymbouridou, R., Routier-Kierzkowska, A.-L., Wilson-Sánchez, D., Jenke, H., Galinha, C., Mosca, G., et al. (2019). A Growth-Based Framework for Leaf Shape Development and Diversity. *Cell* 177, 1405-1418.e17.

Vilches Barro, A., Stöckle, D., Thellmann, M., Ruiz-Duarte, P., Bald, L., Louveaux, M., von Born, P., Denninger, P., Goh, T., Fukaki, H., et al. (2019). Cytoskeleton Dynamics Are Necessary for Early Events of Lateral Root Initiation in Arabidopsis. *Current Biology* 29, 2443-2454.e5.

CORE FACILITIES

4

4.1 BOTANIC GARDEN AND HERBARIUM

BOTANIC GARDEN AND HERBARIUM

Director: Prof. Dr. Marcus Koch
 Curator Living Collection: Dr. Andreas Franzke
 Curator Herbarium HEID: Dr. Peter Sack
 Technical Manager: René Bruse
 Centre for Organismal Studies COS Heidelberg
 Heidelberg University
 Im Neuenheimer Feld 361
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 E-Mail: bgsekretariat@cos.uni-heidelberg.de

History

Heidelberg University Botanic Garden, established in 1593 and therefore among the world's oldest botanical gardens, was originally located in the vicinity of Heidelberg's famous castle as a garden of medicinal plants long before the advent of biological and life sciences.

After six relocations, the Garden was reopened in 1915 at its present site. Following World War II and the loss of all greenhouse collections, the plant collections were continuously enlarged, especially under the directorship of Werner Rauh from 1960 to 1982. These historic collections—succulents, xerophytes from Madagascar, bromeliads and tropical orchids—still form the basis of the indoor Garden's specimens. The affiliated Herbarium HEID encompasses at least 50,000 species, represented by approx. 350,000 specimens with a particular focus on South American taxa, especially from the Andes, and African taxa, mainly from Madagascar and Kenya. The »old herbarium« collections originate from the early 19th century. Significant parts of the »new herbarium« contain 50,000 specimens, particularly cacti, bromeliads, orchids and tropical ferns, collected by Werner Rauh and colleagues. Approximately 40,000 vouchers can be attributed to the research activities of its current director, Marcus Koch. HEID encompasses approx. 2,500 vouchers representing type material of nearly 1,600 taxa. However, discoveries of type specimens believed to be lost or not yet identified as such, still occur every year. A major research and curatorial focus in HEID is on the Brassicaceae family with its 4,000 species, encompassing various important crops and several of the most important model organisms of contemporary plant research programmes.

Mission, Objectives & Vision

The Mission of Heidelberg Botanic Garden is the conservation and development of its collections and promoting the discovery, understanding, responsible use and enjoyment of plant biodiversity. The living collection with approx. 12,000 accessions and the 350,000 specimens in the Herbarium are among the most important plant biodiversity archives in Germany, actively used in internationally recognized scientific research programs. Being one of the University's leading visitor attractions, the Garden is also dedicated to making biology as accessible as possible to the wider public. It does so by means of its exhibitions, as well as teaching and outreach programs. The Garden's vision is to be widely acknowledged as an outstanding plant collection in Germany, valued by stakeholders as a major scientific research facility and as a centre for innovative public engagement with plant science via its collections and expertise. Likewise, the Herbarium HEID is an active research facility, regularly visited by international scientists to support their research activities and loaning specimens for external research programs. Moreover, supporting loan programs from other international Herbaria via HEID ensures that Heidelberg remains a centre of evolutionary and biodiversity research. To illustrate the importance of modern and well-equipped herbaria we contributed to a joint mission statement of German herbaria (Borsch et al. 2010).

General Collection Management & Development Policy—Living Collection

The collections—kept in greenhouses, outdoor gardens, and germplasm archives—meet the full spectrum of research, educational, cultural, and conservation needs and can be divided into specialized and non-specialized collections, in total representing nearly 5,000 species. Our specialized collections are of a size and significance that merits national and international recognition, ideally suited to research: tropical orchids, bromeliads, succulent plants, and Brassicaceae. Smaller non-specialized collections contribute to the diversity of the collections in general and are primarily used for teaching and display purposes (e.g. insectivorous plants, the arboretum). The main acquisition methods of the Garden are plant or seed exchanges with other Gardens, and field collections. New plant material should generally be from a collection in the wild or, if cultivated, from a known wild origin. Provenances of newly included specimens must be known and must respect the Convention on Biological Diversity (CBD) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and other laws. The documentation of all accessions is recorded in the database »Gartenbank« which is freely accessible. The collection is continuously monitored for specimens that are in conflict with the collection's criteria and for unnecessary duplicates. Plants of garden or unknown origin—especially in the core collection—are replaced with specimens of known wild origin, preferably of direct wild origin. These processes are under permanent internal evaluation and supervised by Andreas Franzke, curator of the living collections.

Selected Activities & Achievements 2018-2021

Living Collection Development

- ≈ 800 accessions corrected and removed
- ≈ 350 new accessions
- ≈ 350 taxon identifications (determined/confirmed)
- ≈ 1,600 nomenclatural changes
- ≈ 8,500 curator-revised data sets

Herbarium Collection Development

- ≈ 1.700 new specimens
- ≈ 3.000 specimens digitised
- ≈ 500 taxon identifications (det./conf.)
- ≈ 200 newly identified/rediscovered type vouchers

The herbarium is actively curated by Peter Sack, technical assistant and a team of six volunteers kept together by Helmi Medda.

During the report period, the research department, Marcus Koch, discovered and described a new genus (*Zahora*), and two new taxa from the genera *Dianthus* and *Aubrieta* (e.g., Aydin et al. 2021, Koch & Lemmel 2019)

A major challenge was an insect infection with the herbarium in 2019/2020. We decided to deep-freeze the entire collection during an 8-weeks dual freezing rotation; and the majority of fascicles (bundle of vouchers) are now sealed in plastic bags.

Wild Plant ex-situ Conservation Projects

In cooperation with the Stuttgart and Tübingen Administrative Districts we continued our ex-situ maintenance and resettlement activities, along with fitness parameter studies for endangered Cheddar Pink populations from the Swabian Alb Biosphere Reserve. Some financial support for these ex-situ-populations efforts was provided by the WIPs-De programme, a national network for the conservation of endangered plant species for which Germany has special responsibility. WIPs-De is in turn funded by the German Federal Agency for Nature Conservation (BfN) in the context of Germany's National Biodiversity Strategy. The scientific monitoring and respective research has been supported by the Stiftungsfond Naturschutz Baden-Württemberg.



Prairie at the main entrance of the Botanic Garden

Rauh Archive Project

Werner Rauh (1913–2000) was a former director of the Garden and many specimens of the Gardens historic collections—succulents, xerophytes from Madagascar, bromeliads and tropical orchids—were collected on »plant hunter« Rauh's numerous expeditions. A large part of his scientific legacy went to his scholar Wilhelm Barthlott (Bonn University) and was transferred back to Heidelberg in 2016. United with Heidelberg documents, the curated Rauh Archive now comprises 45 meters of shelf space of historical biodiversity data and is accessible for research purposes. Major and important parts are photos and field records. The Klaus-Tschira-Foundation financed the project, which actually found its first end early 2019.

Evo-BoGa Project (2017–2020)

Joint project »Plant collections of Botanic Gardens: living resources for integrative evolutionary research« funded by The Federal Ministry of Education and Research (BMBF) in the context of the initiative »Networking–Development–Research. Alliance for University Collections«. Affiliated partners: Botanic Garden Berlin (BGBM) and Senckenberg Research Institute Frankfurt. Our Garden led the subproject Bromeliaceae DNA barcoding (Heller et al. 2017, Bratzel et al. 2020) and was involved in pilot studies for cross-linking inventory databases of university living plant collections. The latter activities resulted in the gardens-4science online portal (<http://gardens4science.biocase.org/>).

Atacama Projects (2017–onwards)

In the period under review, growth measurements with tillandsias (bromeliads) from the Atacama Desert were carried out in the Botanic Garden in the context of a BMBF-funded German-Chilean research project as well as in an ongoing DFG (German Research Foundation) funded project (CRC1211 – Earth – Evolution at the Dry Limit) (e.g. Koch et al. 2020, Möbus et al. 2021). For these desert plants, fog is de facto the only source of water and nutrients and the decline in abundance of such tillandsia stands over the last 50 years is an indicator of climate change.

Material Transfer for Scientific Purposes & Support of Local Research Programs

As a member of the International Plant Exchange Network (IPEN), the Garden supplies material for international research programs conforming with the Convention on Biological Diversity (CBD). In the period under review, material from over 2,400 accessions was transferred. A list of external publications that made use of such transfers can be found on the gardens homepage. The Botanic Garden supported also Heidelberg-based research programs with the provision of plant material, test areas and horticultural expertise. The Garden's Scientific Plant Cultivation Service (SPCS) cultivated around 7,000 individual plants annually from a great variety of wild species. This also led to a substantial number of SPCS-based publications, listed in the gardens annual reports.

Academic Teaching & Theses

The Botanic Garden plays an important role in the academic teaching of COS Heidelberg: Each year the Garden provides plant material for ≈ 80 course days with ≈ 400 students, and the Garden's collections hosted academic courses with ≈ 500 students on ≈ 50 days each year. The Herbarium is also integrated into education programs. The Student's Herbarium of plants, collected during field excursions during the last ten years, incorporates more than 10,000 (fully digitised) specimens. The Garden provided plant material or test areas for a variety of academic works. Garden-related, Heidelberg-based theses submitted from 2017 to 2020 include 3 PhD theses, 9 master theses, 18 bachelor theses, and 8 state examination theses.

Vocational and Public Education Program, Visitors & Public Events

Excellent horticulturists are a prerequisite for the maintenance of scientific living plant collections. Therefore, the training of gardeners is another prominent function of botanical gardens. In the period under review, eleven gardener apprentices have successfully completed their training in our garden. In addition, we also provided gardening work experience placements for ≈ 50 pupils.

