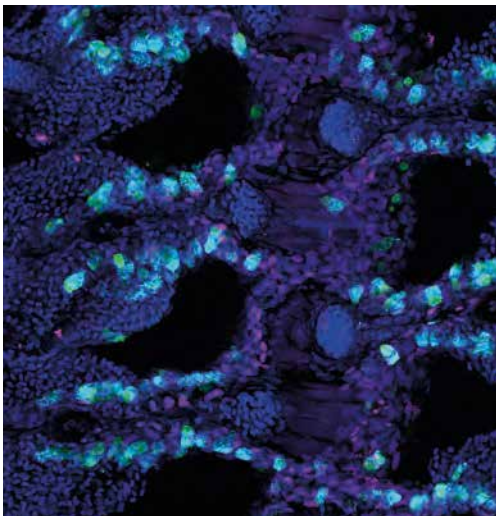
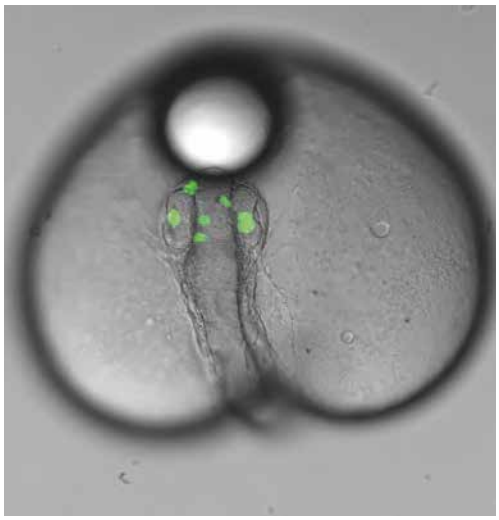
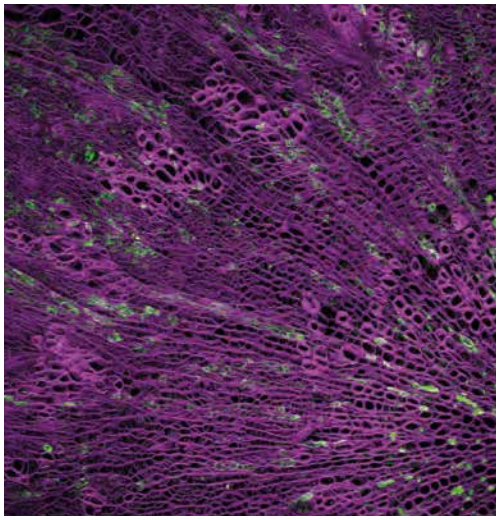
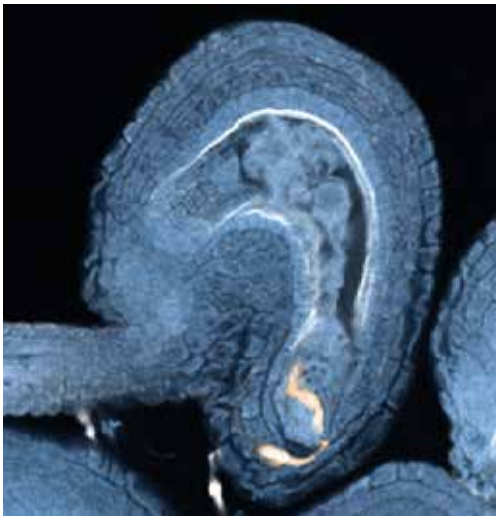
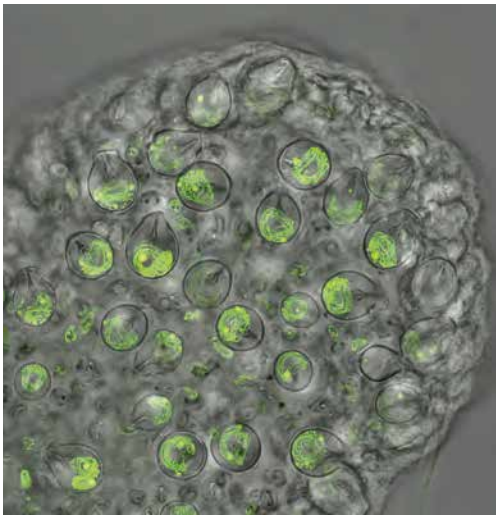
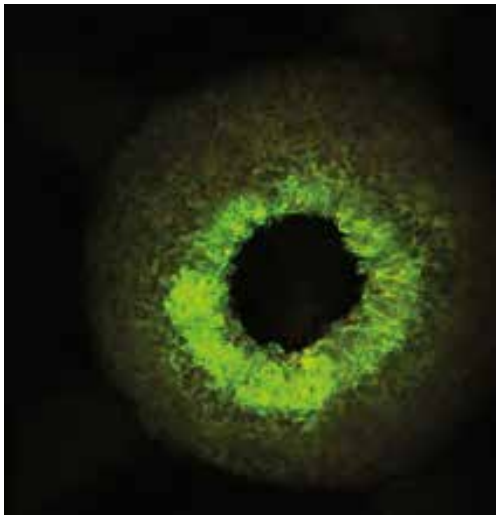
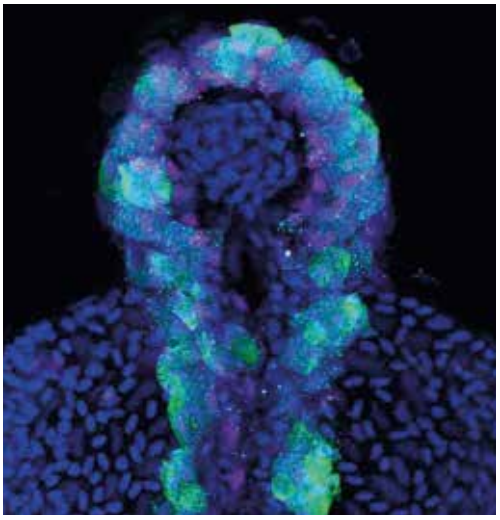
CENTRE FOR
ORGANISMAL STUDIES

UNIVERSITÄT
HEIDELBERG
ZUKUNFT
SEIT 1386

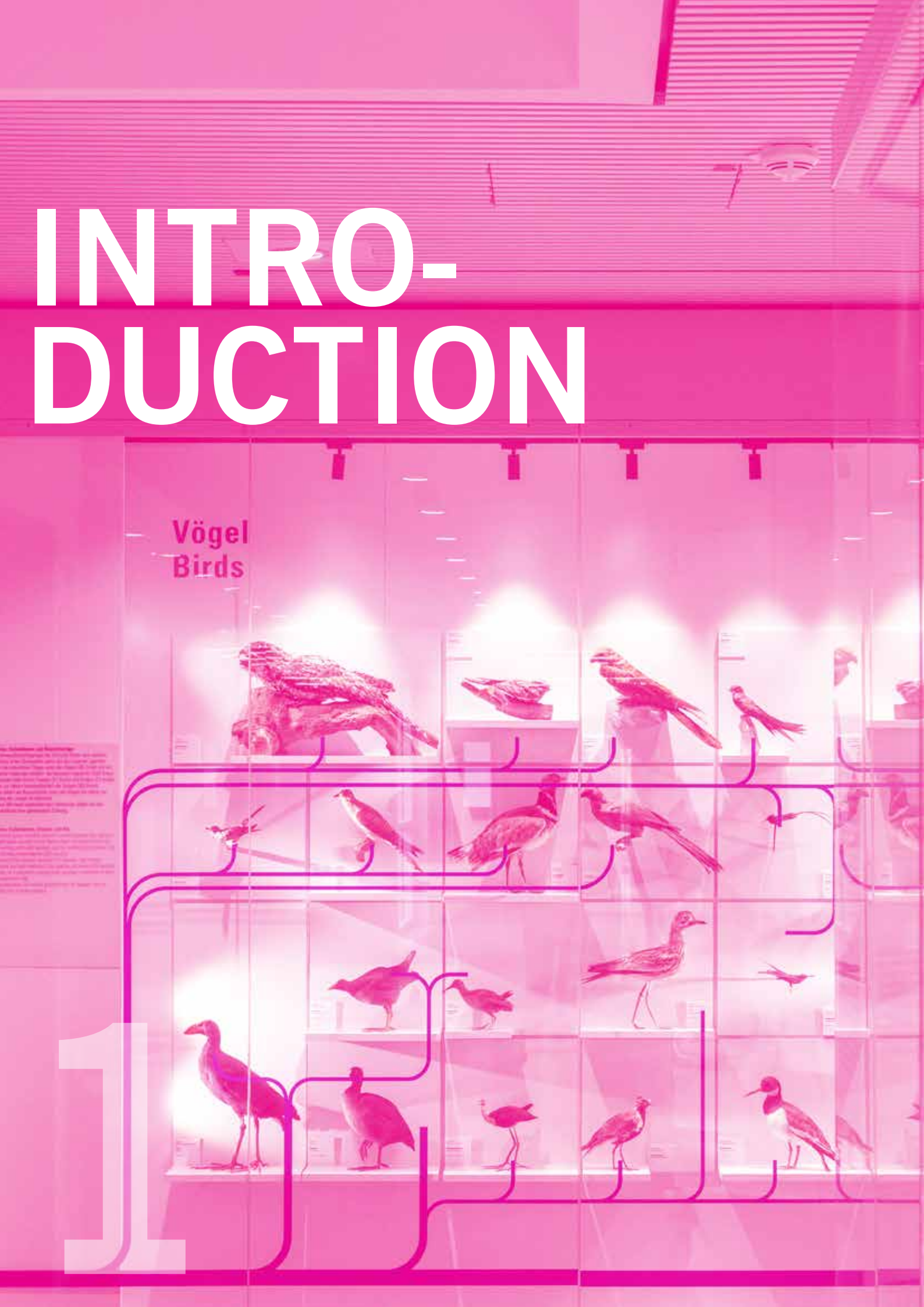
REPORT 2022-2024

CONTENT

INTRO-DUCTION



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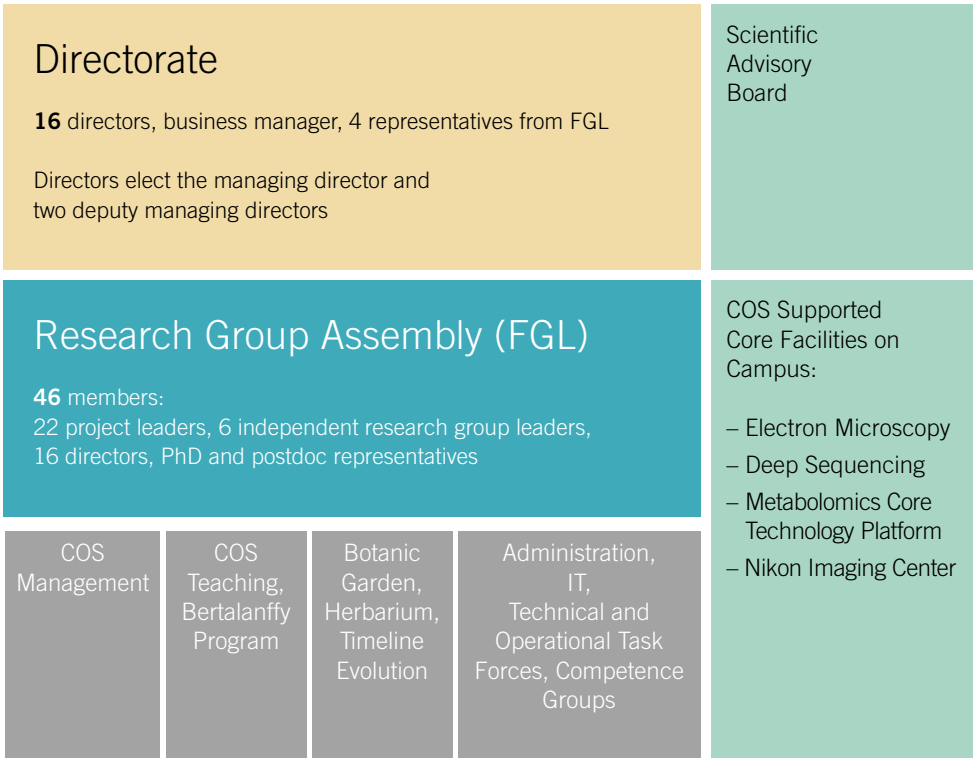
1.1 PREFACE

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 in all kingdoms of life. 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 being completed, this reporting period covering the years 2021-2024 represents a transition phase, during which we further strengthened our portfolio in research and teaching and thus solidified our role as a major player in the Heidelberg Life Sciences.