The »Green School« of the Botanic Garden represents a comprehensive outreach program that reached about 5,000 children and adults in the period under review. Activities for children and teenagers are part of the Heidelberg Young University educational program. The Botanic Garden (gardens and greenhouses) is free and open to the public. The gardens are accessible at all times and the 2,000 m² greenhouses are open six days per week. (In pre-pandemic times) more than 50,000 visitors yearly enjoyed all that Heidelberg Botanic Garden has to offer and the annual »Garten-Fest« alone attracts 1,000 visitors.



Raised Bog in the western part of the Botanic Garden opened in 2021

A Challenging Future – A Garden for the Future

The concept and planning for the restoration and refurbishment of the entire Botanic Garden and most of its ailing infrastructure is finalized and we expect to have a chance to start the construction activities in 2023/2024. This would mean many years of intensive work and plant care in front of us to guarantee that the collections are carefully handled and will find a new, old home.

We are also facing increasingly the problems of climate change. Heat, draught and high solar radiation demand new approaches and solutions in plant cultivation and maintenance often not tried before. But we are optimistic to face the next years with a great and enthusiastic team.

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CURATORIAL RESEARCH ACTIVITIES IN LIVING COLLECTIONS

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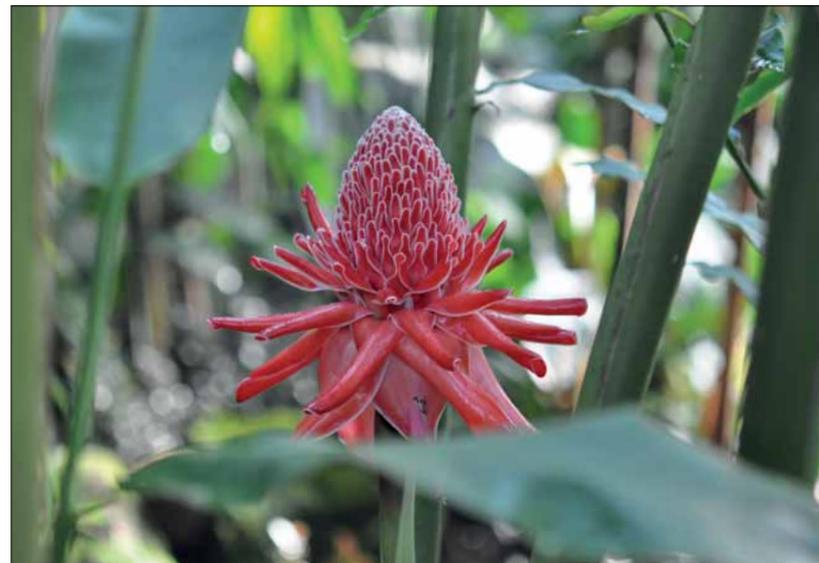
Fields of Interest

Living collection management, evolution of
Brassicaceae, evolutionary biology



Brief Summary of Work & Major Contributions Since 2017

Botanic gardens maintain documented collections of living plants for the purposes of scientific research, education, conservation and display. To this end, curatorial practice has developed to include the topical fields of acquisition, documentation and preservation, and the use of these collections. Since the specimens are living organisms, our collections require constant and diligent attention. Documenting collections is a facet of botanical gardens that fundamentally distinguishes them from other plant collections like parks, which have very limited reference value. My own curatorial work over the last several years has included a focus on significantly improving the documentation of our 12.000 living accessions and in boosting the impact of our collections on research and society through improved online access to this data. One part of this ongoing »provenance research«



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includes the critical (re)evaluation of data utilising our own archives of old entry books, fieldbooks, index cards, lists, literature etc. (cf. also the Rauh Archive Project in the botanic garden chapter). Another elaborate part of this work—especially in the period under review—includes efforts to cross-reference (retrospectively) our accession numbers with corresponding numbers and data of other Botanical Gardens that donated material in the past. The focus here has been on our Bromeliad collection with over 2.000 accessions in the context of the Evo-Boga Project (cf. botanic garden chapter). The public display is another of my fields of work, of which a major part has been the elaboration of new concepti-

ons for our eco-geobotanical sections including corresponding information boards (heath, inland dune, raised bog, vineyard, prairie). In my research, I am focused on Brassicaceae evolution.

Selected publications since 2017

Number of peer-reviewed articles 2017-2021: 4

Number of author peer-reviewed articles as first or last/corresponding 2017-2021: 2

Number of citations 2017-2021: 526 (Web of Science)

Borsch, T., et. al (2020) A complete digitization of German herbaria is possible, sensible and should be started now. Res. Ideas Outcomes 6, e50675.

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4.2 DEEP SEQUENCING CORE FACILITY

DEEP SEQUENCING CORE FACILITY

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Introduction

The CellNetworks Deep Sequencing Core Facility was opened in September 2010 to provide access to Next Generation Sequencing technology (NGS) for the Heidelberg University research community. The core facility was previously supported by the Excellence Cluster CellNetworks, the Centre for Organismal Studies (COS Heidelberg) and the Heidelberg Molecular Life Sciences (HMLS) research council. Since October 2019 the facility has been supported by the Research Council and Heidelberg University, and is embedded into the CellNetworks Core Technology Platform.

Personnel

Since the previous SAB Report in 2017, the Deep Sequencing Core Facility has again been forced to make changes to its personnel structure due to the funding uncertainties with the end of the CellNetworks cluster funding. This meant the unfortunate loss of the technical staff Hannah Hännisch. As such the facility is now operated and ran solely by David Ibberson (MSc). This leads to times when the facility is fully booked or closed and thus processing times are longer than usual.

Core Facility Concept and Services Provided

The facility was founded with the idea not only to provide library preparation and sequencing for users on campus but also to offer professional advice on how to implement NGS into its users' research. This advice has been widely taken up and has in some cases lead to development of new protocols or the refinement of current "standard" protocols. Examples include designing of oligos for SELEXseq for AG Jäschke (IPMB), or the designing and optimization of oligos for the targeted amplification and sequencing of AAV barcodes (University Clinic Heidelberg, AG Grimm and AG Most).

The core facility offers a large spectrum of services for its users. The main day to day activities revolve around standard library preparation methods for Illumina sequencing. However, due to the high diversity in sample genome origin on campus, and the fact reagents are tailored to samples originating from human and mouse, the library preparations are often tailored to the individual group from which the samples originate.

"Standard" Library Preparations on Offer:

- Stranded RNA-Seq
- Small RNAseq
- Chip-Seq
- gDNA-Seq (de novo, resequencing)
- Target Enrichment
- Methyl-Seq / Bisulfite Sequencing

Single Cell Sequencing

Since 2019, the facility has introduced single cell analysis on the 10x Genomics Chromium platform. This instrument had been previously jointly purchased through CellNetworks via a funding application in conjunction with DKFZ and EMBL. The platform can be used to generate libraries from single cell suspensions for 3' Gene Expression or 5' Gene Expression, ATACseq and Immune Cell Profiling. To date the facility has established all but the ATACseq protocols. When compared to "standard protocols" these protocols have a

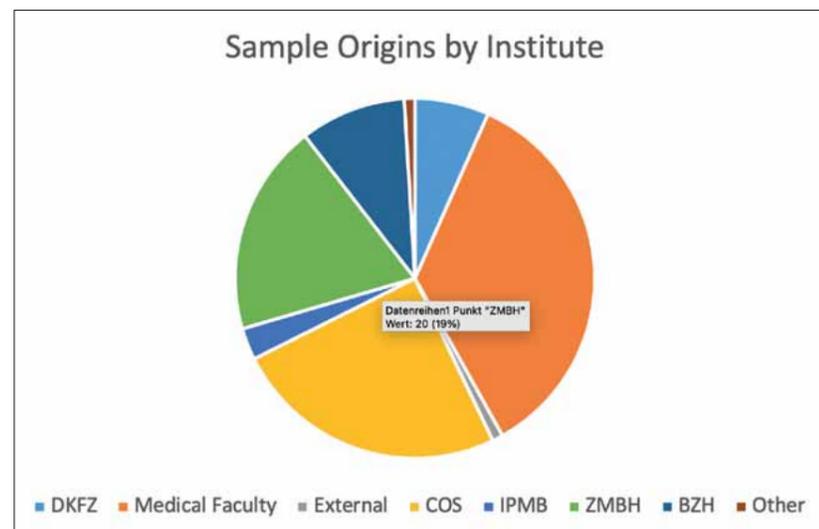


Figure 1
Chart showing sample origin by institute.

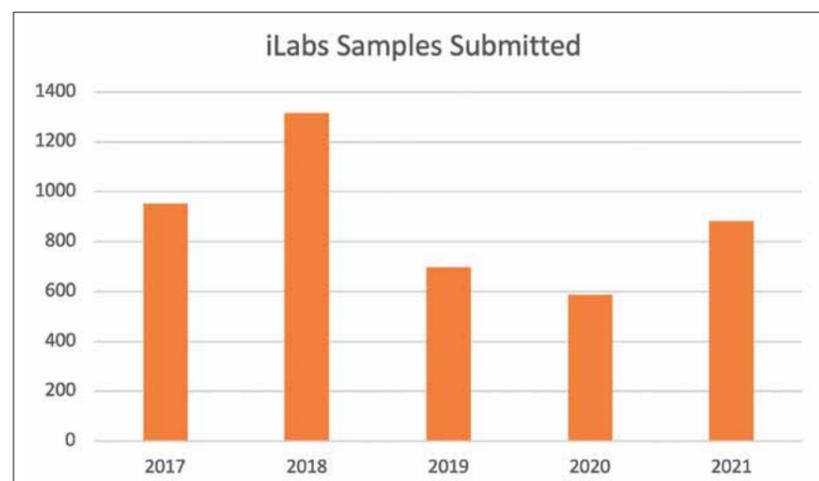


Figure 2
Chart showing the number of samples submitted over the years as registered in iLabs. It does not contain those samples which have been submitted and processed without iLabs. 2021 contains data until July of this year.

significant lower throughput for the time required for the sample preparation. Furthermore, the establishment of stable single cell suspensions require more thought and testing than for other methods. This has led to more cross-talk between groups and institutes, each sharing their experience with others.

In 2018, the facility also established a more traditional single cell method based upon published home brew methods. These have a higher throughput (tens of samples as opposed to individual samples), but is more costly method per cell than the 10x Genomics option.

With both these methods as basis, the facility is able to offer its users a range of techniques in the new field of single cell genomics, which is extremely well received.

Sequencing Service

As mentioned in the previous report, the core facility entered a successful collaboration with the Genomics Core Facility, EMBL in 2003. This has so far been a mutually beneficial collaboration, which has resulted in improved turnaround times for sequencing, and an optimal load on the sequencing instruments, which were exclusively run by the EMBL. This has in turn allowed us to keep prices low and thus keep our facility attractive also to junior groups which usually lack core funding. In addition, there the open communication between the two facilities has allowed the exchange of protocols, new product information and streamlined troubleshooting to the benefit of our users.

While this setup is extremely efficient for standard applications, the requirement to enter the regular EMBL sequencing queue severely limited the options for fast turnaround runs for methods development. To alleviate this issue, an Illumina NextSeq 550 was purchased in 2019 with joint funds from the ERC DECODE project of Jan Lohmann (COS), as well as the CellNetworks Cluster. This instrument is exclusively used for protocol development and not for everyday sequencing, which either goes to the EMBL GeneCore or external companies. This arrangement has been instrumental for groups establishing advanced single cell protocols and who are therefore dependent on short turnaround times.

Sequencing options currently on offer using in house instruments:

1. NextSeq 550:
 - a. Mid-Output (ca 120 million reads raw data)
 - 75 and 150 PE (150 cycle and 300 cycle reagents)
 - b. High Output (ca 300 – 400 million reads raw data)
 - i. 75 Single End (SE)
 - ii. 75 and 150 PE (150 cycle and 300 cycle reagents)
2. MiSeq (ca 12 million reads raw data)
 - 36, 150, 250 PE

User Base, Access and Fees

2018 saw the busiest year of the core facility with over 40 active users and over 1300 samples. Users came from a spectrum of institutes (see fig. 1). Sample numbers dwindled in 2019 and 2020 but have started to return to previous levels in the first half of 2021 (see fig. 2). Part of this drop in numbers can be explained by the introduction and establishment of the more time and cost intensive single cell analysis, which as previously mentioned has a low throughput when compared to more traditional library preparation methods. It is anticipated that sample numbers will continue to increase, but there will also be periods where the single cell requests will dominate leading to lower throughput.

The following clientele can enroll as user of the facility:

- Members of the Heidelberg University, including the Medical Faculties Heidelberg and Mannheim
- Members of the CellNetworks Core Technology Platform
- Graduate school HBIGS (Hartmut Hoffmann Berling International Graduate School of Molecular and Cellular Biology)
- Institutions represented in the Field of Focus 1 Research council (Heidelberg Molecular Life Sciences) members including DKFZ, EMBL and MPI.

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

User Fees

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

Conclusions and Future Direction

The core facility has become a well utilized facility, and we anticipate a continued growth and demand for NGS on campus. Improvements do need to be made in communication between the facility and its users, and more importantly in processing times. This can be partially achieved by improved usage of features within the online registration system iLabs, but ultimately requires staff recruitment.

In the previous two years single cell transcriptomics has become a hot topic, and the facility has been able to provide its users access to this technology. In the future this field is expected to further expand, and is already starting to go into the next phase of spatial transcriptomics where both spatial positioning of a few cells can be linked with their expression profile.

In the concluding remarks the facility's main area of expertise is not in the library preparation and sequencing per se, but in the open attitude we have towards our users. We encourage an open dialogue and are able to provide input to the experimental design required, including the design of novel solutions. This we wish to further build upon in the future.

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4.3 ELECTRON MICROSCOPY CORE FACILITY

ELECTRON MICROSCOPY CORE FACILITY

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The Electron Microscopy Core Facility (EMCF) provides expertise in electron microscopy for imaging needs at a sub-cellular level. The facility was founded in 2009 by the medical faculty to provide electron microscopy support for all researchers of the Heidelberg Life-Science community. To further streamline development and application of electron microscopy and to leverage existing resources, COS decided to merge its own electron microscopy unit with the EMCF in 2014. Since then, COS contributes equipment, a scientific as well as a technical personnel as well as administrative support to the EMCF, ensuring that it can continue to offer attractive services to our community.

The EMCF currently operates a suite of electron microscopes, which cover a broad range of applications. With the scanning electron microscope (SEM), we can image surface structures of cells and tissues, which is useful both for fine structures on single cells as well as for looking at details from the surface of tissues or whole (mm size) organisms. To investigate intracellular structures by scanning electron microscopy, samples have to be resin embedded and sectioned in ultrathin slices with a thickness of about 70 to 200 nm. In contrast, with transmission electron microscopy (TEM), subcellular structures can be visualized at maximum resolution using ultrathin sections. In addition to a standard TEM, the EMCF also operates an instrument with high accelerating voltage (200kV), which enables 3D tomography. This is achieved by taking a series of images of the section at different tilt angles. This technology allows to examine the spatial arrangement of small structures within cells. In cases where the 3D structure of a larger volume is required, the EMCF can offer array tomography of serial sections using transmission electron microscopy or scanning electron microscopy. For extracted protein complexes or extracted cellular components we use negative stain and transmission electron microscopy to gain insights in their molecular structure. In summary, the EMCF offers EM techniques covering scales from mm of surfaces to ultra structures of protein complexes.

Many biological questions require that structural information is combined with information on protein expression. To support projects in this domain, the EMCF has ample expertise in applying immunogold labelling using antibodies to identify areas of protein localization on sectioned material. Importantly, the EMCF also offers correlative light- and electron microscopy (CLEM) methods, which make use of the expression of a fluorescently tagged protein to find a small structure or a rare event.

With all the different options available for imaging and sample preparation it is important to find the most suitable technique for every project and specimen. The EMCF has ample experience in applying a wide variety of technologies and thus can help to develop protocols that suit different samples and scientific questions. The EMCF workflow hence is based on a close and ongoing collaboration between scientists and facility staff. This starts off with a first meeting with EMCF staff scientists Stefan Hillmer [head, COS member] and Charlotta Funaya [Field of Focus I position] and continues until the data is successfully published. Publication records with papers acknowledging support or authorship of EMCF staff members [38 during the current COS reporting period] from the current reporting period show that many groups on campus benefited from the EMCF. The number of co-authorships of

EMCF scientists [12 during this period] also emphasizes the importance of scientific consulting, which is an important aspect of our mission and can be a valuable asset for groups who only rarely use electron microscopy.

The broad spectrum of applications and technologies offered and supported by the EMCF is essential for the scientific success of our community. Pooling resources into an open campus facility represents the most efficient form of delivering technologies that require costly equipment and dedicated expertise, as it increases instrument use and improves user support. Importantly, training users involves a lot of hands-on training and continuous support at the various instruments, which is only possible in a facility framework. The decision to combine the EM facility of COS with the EMCF to create a central community EM lab, therefore was a very important step and has already paid great dividends. For the future, we are looking forward to the imminent start of construction of microscope rooms at the EMCF location at INF 345 to finally be able to also have the electron microscopes at our location. Along with investments to replace aged instruments, this will create a solid foundation for the EMCF, to ensure that many users can actively learn and use electron microscopy.

4.4 METABOLOMICS CORE TECHNOLOGY PLATFORM

METABOLOMICS CORE TECHNOLOGY PLATFORM

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Summary and outlook

The Metabolomics Core Technology Platform (MCTP) was established in 2013 by the Institutional Strategy of the University's national Excellence Initiative II program. Its support is partially continued by the University's Excellence Strategy program since 2019 but shifted to about 90 % third party funding. It is located in COS within the Department of Molecular Biology of Plants, directed by Rüdiger Hell and managed by Gernot Poschet.

The mission of MCTP is the development and provision of analytical services across the strategic Field of Focus I of Heidelberg University, including the two Medical Faculties and the non-university institutions DKFZ, MPIImF, EMBL and KIT (Fig. 1). MCTP has established scientific metabolomics approaches and developed novel analytical tools enabling custom-made services for research projects. MCTP accompanies projects in close cooperation from early development to publication and funding applications. The strong leverage effect of MCTP is reflected by contributions to 43 publications including top journals (Cell, Nature) and acquisition of about 3 mio. € of funding.

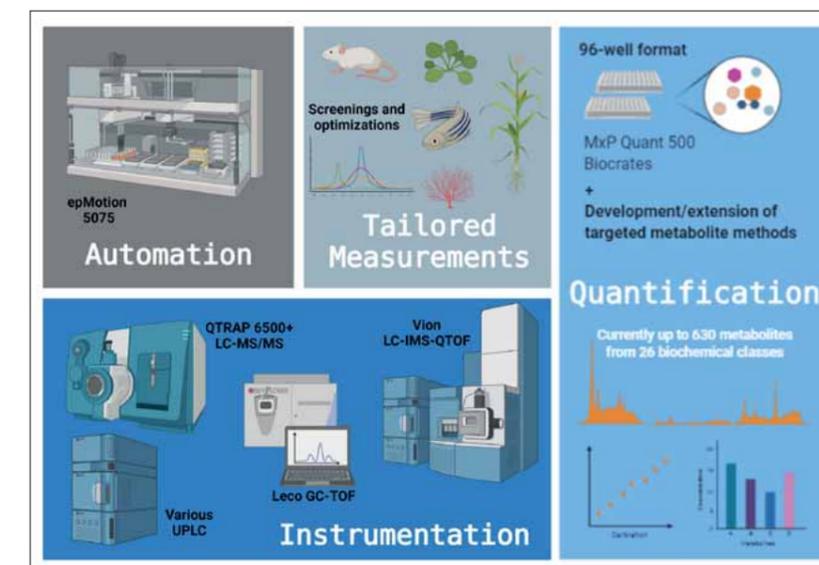


Figure 1
 Overview of MCTP's analytical systems and services. Targeted and untargeted metabolite and flux analyses can be quantitatively assessed from different biological and medical matrices.