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 now consists of 16 tenured or tenure-track 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. During the reporting period, COS went through a substantial turnover of permanent professors with one professorship being added in total to COS by Heidelberg University. Thomas Holstein, Stefan Frings and Elisabeth Pollerberg retired and COS was successful in recruiting Gáspár Jékely, Lázaro Centanin, and Gilles Storelli, respectively, as their successors at the full professor level. By focusing on evolutionary neurobiology, stem cell biology and the host-microbe crosstalk in the invertebrate gut, respectively, our new colleagues nicely complement the COS research portfolio and further strengthen organismal biology at the centre. Conversely, Ursula Kummer moved to the newly founded BioQuant centre and has joined the Faculty of Engineering Sciences. Importantly, COS has been able to secure two new tenure-track positions from the University, which were filled by Lauren Saunders and Britta Velten representing the first tenure-track professors at COS. Lauren Saunders brings in developmental genomics on the single cell level whereas Britta Velten, trained mathematician, holds expertise in biological data science and is co-affiliated with the Interdisciplinary Center for Scientific Computing (IWR). In addition, COS was successful in recruiting Yasin Dagdas as a Heisenberg professor who will join COS in 2025 and take over the department of Plant Physiology from Rüdiger Hell in 2026. Yasin Dagdas' research focuses on the cross-kingdom evolution of autophagy providing multiple opportunities for productive interactions within the centre and the Heidelberg life sciences in general. 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 15 young group leaders with funding from the Emmy Noether program of the DFG (Annika Guse, Amal Johnston, Steffen Lemke, Sebastian Wolf, Michael Raissig, Kasper van Gelderen,



Britta Velten, Lauren Saunders), the Excellence Initiative (Emmanuel Gaquerel, Guido Grossmann), the CRC1324 (Sergio Acebrón, Josephine Bageritz), the CRC873 (Lázaro 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. Highlighting our success in this direction, most junior PIs have secured professorship positions at renowned European universities after their initial phase of scientific independency at COS. During the reporting period, the success of our junior group leaders continued with Michael Raissig moving to the University of Bern where he was promoted recently to an Associate Professor and Sergio Acebrón who secured a professorship at the University of the Basque Country in Bilbao. At the same time, Kasper van Gelderen started his scientific independency as a new Emmy Noether fellow at COS. The development of COS faculty is a continuous process, and with ongoing applications to junior group leader positions and to broader structural programs, we expect that we will continue to host a highly dynamic and stimulating group of scientists conducting research at the forefront of organismal biology. A major roadblock to the development of COS is space constraint. In the reporting period we have undergone an official resource audit that came to the conclusion that COS is lacking 3000 square meters of lab and office space. This shortage has forced us to turn away a substantial number of promising candidates for junior group leader and thus already has severely impacted the development of COS.

Biodiversity and Plant Systematics M. Koch	Evolution of Morphogenesis S. Lemke §	Cell Biology of Endosymbiosis A. Guse §	Developmental Genomics L. Saunders $
Molecular Evolution and Genomics T. Holstein	Circadion Clock Biology N. Foulkes	Evolutionary Neurobiology G. Jékely $	Biological Data Science B. Velten $
Stem Cell Niche Heterogeneity J. Bageritz	Animal Evolution D. Arendt	Developmental Biology I. Lohmann	Developmental Biology/ Physiology J. Wittbrodt
Cell and Developmental Biology A. Maizel	Clonal Analysis of post-embryonic Stem Cells L. Centanin	Stem Cell Biology J. Lohmann	Developmental Physiology T. Greb
Plant Molecular Biology R. Hell	Cytoskeleton, Cell Division and Signalling G. Pereia	Stomatal Biology M. Raissig §	Modelling of Biological Processes U. Kummer §
Cell Biology K. Schumacher	Light Signalling and Cell Biology K. van Gelderen $	Cell Signalling S. Acebrón	Glycobiology S. Strahl

From Molecules to Organismal Interaction

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

1.3 RESEARCH AND COMMUNITY INTEGRATION

With its 24 PIs, COS covers a wide range of excellent and innovative research topics ranging from the molecular to the cellular and organismal level. Our success is visible at multiple levels, such as awards, publications, third party funding, participation in research consortia, or appointments. Highlighting our standing in the scientific community, Ingrid Lohmann, Jochen Wittbrodt, and Gáspár Jékely were elected as EMBO members during the reporting period, which, together with the memberships of Jan Lohmann and Detlev Arendt, brings the number of EMBO members among COS PIs to five. Since other aspects are described in detail in the contributions from individual PIs, we only give a brief overview of some of the key facts reflecting our performance. Regarding publications, COS PIs have pledged to adhere to open access publication wherever possible, and we are proud that during the reporting period, more than 100 manuscripts with COS participation have been posted on bioRxiv. In total, PubMed reports more than 340 publications with COS affiliation published from 2021 to 2024 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 since its inauguration, the funding portfolio of COS 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. During that time, five ERC grants were secured by Detlev Arendt, Jan Lohmann, Jochen Wittbrodt, Thomas Greb and Annika Guse. Due to the large turnover of professors and the promotion of many junior group leaders to professors in other universities since the last reporting period, the amount of external funding decreased to 8.9 M€ in 2023. Beside the turnover of research groups, and the COVID-19 pandemic, space constraints have contributed to this development, since COS has had to turn away two candidates with funded Emmy Noether grants worth more than 3.5 M€. Another important transition was the expiration of COS-driven research consortia, mainly the Collaborative Research Center (CRC) 873 led by Jan Lohmann and the Research Unit (RU) 2581 led by Alexis Maizel. Consequently, the establishment of a new COS-centred CRC integrating the broad spectrum of organismal biology at the centre and including the research of our new colleagues will be one of the major focuses for us in the next two years. Currently, the main drivers of our external funding are two ERC Synergy grants held by Jan Lohmann and Jochen Wittbrodt, and two ERC Advanced grants held by Gáspár Jékely and Detlev Arendt, the latter administered at EMBL. Moreover, Kasper van Gelderen's group is funded since 2022 by a prestigious Emmy Noether grant of the DFG and our two new tenure-track professors Britta Velten and Lauren Saunders secured the same funding in 2024. It is also worth mentioning that the two new full COS professors Gilles Storelli and Yasin Dagdas bring in two additional ERC grants which will further boost our funding situation. In addition to this more group-centered funding, COS PIs take a lead in existing research consortia, like the CRC1324 in which Thomas Holstein is acting as co-spokesperson and the RU2509 which is led by Sabine Strahl. Overall, we are proud that COS representation in DFG-funded collaborative networks is strong with eight of our PIs being active members in the CRC1324 (Wnt Signalling, coordinated by COS), five in the CRC1101 (Specificity in Plants, coordinated by Tübingen University), one in the CRC1211 (Evolution at the dry limit, coordinated by Cologne University), three in the excellence cluster "3D Matter Made to Order", and two in RU2509 (Glycosylation, coordinated by COS). In total, more than 80% of our independent PIs are part of nationally or internationally funded consortia, highlighting the excellence of our research and the visibility of COS PIs. Collectively, we are proud to say that, on average, for every Euro invested in COS by the state, we secure more than two Euros of outside funding, underlining the commitment and competitiveness of our PIs.