Since 2017 metabolite analyses for more than 80 research groups all over Heidelberg Molecular Life Sciences have been carried out. This was accompanied by strong acquisition of third party funding going along with personal and equipment. Highlights are the routine targeted measurement of more than 600 compounds, non-targeted metabolomics, and tracer/flux analyses. Future developments aim at the consolidation of high-end instrumentation and novel analytical tools, training of postdoctoral researchers in metabolomics and extended bioinformatics approaches.

Research Highlights since 2017

With the beginning of this report period a substantial improvement and enlargement of personal and analytical instrumentation based on third party funding took place. To streamline the operation of MCTP and face increasing demands for metabolomics analyses a professional web-based service booking and management system (iLab, Agilent) was implemented in 2017 as joint initiative together with several other Heidelberg life sciences core facilities. The first addition to the existing analytical equipment was an UPLC system coupled to a Vion IMS-QToF MS (2017) providing excellent mass accuracy for untargeted/explorative metabolite analyses that was co-financed by the excellence initiative II, COS and CRCs at the Heidelberg campus. A QTRAP 6500+ Selexion for ultra-sensitive targeted analyses was acquired in 2019 by shared funds from the CellNetworks excellence cluster, life science research council and COS (50%). A five-year cooperation with DKFZ was initiated in 2019 and included the acquisition of a GC-TOF system suitable for enhanced screening of central metabolism and tracer studies. The second QTRAP 6500+ Selexion instrument with an UPLC and microLC system was purchased, together with an automated sample preparation robot, in the framework of the BMBF funded consortium "SMART-CARE" in 2020. In this cross-campus initiative with several clinics, DKFZ and Hochschule Mannheim, deep metabolic phenotyping based on a multi-omics approach of simultaneous targeted detection of >600 metabolites and untargeted metabolomics is combined with proteomics towards a translational approach for prediction of recurring cancer entities. The mentioned analytical instrumentation sums up to 1.67 mio. €.

Together with our contribution to CRC1118 (Reactive Metabolites as Cause of diabetic long term damages) these projects went along with an increase in personal to enable routine projects and analytical developments. At present MCTP comprises Gernot Poschet as manager, three full time researchers, two part-time engineers and two part-time technicians (all but one scientist are third party funded). As examples, within 2.5 years more than 4.500 samples were assayed for 13 different research groups in CRC1118 and more than 6.000 analyses were conducted for 30 DKFZ groups. In qualitative terms special applications for answering dedicated scientific questions have been developed for diverse research projects from Heidelberg life science institutes, such as quantification of major redox coenzymes, coenzyme A derivatives, retinoic acids, acetyl-spermidines, polysulfides, phytohormones, dipeptides, and many others (Fig. 2). A major technological achievement was the establishment of tracing analyses of central carbon and nitrogen metabolism using heavy labelled isotope tracers (¹³C or ¹⁵N) in cooperation with one of the leading experts in Germany, Prof. C. Hiller, TU Braunschweig. Several projects based on tracing studies are underway and future developments will aim at further targets covering additional metabolic pathways not only in human cell lines but other biological model systems.

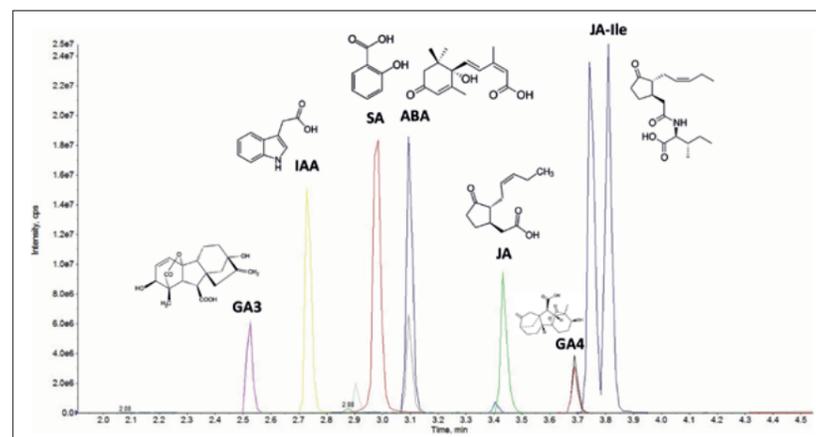


Figure 2
Chromatogram of major phytohormones determined via UPLC-MS/MS. Method established upon request of COS groups.

The leverage effect of MCTP scientific services enable quite a number of publications by institutions all across campus as well as several international cooperations. MCTP's branched-chain keto acid (BCKA) analyses supported a manuscript that demonstrated how enhanced BCKA metabolism mechanistically causes DNA hypermethylation in stem cells of certain aggressive cancer entities (Raffel et al., 2018). Development of a comprehensive assessment of tryptophan degradation products such as kynurenine contributed to the elucidation of how aryl hydrocarbon receptor activation by tryptophan catabolites enhances tumor malignancy (Sadik et al., 2020). The same technique was applied in a study where evidence of a glioma genotype-dependent intra-tumoral network was analysed and tryptophan metabolism identified as a target for immunotherapy of IDH-mutant tumors (Friedrich et al., 2021). Many more standard services were provided to publications in addition to the 43 ones in the supplement where MCTP scientists are not listed as co-authors. A major impact can be expected from the establishment of the newly developed UPLC-MS/MS MxP Quant 500 (Biocrates) kits. These allow highly standardized deep metabolome phenotyping of human matrices and are being integrated in many projects of MCTP clients. Analyses on human disease states already contributed to several basic research and applied biomedical publications (e.g. Erben et al., 2021; Mock-Ohnesorge et al., 2021). Currently, we are participating in an international ring trial to validate MxP Quant 500 for large scale comparative and quantitative medical studies where MCTP is placed in the uppermost quarter according to preliminary assessment. Within the framework of CRC1118 we are in the process of establishing the MxP Quant 500 kit system for animal models (mouse, fly and fish; Fig. 3).

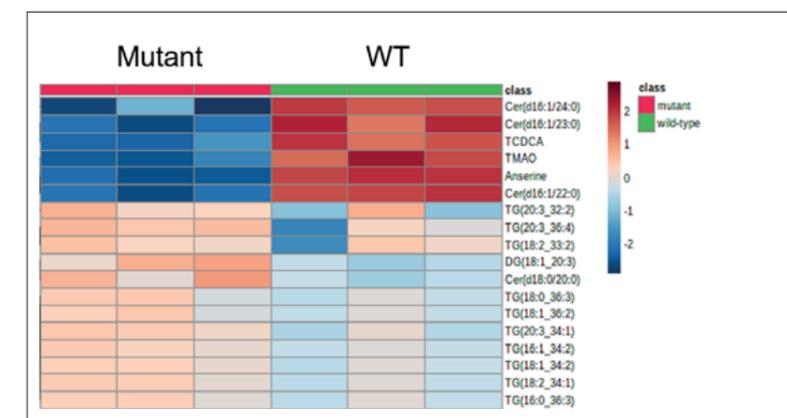


Figure 3
Heatmap showing metabolic differences in Zebrafish mutant in comparison to WT control. Data was generated via UPLC-MS/MS using MxP Quant 500 metabolic kits (Biocrates) enabling the determination of up to 630 different metabolites.

Future directions

The development of MCTP will focus on 1) renewal of running and opening of new funding sources, 2) training of young scientists and scientific development, and 3) addressing bottlenecks in bioinformatics and laboratory space.

Point 1) is clearly defined: First, by the currently prepared renewal of the metabolomics service project in CRC1118 (next funding period 7/2022). Second, by the evaluation for the second period of SMART-CARE by BMBF in 2023; Third, by re-negotiating the contract with the German cancer research center (DKFZ) Heidelberg as associated external core facility due 2024.

2) The scientific excellence of MCTP is part of our mission and depends on young researchers. However, standard analyses based on user fees are usually not attractive for young researchers at the postdoctoral stage, who, based on the funding structure of MCTP, nearly all have limited contracts. Since user fees cannot be coined into positions (including technicians) according to law (and are required for instrument maintenance and replacement), the request for services and scientific development can hardly benefit from each other and strongly impair the long-term development of MCTP.

3) The analysis of primary data in mass spectrometry has to be curated by scientists. The individual and often tailor-made processing is highly time consuming for MCTP and can usually not be transferred to users. MCTP would need at least a 50% position for a bioinformatician who would take care of software and workflows for data analyses and implement algorithms for further data processing to improve data mining and trim down turnaround times for results. A long-term issue is the limited laboratory and office space which prevents any further growth. MCTP currently occupies about half of the space of the Department of Molecular Biology of Plants without contributing much to its original tasks in research and teaching. On top, the technical capabilities of INF 360 have reached the limit (media supplies, safety aeration, air conditioning).

Nevertheless, MCTP thrives (and has to thrive) for opportunities that enable technological and scientific improvement. A current proposal within the REACT-EU funding scheme for innovation and sustainable energy aims to establish a high-end MS-imaging platform for biomedical and translational applications for life sciences in the Rhein-Neckar region.