Research consortia not only represent prominent funding lines, but more importantly, foster collaboration and interaction of COS scientists in house, as well as with colleagues in the Heidelberg scientific community and beyond. Especially, after having overcome the COVID-19 pandemic, re-initiation of personal exchange and interaction, especially among early career scientists, is one major goal of COS and still an ongoing process. In this regard, 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. Here, we follow 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), “Genetics 2019 – old questions and new frontiers” (2019), “Building functionality - the relevance of form across biological scales” (2022), and “Life in Context” (2024). 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. Building on this, major initiatives have emerged, grounded in the cornerstones of our research portfolio, namely the planning of a new CRC on Growth Control and Coordination across Scales and Phyla, and an excellence cluster initiative named GreenRobust focusing on the robustness of plant systems from molecules to ecosystems in light of environmental changes. In this regard it is noteworthy that from four draft proposals for excellence clusters which have been put forward by COS PIs following Heidelberg University´s internal call in early 2021, our GreenRobust initiative made it into the final round of selection on the national level together with 40 proposals for new excellence clusters. The GreenRobust proposal was drafted in collaboration with colleagues from the Universities of Tübingen and Hohenheim and, if successful (final decision in May 2025), the cluster will profoundly boost integration of Heidelberg University into broader scientific activities in this context and secure two more professorships focusing on data-based modelling and on environmental interactions for our centre. We also expect that this initiative will further enhance the embedding of COS into the Heidelberg life science community and act as a motor for the development of our research and infrastructure portfolio.

Renewal of the Memorandum of Understanding between COS and NIBB, October 2024

Further highlighting our development into a leading research and teaching institution on campus, many COS PIs hold leadership positions within our community and serve in manifold strategic roles for our University. Karin Schumacher is the Vice-Rector for Quality Development and Sustainability of Heidelberg University, Rüdiger Hell is the Dean of the Faculty of Biosciences, and Alexis Maizel acts as the Vice-Dean for research and is the faculty representative in the University Senate. Furthermore, Jan Lohmann is the elected 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. In addition, Jan Lohmannn serves in the strategy advisory boards of our University and the Health + Life Science Alliance Heidelberg Mannheim. Overall, we feel that COS is well positioned in the local community and in a strong position for further strategic developments.

In the reporting period, COS has also been successful in extending international interactions. Building on our core mission in organismal biology and the research direction in organismal plasticity and adaptation, COS PIs have built increasingly strong ties with members of the National Institute for Basic Biology (NIBB) 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 renewed in 2024 laying 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. After several online meetings during the COVID-19 pandemic, an onsite satellite workshop was held at COS in the context of the COS symposium in 2022 for which a strong delegation of NIBB PIs traveled to Heidelberg. In turn, a group of seven COS PIs and four students traveled to Okazaki in October 2024 taking part in another NIBB-COS workshop during which scientific projects were presented reciprocally. The exchange of scientists between the NIBB and COS, let it be in the context of short-term visits or by taking up positions at the respective sister institution has been, and will continue to be, a rewarding interaction between two world-leading institutions focusing on organismal biology across phylogenetic kingdoms.

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 has 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. With the recruitment of Gáspár Jékely and his focus on electron microscopy, especially the EMCF experienced a boost on the side of equipment, personnel and technologies. 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 maximize 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 without asking for any user fee. 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, fluorescence lifetime imaging or light sheet microscopy. In the reporting period, large equipment grants have been awarded to Thomas Greb, Jochen Wittbrodt and Karin Schumacher, which allowed them to invest into high end microscopy. In the last two years, most of our microscope systems have been gathered in a common custom-tailored space in the main COS building further facilitating access and operation. 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 or photosynthesis analysis systems. These systems are usually built by a dedicated workshop equipped with state-of-the-art tools and enthusiastic personnel. 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. All campus facilities have recently been assembled under the roof of the CellNetworks Core Technology Platform (CCTP) providing an organizational umbrella further increasing professional administration, up-to-dateness of equipment and fair access. 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 the integration of new colleagues, gives 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

Beside research, teaching is a central mission of COS and reflects our aim to convey organismal biology from Bachelor to PhD level following the same philosophy that drives our science. In our teaching we cover fundamental biological topics ranging from molecular biology, biochemistry, and evolution over the central principles of development and physiology all the way to topics like neurobiology and ecology. With 16 tenured and tenure-track professors and other staff involved, COS is the major contributor to the teaching program within the Faculty of Biosciences at Heidelberg University. COS is involved in the bachelor's degree programs in Biosciences and Biology 50%, where it fully organizes four of seven practical courses and two of four lectures in the compulsory program, while contributing to all other lectures. Of the compulsory elective courses during bachelor studies, COS provides about 50% of the overall offer. For the Master of Molecular Biosciences program COS organizes two of seven majors (Developmental and Stem Cell Biology and Molecular and Applied Plant Sciences) and substantially contributes to a third one (Neurosciences).

In the period from 2020 to 2022, we faced major challenges with regard to our teaching efforts, due to the restrictions during the COVID-19 pandemic, ongoing renovations of the lecture hall and practical rooms and a shooting incident in January 2022. Despite of these significant challenges, we were able to provide the full course program, including practical courses essential for biological studies and supported our students in overcoming personal and study-related challenges. Success in this regard is reflected not only in the consistent number of graduates and very positive feedback in student evaluations but also by the ever rising number of applicants to the bioscience bachelor program in Heidelberg, underlining the attractiveness of our study program. Taking advantage of the necessary innovations made during the pandemic, we successfully integrated some of those inventions into our ongoing teaching efforts. These include high-quality recordings of lectures and tutorial videos for practical courses, online safety briefings, newly established bioinformatics courses and the opportunity to conduct self-organized one-day field trips on biodiversity. With the completion of the teaching area in the main COS building (INF 230/231), COS now offers an excellent environment for students, not only regarding lecture halls and course rooms but also common areas inside and outside the building. One highlight in this context is certainly the new exhibition "Timeline Evolution" displaying our zoological collection, providing much-needed opportunities for studying, learning and interpersonal exchange. Our efforts to improve our teaching infrastructure are currently continued with the ongoing renovation of the lecture hall in INF 360.

Legende: HP-E practical course in the newly renovated and equipped course room.

A highly appreciated opportunity for our centre is the integration of new professors and research groups into the teaching program which provides the exciting opportunity to integrate new technologies, topics and ideas. The last years have therefore seen some innovative new courses reflecting how COS continuously develops in general. Some highlights are presented in the following. Because developmental biology bridging different organisms and organizational levels is at the heart of COS, we reorganized and expanded our mandatory basic course "Experimental Developmental Biology" in our bachelor program. The course now includes five of the most important model species investigated at COS (Arabidopsis, Hydra, Drosophila, Xenopus, Medaka) and addresses topics from environmental and genetic regulation, early embryonic development, birth defects up to early organ development (Greb, Gromes, Domsch, Acebrón, Thumberger). The course is complemented by the new elective lecture „Principles in Developmental and Stem Cell Biology" (Bageritz, Boutros, Centanin, Foulkes, Greb, I. Lohmann, J. Lohmann, Saunders, Wittbrodt), which addresses both plant and animal development. Moreover, new and expanded offers in data analysis and statistics include a practical course for bachelor students on "Data Analysis for RNA-seq data" (Velten) and courses on "Data Analysis for the Life Sciences", "Computational analysis of single cell data" (Bageritz, Saunders, Velten), and "Biological data science for high-throughput omics data" (Velten). A new practical course for bachelor students organized by the group of Gáspár Jékely starting in 2025 will furthermore include a one-week field excursion to the Roscoff Marine Station (France). Another important topic is the ongoing evaluation and improvement of the study program for prospective high school teachers (Biology 50% and Master of Education). Here, we aim to increase the relevance of our study program for students working as teachers after their studies beyond simply conveying knowledge in biology. One way to achieve this aim is the integration of high school teachers as guest lecturers for our introductory biology didactics seminar. To reflect recent developments of the official school curriculum which focuses stronger on biochemistry, a new course for Master students entitled „Bildungsplanbezogene Biochemie für das Lehramt" (Strahl) has moreover been established conveying not only theoretical knowledge of biochemistry but also practical experiments which can be integrated into school lessons. Support of school education is in addition a core mission within the outreach activities at COS. With our newly reopened exhibition "Timeline Evolution" and the Botanic Garden, we provide two prime destinations for school trips holding exceptional educational value. In addition, we offer high school students and their teachers opportunities to join COS in high profile lectures and summer practicals in the context of the Bertalanffy program.


Bedrohte Vielfal
WC

1.5 FUTURE PERSPECTIVES AND CHALLENGES

Having just passed a transition phase during which we were able to make major topical adjustments by the recruitment of new professors, COS has the opportunity to leverage its invigorated structure to consolidate its long-term strategic development. 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 within Heidelberg University and in Germany´s excellence strategy.

Continued integration into Heidelberg life sciences
By having recruited Gilles Storelli focusing on the role of the microbiota in the fly gut and Gáspár Jékely focusing on evolutionary neurobiology, COS accomplished two of its major strategical goals. These were strengthening the aspect of the interaction between metabolism and development within COS and to keep strong ties with the Interdisciplinary Centre for Neuroscience (IZN). Together with existing expertise within our centre, which also includes the Metabolomics Core Technology Platform (MCTP), the work of Gilles Storelli will be one cornerstone for shaping a new COS-centered CRC on growth control and coordination bridging animal and plant biology. Strategic discussions in preparation of the CRC application have started and possible projects have been collected throughout COS groups. Our vision is that such a CRC will integrate and stimulate research activities and generate additional synergies across our centre and take advantage of the existing strong research in this area. The recruitment of Yasin Dagdas focusing on autophagy across phylogenetic kingdoms and of Lauren Saunders focusing on developmental regulation on the single cell level are additional strategic moves supporting this initiative. By also integrating colleagues from the DKFZ and the EMBL working on the interface between development and metabolism, we believe that we will be able to make a convincing point in front of the DFG and the reviewers involved. In this context it is important to note that Aurelio Teleman (DKFZ), a world leader investigating signal transduction in cancer and metabolism, holds a co-affiliation with COS since September 2024.

With the recruitment of Gáspár Jékely, we furthermore provide now a fresh interface for connecting COS with the Interdisciplinary Centre for Neuroscience (IZN), a stronghold of mechanistic organismal biology in Heidelberg. Such a connection is particularly interesting considering upcoming retirements within the IZN in the next years, providing the opportunity for tailoring neurobiological research within the Heidelberg life sciences and for an even stronger integration of COS and IZN.

Our new colleagues at the professorial level had also a major impact on how we positioned ourselves in the ongoing competition for excellence clusters in the framework of the federal excellence strategy. As laid out above, together with colleagues from the universities of Tübingen and Hohenheim, COS was successful in placing the cluster application GreenRobust into the final round of selection which will be completed in May 2025. The cluster proposal addresses the robustness of plant systems from molecules to ecosystems considering environmental fluctuations in temperature, water availability, and pathogens and aims for providing strategies for a science-based management of plant-based ecological and agricultural systems. Thomas Greb, Alexis Maizel, Jan Lohmann, Marcus Koch, Karin Schumacher, and Britta Velten are the current COS PIs within the cluster, with Britta Velten substantially carrying the data-driven activities within GreenRobust. Together with the existing cluster “3D Matter Made to Order”, which is up for a renewal and of which the COS PIs Jochen Wittbrodt, Gislene Pereira and Gáspár Jékely are part, COS is thus shaping two major initiatives within Heidelberg University in this competition. If successful, GreenRobust will allow COS to recruit one full and one tenure-track professor on “Data-based Modelling of Complex Biological Systems” and on “Organismal Plant-Environment Interactions”, respectively. Together with the envisioned new Master program “inPLANT – Integrative Plant Biology: from molecules to ecosystems” bridging the three participating universities, GreenRobust will therefore substantially strengthen large data-driven organismal research and teaching with an environmental focus at Heidelberg University. We

believe that this development is highly relevant in the context of the ongoing fusion of the Medical Faculties of Heidelberg and Mannheim universities creating an unprecedented center of gravity in medical research within Germany, possibly outshining and, in the long-term, outcompeting the non-medical life sciences within the Heidelberg-Mannheim area.

Building infrastructure
Whereas the decisions connected to new colleagues were in our hands, 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 was finally finished in 2022 after more than ten years of grueling construction work, COS will remain segregated into at least four locations and suffering a space deficit exceeding 3000 sqm. This situation not only hampers development of existing research groups but also forces us to frequently reject highly competitive candidates for junior group leaders, who approach COS to serve as their future host. 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 University's developmental plan projects that COS will gain a large amount of space in INF234, adjacent to our main building, but 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 restructuring the economy in face of the climate crisis and larger political challenges 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.