Figure 4
Group photo MCTP, from left to right: Michael Schulz, Nina Kunze-Rohrbach, Dr. Michael Büttner, Elena Heidenreich, Dr. Glynis Klinke, Dr. Hagen Gegner, Eva-Maria Käshammer-Lorenz, Dr. Gernot Poschet

Selected publications since 2017

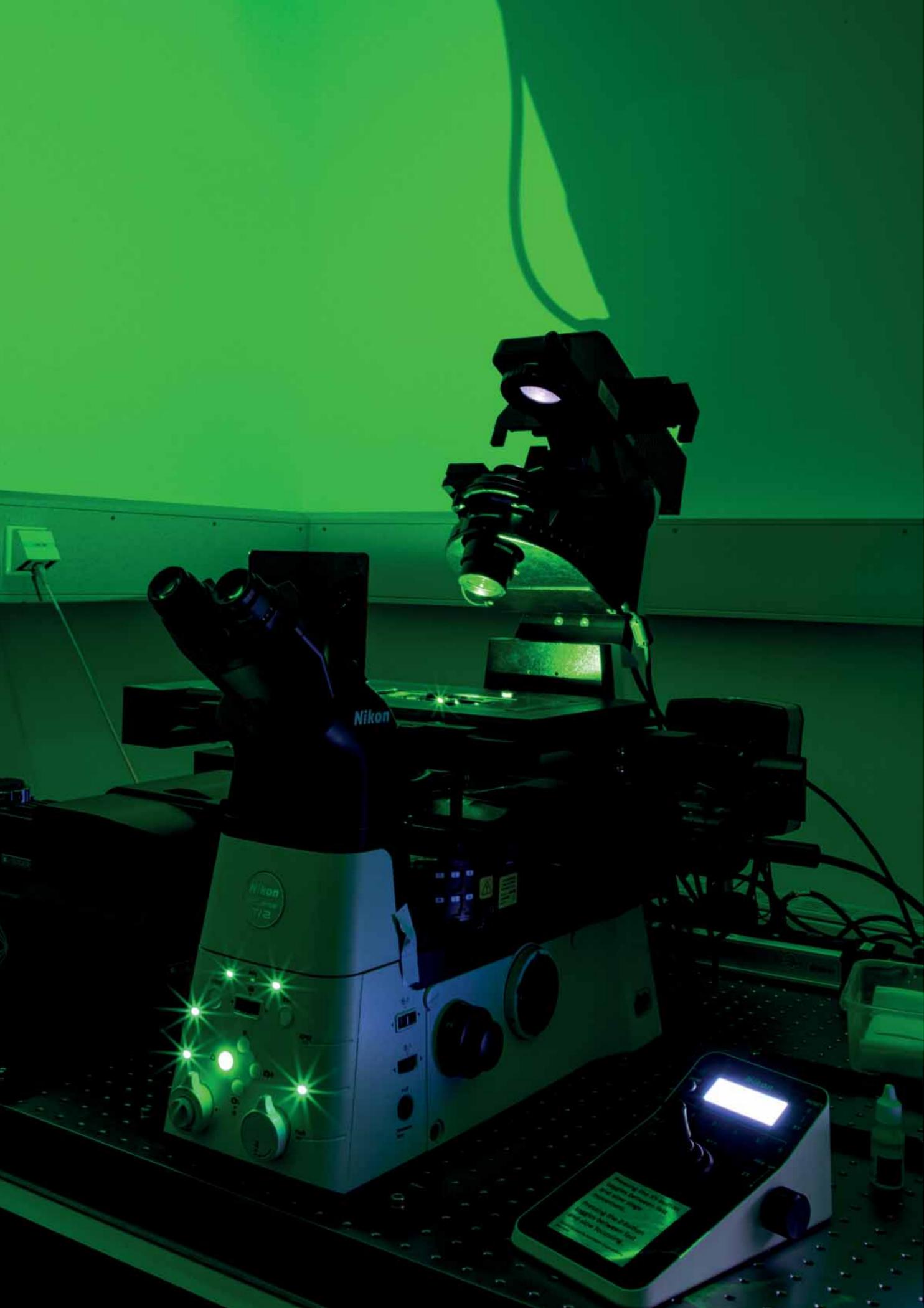
Friedrich M, Sankowski R, Bunse L, Kilian M, Green E, Guevara C, Pusch S, Poschet G, Sanghvi K, Hahn M, Bunse T, Münch P, Gegner H, Sonner J, von Landenberg A, Cichon F, Aslan K, Trobisch T, Schirmer L, Abu-Sammour D, Kessler T, Ratliff M, Schrimpf D, Sahm F, Hopf C, Heiland D, Schnell O, Beck J, Böttcher C, Fernandez-Zapata C, Priller J, Heiland S, Gutcher I, Quintana F, von Deimling A, Wick W, Prinz M, Platten M (2021) Tryptophan metabolism drives dynamic immunosuppressive myeloid states in IDH-mutant gliomas. *Nat Cancer* 2, 723–740.

Sadik A, Somarribas Patterson LF, Öztürk S, Mohapatra SR, Panitz V, Secker PF, Pfänder P, Loth S, Salem H, Prentzell MT, Berdel B, Iskar M, Faessler E, Reuter F, Kirst I, Kalter V, Foerster KI, Jäger E, Guevara CR, Sobeh M, Hielscher T, Poschet G, Reinhardt A, Hassel JC, Zapatka M, Hahn U, von Deimling A, Hopf C, Schlichting R, Escher BI, Burhenne J, Haefeli WE, Ishaque N, Böhme A, Schäuble S, Thedieck K, Trump S, Seiffert M, Opitz CA. (2020) IL411 Is a Metabolic Immune Checkpoint that Activates the AHR and Promotes Tumor Progression. *Cell* 182(5):1252-1270.

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4.5 NIKON IMAGING CENTER

NIKON IMAGING CENTER

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The Nikon Imaging Center at the University of Heidelberg (NIC@Uni-HD, or NIC) offers advanced light microscopy on campus since 2005. We support scientists across the campus on their imaging research with a team of 3 postdoctoral scientists. This relatively small team trains scientists on currently 15 instruments. The expertise of the team includes: FLIM-FRET, FRET by acceptor bleaching, FRAP, imaging of cytoskeletal dynamics, single molecule detection in TIRF, 2-photon including 2-photon ablation, light sheet microscopy, large volume imaging and stitching, structured illumination microscopy.

Summary and outlook

The Nikon Imaging Center is based on a collaboration between the University and the company Nikon, where Nikon supports the imaging facility with instrumentation. The techniques offered have diversified as new technologies emerged and we offer now two types of light sheet microscopes: One for cleared tissue and one for live imaging of organoids. Laser scanning and spinning disk microscopy is still taking the main load of experiments (see Figure 1) while we also offer several advanced wide field imaging setups (Table 1 shows major instrumentation). The light sheet microscope for cleared specimen acquired in 2017 bridges the gap between low resolution whole animal imaging and laser scanning confocal microscopy.

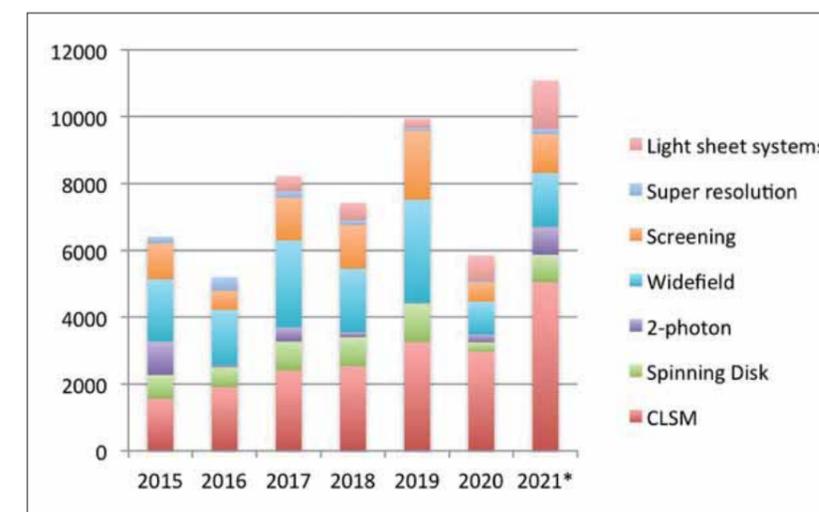


Figure 1
Development of usage (hours booked by users) shown for different techniques/ systems. Note that in CLSM, wide field and light sheet more than one system is summarized. For 2021, the data collected from January to June was used to extrapolate for the whole year. During 2020 access had to be restricted because of Covid-19.

Our user base is spread across 80 groups in institutes across the Biosciences and the Medical Faculty (See Figure 2). We have so far trained approx. 1700 researchers and supported them in their projects – 500 in the last 4 years. We are part of two research consortia, which are solidly anchored in COS: the CRC 873 on stem cells (speaker Prof.

Jan Lohmann) and the CRC 1324 on Wnt-signaling (speaker until now Prof. Th. Holstein, future Prof. Michael Boutros). We work closely together with researchers of this consortia and are involved in the individual projects. We cover a wide range of fluorescence techniques on currently 15 instruments (Table 1), which researchers can work on independently once they have received training. The team also supports users in basic image analysis and provides image restoration by deconvolution (SVI Huygens remote Manager on dedicated server) and has started implementing artificial intelligence (AI) modules.

Table 1: Major Instrumentation at Nikon Imaging Center

Type	Number of instruments	Short description
Light sheet microscope for cleared samples	1	Light sheet microscope for specimen up to 10mm with dipping lenses equipped for clarity, BABB or for living samples in aqueous medium. FOV maximal 3mm, with stitching 10mm samples can be imaged. Laser lines: 490 nm, 560 nm and 640 nm
Light sheet microscope for live imaging	1	Inverted light sheet with subcellular resolution to image organoids. Luxendo InVi-SPIM. FOV 200 or 400µm. Laser lines: 490 nm, 560 nm and 640 nm
Inverted wide field fluorescence microscope	2	Automated inverted microscope for time lapse acquisition with perfect focus system (Nikon Ti2), 7 channels (multiple fluorescent proteins, FRET, Fura). Cameras include EMCCD (Andor iXon) and dual sCMOS (2 Andor sNEO on TuCAM)
TIRF	1	Total internal reflection fluorescence (TIRF) microscope with triggered acquisition and single molecule sensitivity. FRAP with 10 ms switching time to acquisition. Laser lines: 405nm, 440 nm, 488 nm, 514 nm, 561 nm, 640 nm
Laser scanning confocal microscope	2	Laser scanning confocal systems (Nikon C2 and Nikon A1R) on an automated inverted microscope (Ti2) with perfect focus and multipoint acquisition. On A1R resonant mode as well as GaAsP-detectors and spectral detector are available. Laser lines: 405, 488, 514, 561 nm.
FLIM	1	Fluorescence Life Time Imaging Microscopy (FLIM) module (Picoquant TimeHarp) on A1 in the time domain (TCSPC). Pulsed lasers 440 and 490 for FRET pairs CFP-YFP and GFP-mCherry or similar.
Spinning disc confocal Yokogawa X1	1	Spinning disk confocal systems with sensitive EM-CCD detection on inverted microscope for high resolution cellular dynamics. Fast z-acquisition with objective piezo and dual camera mode. Laser lines: 405, 440, 488, 514, 561, 640 nm
Spinning disc confocal CREST V3	1	Spinning disk confocal systems with large field of view (FOV 25 mm) combined with digital mirror device (DMD) for optogenetic stimulation. Laser lines: 405, 440, 488, 514, 561, 640, 750 nm Simulation lines: 395, 440, 488, , 550, 640, 750 nm
Upright 2-photon	1	2-photon system (LaVision Biotec) on upright fix-stage microscope (Nikon FN-1) for physiological deep-in-tissue observation. Detection on non-descanned all-GaAsP ultra-sensitive-PMT port (4 channels). Excitation of UV-dyes (fura) up to red fluorescent proteins (e.g. mCherry). Dipping lenses.
Structured illumination for superresolution	1	Nikon structured illumination microscope (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

During 2019 we had to restrict access to microscope to allow for safe operation using distancing - and at the beginning of the pandemic also temporal gaps between user sessions. While already trained users were able to continue working, after a short shut down period, trainings were much more difficult to realize. Some of the trainings were conducted remotely but luckily the availability of tests in the framework of a study COS participated in (LAMP tests ZMBH University of Heidelberg) allowed us to take up trainings early on. Since the beginning of 2021 we experience a very high demand, especially in CLSM (see Figure 1). We are currently applying for funds to acquire a new CLSM with a large field of view.