Support of early career scientists
Students and early career scientists experienced exceptional challenges during the COVID-19 pandemic and the impression is that the impact of these challenges have not yet been overcome completely even after several years of recovery. This impression goes hand in hand with a continuous reduction of applications to PhD and Master theses and reduced peer activities among students within the centre. Because the success and wellbeing of COS heavily depends on the enthusiasm and drive of its young researchers, the continuous supportive interaction with bachelor, master and PhD students is therefore a central mission, which we expect to become even more important in the future. COS has substantially invested into the support of young researchers with activities, such as student retreats, COS parties, and opportunities for our students to invite seminar speakers. In addition, we have recently organized PhD and postdoc assemblies and established a liaison officer to ensure better communication between faculty and young scientists, as well as help to improve organizational continuity within student representatives. We are aware that the support of early carer scientists requires constant effort and an open ear for problems of the next generation of scientists to ensure their well being, career progression and continuous scientific performance on a high level.

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 support of young scientists remain continuous challenges.

RESEARCH GROUPS

2

2.1 PROF. DR. SERGIO P. ACEBRÓN
CELL SIGNALLING & GENOME STABILITY

PROF. DR. SERGIO P. ACEBRÓN
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Fields of Interest
Cell signalling, mitosis, stem cells, genome stability, microenvironment, tissue homeostasis, cell lineage specification, WNT signalling

Summary and outlook
The architecture, homeostasis and function of the organs is defined at the cellular level by the integration of a myriad of molecular signals. In our lab, we study how different cellular lineages integrate microenvironmental signals, as well as how the underlying cascades define cellular identity, behaviour and resilience. We focus on unravelling molecular mechanisms in signal transduction, especially in the context of WNT signalling, DNA replication, DNA repair and mitosis, which misregulation often leads to disease, notably cancer. To address these questions, we integrate cutting edge live cell imaging, single-cell replication, genome & transcriptome sequencing, and genome editing techniques with detailed molecular analyses in 2D/3D stem cell models of development, organoids, and mouse models.

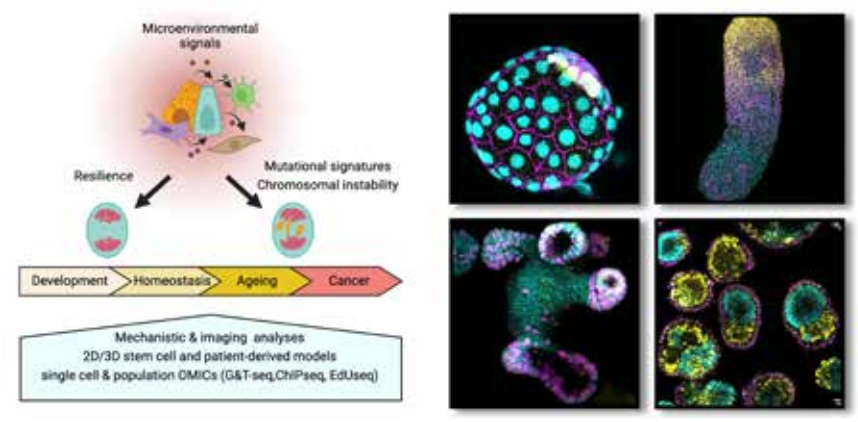

Figure 1:
We aim at understanding the roles of microenvironmental signals in regulating genome integrity across the lifetime. We leverage 3D imaging techniques with different OMICs approaches in 3D models for development (gastruloids, EiTiX) and organoids to map those processes during development, tissue homeostasis, organ ageing and disease.

We are especially interested in understanding why genomic mosaicism arises during specific stages of development, as well as during organ ageing. We have identified that several embryo patterning signals have moonlighting roles in genome maintenance from monitoring DNA replication dynamics to ensuring chromosome segregation fidelity. Intriguingly, we identified that cell signalling control of genome stability is tightly linked to cell identity. In particular, we uncovered early lineage specification and neurogenesis as developmental bottlenecks where signalling gradients put genome integrity at risk, which could provide a rationale for the high prevalence of genomic mosaicism during embryo implantation and brain development. We plan to study the bidirectional relationship between microenvironmental signals and genomic mosaicism across the lifetime, as well as to map the genomic signatures caused by signalling gradients in space and time.

Research highlights since 2021

Development and homeostasis rely on the correct replication, maintenance and segregation of our genetic blueprints. Cells harbour conserved intrinsic molecular mechanisms and checkpoints to protect DNA from damage and to promote faithful chromosome segregation, including molecular hubs that coordinate stemness and DNA damage response (Giebel et al., EMBO R 2021). However, how these intracellular processes are monitored across different cellular lineages, and why the rate and distribution of genomic mosaicism vary through lifetime, remain challenging questions. During the last years, we uncovered that several patterning signals do not only read our genetic blueprints, but they also have a moonlighting role on their maintenance. We have identified two distinct programmes controlled by extracellular signals that contribute to genome maintenance:

First, building on our previous work on cell division, we found and characterised a signalling cascade controlled by WNT ligands that monitors spindle and chromosomal dynamics directly during mitosis (Bufe et al., PNAS 2021, Habib & Acebrón Trends Cell 2022). In particular, we identified that mitotic WNT signalosomes recruit PLK1 to position the minus-end microtubule depolymerase KIF2A at the spindle poles, thereby generating pulling forces on attached kinetochores to ensure the congression and alignment of chromosomes.

Second, we identified that mammalian patterning signals –including WNT, BMP and FGF– form a signalling "rheostat" during pluripotency that monitors DNA replication stress and damage during S-phase, which in turn controls spindle dynamics and chromosome segregation fidelity in mitosis. (van den Berg et al., Nature Methods 2024; De Jaime et al., Nature Communications 2024). We revealed that patterning signals associated with anteriorisation during mammalian gastrulation increase chromosome missegregation during early human lineage specification, while the posteriorising signals WNT and BMP protect pluripotent stem cells from excessive origin firing, DNA damage and chromosome missegregation derived from stalled forks, with important implications to understand how mosaicism arises in peri-implantation embryos: the leading cause of miscarriage in humans. Through epistasis experiments, we found that WNT, BMP and FGF have distinct roles during DNA replication, and demonstrated that WNT signals sit at the helm of this regulatory cascade. We uncovered that cell signalling control of chromosome segregation declines after pluripotency exit and specification into the three germ layers, but re-emerges in differentiating neural progenitors. In particular, we identified that FGF and WNT signalling form a tug-of-war in the regulation of chromosome segregation fidelity in mouse neural progenitors during the onset of neurogenesis, but not during their self-renewal and expansion. In particular, we found that the neurogenic factor FGF2 induces DNA replication stress-mediated chromosome missegregation, which could provide a rationale for the elevated chromosomal mosaicism of the developing brain. Our results highlighted a role for patterning signals and cellular identity in genome maintenance that contributes to somatic mosaicism during mouse and human early lineage specification and neurogenesis.

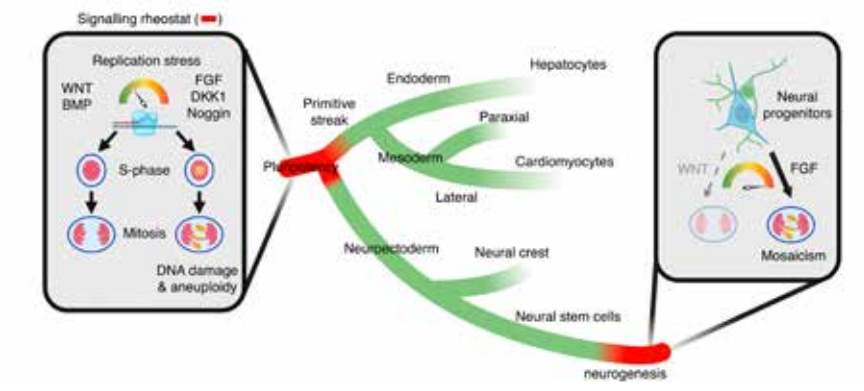

Figure 2:
A signalling rheostat controls chromosome segregation fidelity during early lineage specification and neurogenesis by modulating DNA replication stress

Microenvironmental response to DNA damage and other genotoxic insults
The STING signalling pathway acts as gatekeeper in the innate immune response towards extrinsic and intrinsic sources of cytoplasmic dsDNA by informing neighbouring cells and immune cells about their status. At the core of this pathway is the cGAS-dependent production of the intra- and extra-cellular messenger cGAMP, which activates STING and leads to IRF3-dependent expression of cytokines and interferon. Despite its relevance to monitor infections, cell death, and genome instability, the lack of specific live reporters has precluded spatio-temporal analyses of cGAS-STING signalling. We generated a fluorescent cGAMP biosensor by engineering the functional interaction of activated STING and IRF3 at the Golgi (Smarduch et al., BioRxiv, under review in EMBO J). We showed that cells encoding for the cGAMP biosensor react in a time- and concentration-dependent manner to STING agonists and cGAMP, and demonstrated that it is suitable for single cell characterisation of the dynamics of viral infection, mtDNA release upon apoptosis, and other sources of cytoplasmic dsDNA. Furthermore, we demonstrated that the biosensor sensitivity is sufficient to report microenvironmental cGAMP, allowing to analyse how STING signalling spreads through neighbouring cells. Unlike previously reported, we demonstrated that STING signalling does not monitor ruptured micronuclei, a key consequence of chromosomal instability, suggesting that other cytosolic pattern recognition receptors underlie the interferon response upon chromosomal instability. In summary, we generated a tool to capture the spatio-temporal and heterogenous dynamics of the response to cGAMP across the different scales of life; and provided a proof of principle to identify and engineer other innate immune responses reporting on DNA damage and chromosomal instability.

Future directions
We aim to understand the bidirectional roles of microenvironmental signals in genome maintenance during embryonic development, tissue homeostasis, organ aging and tumour progression (Figure 1). This line of research builds on our research on the roles of morphogens in DNA replication and cell division (Bufe et al., PNAS 2021; De Jaime et al., Nature Communications 2024), and critical techniques that we developed during the last years (van den Berg et al., Nature Methods; Smarduch et al., BioRxiv 2024; and see below). We will map the spatio-temporal roles of microenvironmental signals in genome maintenance of developmental lineages, most notably in periimplantation embryos and the brain, and during tissue renewal, including in the intestine and mammary gland.
To that end, we will employ *in vitro* lineage specification models, engineered mammalian 3D embryoids, gastruloids and organoids, *in vivo* mouse models, and patient-derived cancer organoids to decode the bidirectional molecular mechanisms underlying microenvironmental control of genome stability (Figure 1). We will combine genome editing and 3D imaging of DNA replication and cell divisions with OMICs approaches, including single cell genome and transcriptome sequencing (scG&T- seq) and the newly developed scEdu-seq (van den Berg et al., Nature Methods 2024; De Jaime et al., Nature Communications 2024), to map genomic mosaicism not only across different lineages and signalling gradients but also by unravelling the genomic footprints of different microenvironmental signals. Building on our experience designing a robust cGAMP biosensor (Smarduch et al., BioRxiv 2024), we will develop new tools to study how DNA damage and chromosomal instability are sensed in the microenvironment across space and time, including through the innate immune response.

Piling evidence suggests that the efficiency of the mechanisms overseeing genome maintenance fade down with age, although the underlying causes are not yet well-understood. As a consequence, mosaicism accumulates with time and contributes to organ aging and agedrelated pathologies, including cancer. Intriguingly, the balance between different microenvironmental signals also changes the lifetime. We are particularly interested in determining whether changes in microenvironmental signals strike both genome maintenance and cell fate during physiological aging, as well as how they contribute to tumour genomic evolution.

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Publication statistics

https://scholar.google.com/citations?user=LxZoLhsAAAAJ&hl=en

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Fields of Interest
Animal evolution, evolution of the nervous system, evolution of photoreceptor cells and eyes, cell type evolution, ecology and evolution

Summary and outlook
The cell is the primary unit of life, where the transition from genotype to phenotype is most immediate and mutations and selection manifest first. Our aim is to understand the rules that govern the evolution of cells in response to the environment, at micro- and macro-scale.

With our new multimodal cellular atlas for the marine annelid *Platynereis dumerilii,* which combines gene expression and ultrastructure for hundreds of cell types, we have a unique resource that allows us to study the genotype-to-phenotype transition for an entire body.

First, how did the genotype-phenotype link change in macroevolution? For this, we have built whole-body single-cell atlases for a whole spectrum of animals and developed new means to compare these atlases and reconstruct the complement of cell types that were present in key ancestors. With this information, we can now explore cellular changes that happened along major evolutionary lineages. Here, our main question will be the evolution of the bilaterian CNS. What were the neuron types that assembled into the first sensory-associative and motor centers, how did they originate and how did they diversify? We will thus attempt to reconstruct the urbilaterian brain.

Second, how does the genotype-phenotype link vary in microevolution in response to the environment? For this, we will map into our atlas cellular expression profiles and ultrastructure for *Platynereis* populations that we have collected during the Traversing European Coastlines (TREC) expedition. What are the cellular hotspots of variation, what molecules drive celllular change, and in what time scales does it occur?