Research Highlights since 2017

The Nikon Imaging Center at the University of Heidelberg provides access to advanced light microscopy for a broad range of applications. These range from in-vivo imaging to subcellular dynamics, FRAP and FRET. A highly used application is volumetric tissue imaging on the Ultramicroscope light sheet microscope operated by Dr. Nicolas Dross. This system is particularly well suited for big specimens, which are chemically treated for optical transparency (clearing). We mainly collaborate with groups from Neurobiology (brains), but also with more clinical-oriented groups for detection of tumor cells in various tissues. For much smaller samples and live imaging of organoids the Luxendo InVi-SPIM has been installed in 2018 in the frameworks of the CRC 873 and is especially suited to image several organoids over time (ongoing work with groups of G. Pereira and A. Martin Villalba) but has also been used to image Arabidopsis embryos. Laser scanning microscopy remains an important pillar of the NIC. (See Fig. 1). We make use of novel silicone immersion objectives, which allow for imaging deep into the tissue (Fig. 2). We have also added a new spinning disk with screening capabilities with funding from the Excellence Initiative this

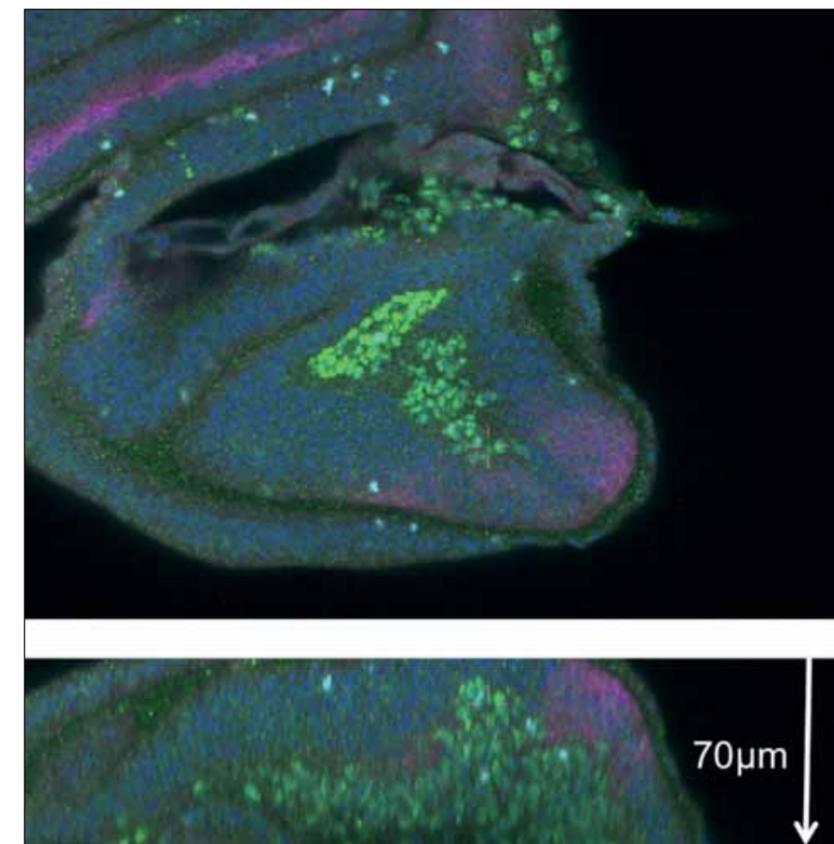


Figure 2
Imaging of Drosophila imaginal disks for wnt expression and other marks with a 40x Si immersion objective NA 1.25. Due to the good match of refractive index of tissue, glycerol embedding and Si-immersion it is possible to obtain good images well over 50µm in the tissue. Sample: Josephine Bageritz, COS.

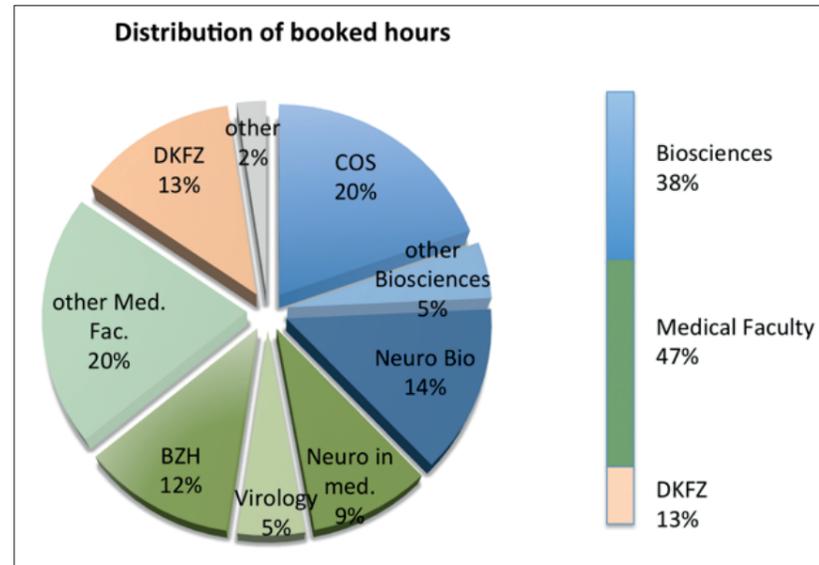


Figure 3
Distribution of booked hours
across institutes and faculties.

year, which replaces a system purchased in 2007. For super-resolution, the NIC offers structured illumination with up to 4 channels (Engel 2017) and was used to study arrangement of centriole components in yeast and mammalian cells (Rüthnick et al. 2017, Hata et al. 2019, Antorino et al. 2020) and cytoskeletal organization (Ciuba et al. 2018).

In collaboration with the Holstein lab, we started investigating the Hydra organizer where wnt-expressing cells form long protrusions called myonemes, potentially important in wnt transport. Also in the framework of the CRC1324 focussed on wnt, we supported the Acebron group in analysing the role of Wnt-signaling in mitosis using live imaging (Bufo et al, 2021 PNAS in press).

In the reporting time, the NIC supported 79 publications. The publication record reflects the wide range of application the NIC supports. Looking at the booked hours sorted across the different research entities (Figure 3), it becomes clear that the medical faculty has the largest share in booking as a lot of biological research is conducted in this faculty.

Future directions

FLIM-FRET for protein-protein interaction: We have evaluated fluorescent protein FRET pairs for high contrast in life time response in the Picoquant FLIM on the A1 CLSM. There are ongoing projects within the wnt-CRC where we will use FLIM to probe protein-protein interaction.

Light sheet microscopy: We are working with several groups on optimizing live imaging on the inverted light sheet microscope (Hydra, organoids, Arabidopsis). On the upright light sheet for cleared specimen, imaging is well established. Analysis of data remains a challenge because of the size of the volumetric data that is acquired.

Implementation of searchable image database. The quest for a searchable imaging database becomes more urgent. While the user base of the NIC is too heterogeneous provide such a database for all of our users, we aim to install OMERO as an image database together with COS and try to integrate it with the introduction of an electronic lab book.

Selected publications since 2017

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Linster, E., Layer, D., Bienvenut, W.V., Dinh, T.V., Weyer, F.A., Leemhuis, W., Brunje, A., Hoffrichter, M., Miklankova, P., Kopp, J., Lapouge, K., Sindlinger, J., Schwarzer, D., Meinel, T., Finkemeier, I., Giglione, C., Hell, R., Sinning, I., and Wirtz, M. (2020). The Arabidopsis N(alpha)-acetyltransferase NAA60 locates to the plasma membrane and is vital for the high salt stress response. New Phytol. 228, 554-569

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4.6 ZOOLOGICAL COLLECTION TIMELINE EVOLUTION

ZOOLOGICAL COLLECTION TIMELINE EVOLUTION

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Due to the necessary extensive renovation of the 50-year-old building INF 230, the “Zoological Collection of Heidelberg University” is currently still not accessible to the public. However, in spring 2022, the old collection will be become available again after a major face lifting and modern set-up. This major approach is financed by the university with a generous support of the Schmeil foundation (125,000 €) and private donators.

The old collection: Zoologisches Museum of the Zoological Institute

The old collection contains specimens allowing insight into zoo-geography, systematics and comparative anatomy. Additional topics covered are domestication, wild-life 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

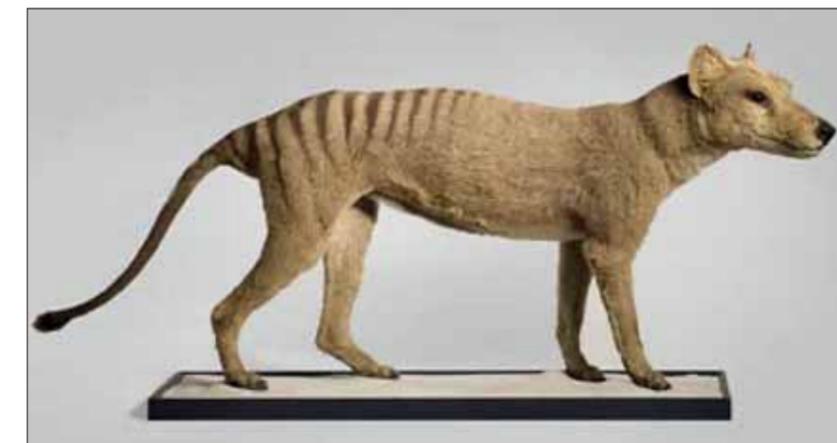


Figure 1
Thylacinus cynocephalus
(Tasmanian Wolfe or Tasmanian
Tiger), extinct since 1936.

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. During the extensive renovation the Zoological Collection is currently safely stored. All exhibits will be checked, cleaned and renovated, when necessary, by an internationally renowned taxidermist. With the completion of the renovation – now expected in 2022 - the collection will be made accessible to the public representing a new and timely concept.

The new concept: Exhibition Timeline Evolution @ 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

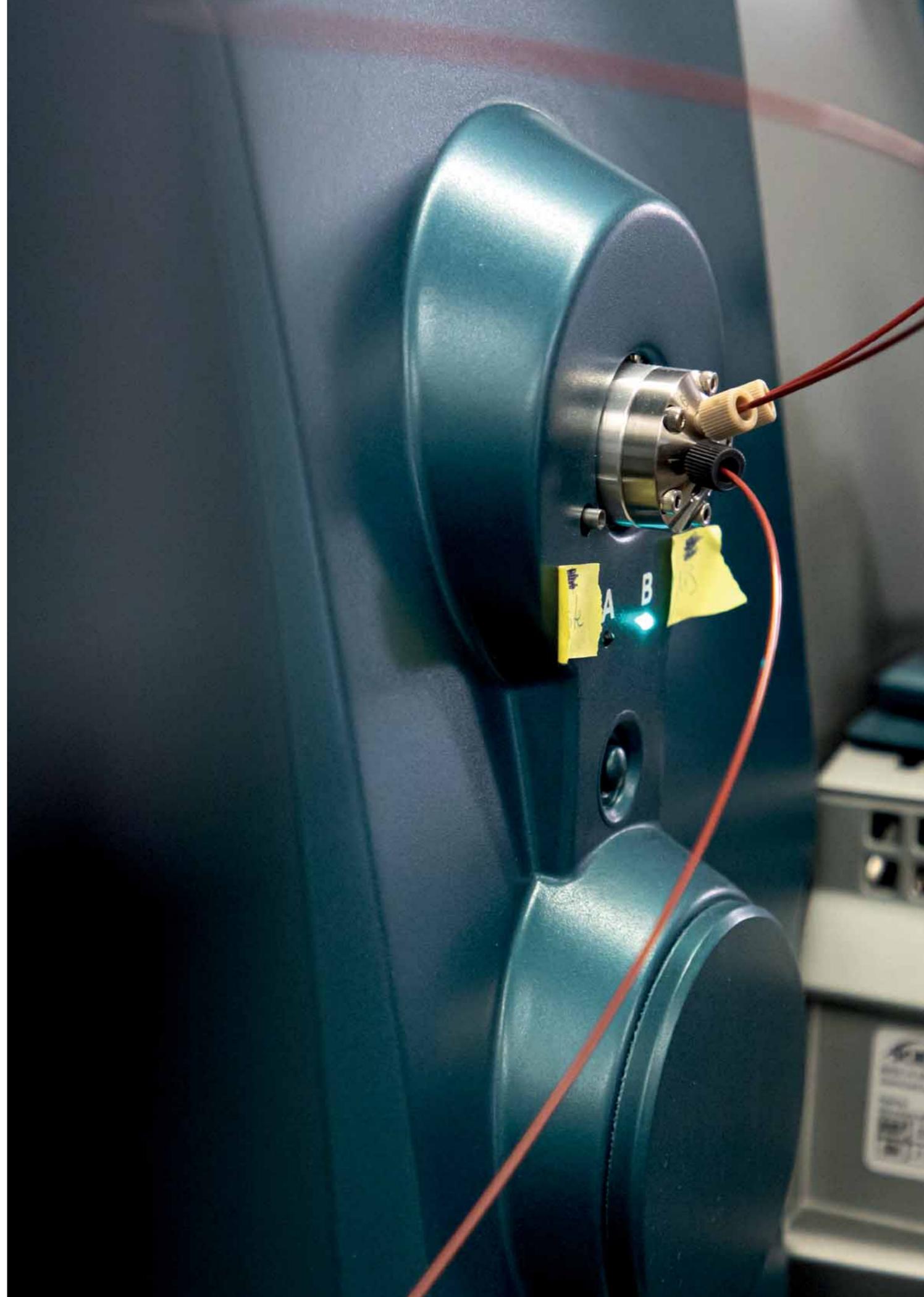
the scientifically meaningful specimens of the original collection have been outsourced to the Senckenberg Research Institute and Natural History Museum in Frankfurt many years ago, there are still some remarkable specimens of the Zoological collection that are of general public interest. Many specimens were part of the "Zoologisches Cabinet" 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 marsupials, 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, *Thylacynus cynocephalus*), the largest carnivore marsupial, which was extinct in 1936 (Figure 1).

The new exhibition Timeline Evolution is designed and realized by Ranger Design in Stuttgart, an experienced team of specialists from the fields of product, graphic, interior, and media design which has already won several national and international awards and is German Design Award Winner 2020. On August 1, 2021, >95% of all exhibits have found their new home in the new showcases of the Timeline Evolution exhibition (Figure 2) and, after their face lift, will move into them by spring 2022.



Figure 2
Staff Members of Ranger Design
finalizing the position of some
exhibits in their new show cases.



APPENDIX

A. 1 COS FUNDING

Finances

The Centre for Organismal Studies Heidelberg (COS) - as a central research institution of Heidelberg University - receives basic funding by the state of Baden-Württemberg through the rector's office (internal funding). Since the founding of COS, research group leaders at COS have been very active in acquiring additional funding from several different funding organizations (external funding). During the reporting period, the internal funding was also increased to support the centre appropriately (Figure 1). Internal Funding mostly provides for staff appropriations. The ratio between internal and external funding varies between 2,18 (2017) and 2,21 (2020).

The main funding organization for external grants is the Deutsche Forschungsgemeinschaft (DFG) through several different funding instruments such as Collaborative Research Centers (CRCs) and Research Units (RuS), Excellence Initiative (ExIni) and Excellence Strategy (EXC) and research grants including the Emmy Noether Programme and individual research grants. Other funding bodies are the European Union (grants by the European Research Council (ERC), the Framework Programme 7 and Horizon 2020), the Bundesministerium für Bildung und Forschung (BMBF), Foundations (Klaus Tschirra Stiftung, Baden Württemberg Stiftung, Lautenschläger Stiftung, Volkswagenstiftung, Alexander v. Humboldt-Stiftung, Schlumberger Foundation etc.) and others (including industry funding, equipment purchases, scholarships). The continuous high level of external funding can be



Figure 1
COS global finances: internal funding versus external funding in € p.a. from 2017 till 2020. Internal funding without individual financial offers of appointment (Berufungszusagen), funds from open positions (Mittelschöpfung) and project-bound state funding (Zweitmittel). Numbers according to the budget of Heidelberg University and SAP expenses.

A

explained such that many research groups participate in research consortia like CRCs and within the Excellence Initiative, that the acquisition of funding from the European Research Council lately increased strongly with two Synergy Grants and that twelve current or former independent junior research groups worked at COS during the reporting period.

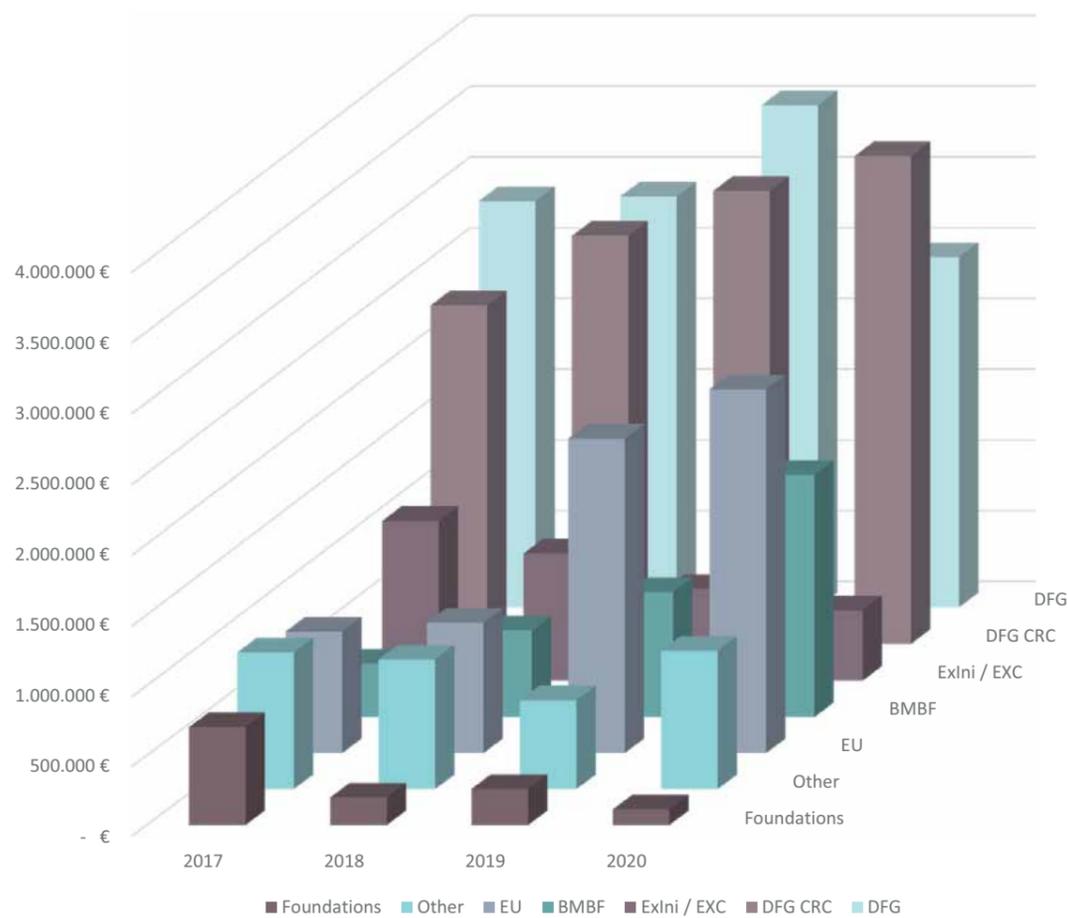


Figure 2
Detailed analysis of external funding 2017 – 2020 based on SAP expenses. Abbreviations: BMBF Bundesministerium für Bildung und Forschung, CRC Collaborative Research Center, DFG Deutsche Forschungsgemeinschaft, EU European Union, ExIni Exzellenzinitiative des Bundes und der Länder, EXC Exzellenzstrategie des Bundes und der Länder.

A. 2 COS EVENTS

Events: Seminars, Symposia and Public Outreach Activities

Researchers at COS engage in events for the scientific community on campus such as lectures, seminar and symposia as well as for events for the interested public. The aim is to provide stimulating scientific discourse on the Heidelberg life science campus reflecting the diverse research interests of COS Heidelberg, and to present selected topics to a general audience beyond the Heidelberg life science campus.

Lectures at COS and COS Keynote

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

COS Lectures 2018 – 2019

Date	Invited Speaker	Title
08.03.2018	Martin Kaltenpoth*	An inordinate fondness for symbionts: mutualist-provided defense, digestion, and desiccation tolerance in beetles
09.04.2018	Lyuba Ryabova	Target of rapamycin (TOR) in translation reinitiation control
26.04.2017	Eric Kemen	Dynamics versus stability - How microbial communities interact to colonise a host
03.05.2018	Sabine Zachgo	Unraveling key transcription factor functions in <i>Marchantia polymorpha</i>
17.05.2018	Niko Geldner	The building of polarised cellular barriers in animals and plants
14.06.2018	Matias Zurbriggen	Plant and Mammalian synthetic biology and optogenetics approaches for the control and understanding of cellular processes
13.07.2018	Yasin Dagdas	Bridging the gap between selective autophagy and endoplasmic reticulum homeostasis
11.09.2018	Takashi Ueda	Diversification and Evolution of Membrane Trafficking Pathways in Plants - How did plants acquire new organelles
25.10.2018	Dorothee Staiger	Ribonomics to identify RNA-binding protein targets in <i>Arabidopsis</i>
08.11.2018	Klaus Theres	Mechanisms in axillary meristem formation
15.11.2018	Caren Norden	From growth to lamination the emergence of the vertebrate retina
13.12.2018	Christoph Englert	Insights into aging and sex determination from a short-lived killifish
24.01.2019	Anja Geitmann	Mastering the maze - how plant males find their partners
5.02.2019	Teva Vernoux	Making flowers over and over again: self-organization at the shoot apex

COS Lectures 2018-2021, continued

Date	Invited Speaker	Title
25.03.2019	Oliver Fiehn	Classifying plant metabolites by mass spectra, organs and plant species
04.04.2019	Holger Puchta	Genome engineering in plants: past, presence, future
16.05.2019	Yrjo Helariutta	Title: Towards understanding the morphogenesis and functionality of phloem
10.10.2019	Jonathan Gershenzon*	Two-component chemical defenses of maize and wheat: more than just protection

* Student & postdoc organized COS Lecture

In 2020 the format was changed to “COS Keynote” and initially planned for with 3-4 COS Keynote lectures per year. For the COS Keynote, outstanding and high-profile speakers are selected who are able to attract scientists working with very diverse systems across the Heidelberg life science community. They work ideally beyond systematic boundaries or establish concepts of very broad impact. So far two events took place and drew large numbers of participants.

COS Keynote 2020-2021

Date	Invited Speaker	Title
20.02.2020	Caroline Dean	Antisense-mediated Chromatin Silencing
18.05.2021	Stein Aerts	From single-cell multi-omics to gene regulatory networks and enhancer logic

Seminars and Seminar Series at COS

Apart from seminars organised by each research group fostering scientific exchange within COS and on campus, the PhD students and postdocs at COS are organising since 2014 a seminar series solely for the COS community, the “COS PhD postdoc seminar” renamed “COS talk” in 2018. This student and postdoc organized series is held on a weekly basis and provides opportunity to present research concepts for PhD students, postdocs and research group leaders. With the pandemic since March 2020, the format was changed swiftly and successfully to an online format being an important anchor and platform not only for scientific exchange but also for social interactions during the weeks to strict lockdown.

Symposia at COS

COS Symposia are organized on a biannual basis reflecting a topic selected by the research group leaders of COS. Renown experts from inside and outside COS are invited, short talks selected from abstracts as well as poster sessions provide a platform for PhD students and postdocs to present their projects. COS symposia have received generous financial support by the Klaus Tschira Foundation, HBIQS, GfE, EMBO, eurofins, elife and Nikon.

During the reporting period, only one symposium was held. The seventh international COS symposium is currently planned for autumn 2022.

6th International COS Symposium**Genetics 2019: Old Questions and New Frontiers, June 06 and 07, 2019**

Session 1: Genetic Variation

Elaine Ostrander	How to Build a Dog in 927, 152, 360 Easy Steps
Diethard Tautz	Testing the omnigenic model for quantitative traits
Trudy Mackay	Context Dependent Effects of Alleles Affecting Quantitative Traits: Insights From Drosophila

Session 2: Genetics and Environment

Claudia Köhler	Epigenetic mechanisms regulating plant reproduction
Ortrun Mittelsten Scheid	Shining light on seeds sheds light on germination
Korbinian Schneeberger	The evolution and natural control of genetic incompatibilities
Duncan Odom	The evolution of transcriptional regulation among mammals

Session 3: Cellular Geneticist

Eileen Furlong	Genome regulation during developmental transitions: a single cell view
Brenda Andrews	From phenotypes to pathways: global exploration of cellular systems using yeast functional genomic
Nikolaus Rajewsky	Design Principles of Gene Expression in Space and Time

Session 4: Molecular Mechanisms

Stacy Harmer	There and back again: from phototropism to heliotropism
Martin Jonikas	Structure, Biogenesis, and Engineering of the Eukaryotic CO ₂ -Concentrating Organelle, the Pyrenoid

Public Lecture

Detlef Weigel	Klima im Wandel – Pflanzen im Wandel
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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 tenth year and with sixteen events having taken place already, the lecture now attracts regularly more than 200 students from high schools in Heidelberg as well as other cities and resonates very well on campus.

In brief, one event is held over two days: Day one is reserved for high school students in their final two or three years. The lecture is followed by a tutor-lead discussion in small groups of 12-15 persons. During this discussion, the invited speaker tours all subgroups to answer questions personally. Students, tutors (recruited from COS research groups) and speaker finally meet for a concluding discussion and remarks. This part of the lecture series is coordinated with the “Stützpunktschulen Molekularbiologie”² 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.

Bertalanffy Lecture Series 2018-2021

Date	Invited Speaker	Title
19./20.04.2018	Kristin Tessmar-Raible	Right timing is crucial in life: investigating rhythms and clocks in biology Light and time: how aquatic animals can inform human biology
18./19.10.2018	Dirk Inze	PLANT ORGAN SIZE: FROM BASIC RESEARCH TO HIGHER CROP YIELD MECHANISM REGULATING THE SIZE OF PLANT ORGANS
04./05.07.2019	Jan Kaslin	Regeneration: animals with super-hero powers Make do and make new: how zebrafish rapidly regenerates CNS injury
14./15.10.2019	Mirana Ramialison	THE SECRET LIFE OF JUNK DNA DECIPHERING CARDIAC GENE REGULATORY NETWORKS IN 3D
14./15.10.2021	Elaine Ostrander	Tip to tail: How to construct a dog Genetics of morphology in the domestic dog

Since 2014, the Bertalanffy Lecture Series is complemented by a 2-week summer course for high school students, in which they will work on small research projects.

In a further step, the program was expanded in 2016 by the third element "Science goes to school". COS PhD students apply to visit biology courses at schools and talk about their research project. This way, high school students gain insights and PhD students train their communication and presentation skills.

In 2020 all formats initially had to pause but in 2021 they resumed stepwise with format changes compatible with the pandemic situation.

One innovation is the project "wissenschaft.leben" which aims at explaining basic research to the public³. The kick-off event was held as a discussion round on the topic "Coronavirus" and two more events did follow already.

2 <http://www.stuetzpunktschulen.de>
3 <https://bertalanffy-live.de/wissenschaft-leben/>

Events "wissenschaft.leben" since 2021

Events	Invited Speaker	Title
Discussion round	Claudia Denking, Andreas Welker, Michael Knop	Universität und Gesundheitsamt: Mit Tests gemeinsam gegen Corona
Discussion round	Barbara Mittler, Johanna Stachel, Ekkehart Reimer	Wissenschaft – ein Team sport?
Podcast	Annika Guse, Sebastian Rupp	Alles schon im Kopf drin, das ist der erste Schritt

Sunday Matinée

The Sunday Matinée was a highly successful lecture series running every winter semester from 1980 till 2017 with the aim to present current topics of life science research and related disciplines to the interested public. The lecture series will be restarted in an appropriate format with the reopening of the exhibition Timeline Evolution.

The botanic garden with its "Grüne Schule"⁴ continues to be very active in public outreach: offering guided tours and courses itself and participating in the "Junge Universität" of Heidelberg University.

In addition to the here mentioned scientific and outreach programs, researchers at COS participate in many other initiatives on campus, notably the KinderUni, preschool education, the courses of the "Tschira Jugendakademie"⁵ and the "International Summer Science School Heidelberg"⁶. All programs have been very creative in adapting to the pandemic situation to continue their offers.

4 <https://grueneschule.cos.uni-heidelberg.de/index.php>
5 <https://www.tschira-jugendakademie.info>
6 <http://www.ish-heidelberg.de/>



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