Research highlights since 2021
Molecular physiology of sponge movement
Investigating the cellular mechanisms that drive sponge movements, we generated mutimodal data suggesting that the bodily “contraction” of sponges in fact involves a relaxation of the sponge epithelia. To this end, we established Optical Coherence Microscopy in collaboration with the Prevedel lab at EMBL. This has allowed a new view on the morphology and shape changes. We next applied a number of pharmacological agents affecting nitric oxide signaling and actomyosin contraction and found that, in each case, the effect is entirely consistent with actomyosin relaxation, reminiscent of smooth musculature. With the help of Mikhail Savitski/EMBL Proteomics core, we also combined thermal proteome profiling (TPP) for an approximation of the sponge interactome; phospho-proteomics to detect downstream effects of the “contraction” initiation; and secretomics to identify secreted peptides and changes in the composition of the extracellular matrix. This revealed an ancient contractile-inflammatory response which is carried out by both pinacocytes and choanocytes of the sponge showing intriguing similarities with the response of vascular endothelial cells to stress (Figure 1).

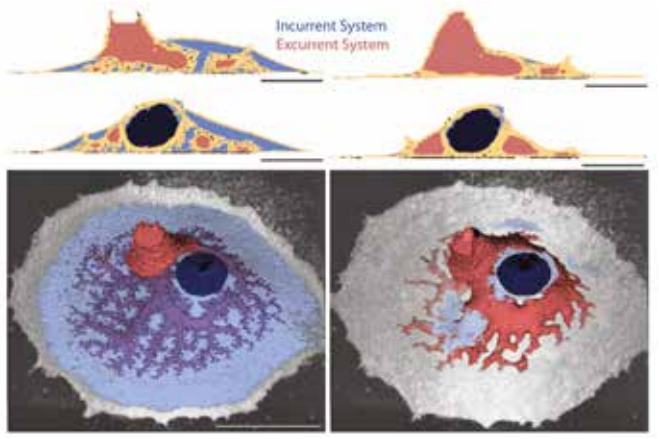

Figure 1:

Sponge deflation movements.

3D reconstruction of inflated and deflated states based on Optical Coherence Microscopy. 2D scale bars, 500 mm. 3D scale bars, 1,000 mm. Image by F. Ruperti

MorphologFinder: AI-driven functional annotation of proteins via structural similarity
The (missing) annotation of proteins in remote species is a key challenge for the cross-species comparison of single-cell transcriptomics datasets. We developed MorF: MorphologFinder, a pipeline for protein annotation using Alphafold-based structural prediction and similarity, exploiting the fact that protein structures can be more conserved than protein sequences. As proof of principle, we applied MorF to the proteome of *Spongilla lacustris* and annotated an additional 50% of the proteome beyond standard sequence-based methods.

MorphoFeatures: Characterizing cellular ultrastructure with AI
In collaboration with Anna Kreshuk's group at EMBL we have developed an unsupervised deep learning pipeline for the analysis of cellular shapes and ultrastructure in the SBEM volume. This yielded a limited set of morphological descriptors, so called MorphoFeatures - that agree with human perception of similarity and quantitatively outperform manually defined features (Figure 2). MorphoFeatures facilitate the detection of cell types by morphological means, via similarity-based clustering in the MorphoFeature vector space. Intriguingly, MorphoFeature clusters show high correlation with genetically defined cell type families, which is indicative of a close correspondence between cellular genotype and phenotype.

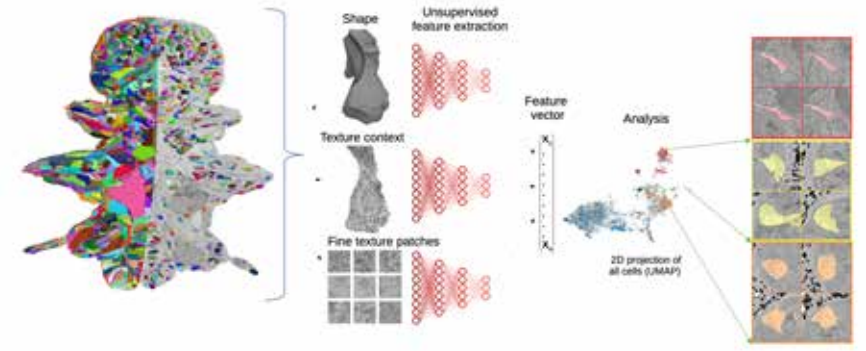

Figure 2:

The MorphoFeatures pipeline.

Deep learning of shape, texture context, and fine texture patches of segmented cells and nuclei yields MorphoFeatures that define one feature vector per cell. These are represented in a UMAP. Neighboring cells in the UMAP are morphologically similar. Scheme by V. Zinchenko.

The Platynereis genome.
Finally overcoming the vast polymorphism of the genome, our lab has now provided a chromosome-resolution genome assembly for *Platynereis dumerilii, P. massiliensis* and *P. megalops* via long-read sequencing, dense transcriptome sampling and chromosome conformation capture (Hi-C). We found > 50% repetitive content and modeled 29,000 protein-coding genes with intron sizes > 2Kb. We also compared the conservation of ancestral chromosomal linkage group across annelids and spiralians.

Building and comparing single-cell atlases
Major aim has been the generation of single-cell transcriptomics datasets for diverse animals, from sponges to sharks – central to our recently terminated ERC AdG "NeuralCellTypeEvo" and MC-ITN "EvoCELL". With this, we attempt to reconstruct the animal cell type evolutionary tree, and to locate the origin of neurons and other hallmarks of nervous system evolution.

For example, we have generated whole-body single-nuclei RNA-seq atlases of multiple developmental stages of *Platynereis dumerilii*; and for heads of adult animals (in collaboration with Stephan Schneider at Academia Sinica/Taiwan). Data from different time points were processed separately and then integrated to identify pseudo-precursors.

For the cross-species comparison of cell types we contributed to the development of SAMap, a method to map cell atlas manifolds across species in cooperation with the Bo Wang lab at Stanford. This method identifies homologous cell types with shared expression programs within and across phyla. Strikingly, SAMap finds many genes with more similar expression to their paralogs than their orthologs, suggesting paralog substitution. We are currently improving SAMap to enable larger-scale comparisons of cellular atlases.

Sampling Platynereis on the Traversing European Coastlines (TREC) expedition
Our laboratory leads a key project of EMBL's Trec expedition, which is to sample *Platynereis dumerilii* along the entire European coast. The European population of *P. dumerilii* shows a stunning richness in alleles, reflecting standing variation that allows for life under highly variable environmental conditions. In the past two years, we have sampled *Platynereis* immature adults at more than 20 sampling spots for genome sequencing and microbiome capture. We have also sampled sexually mature adult worms at 5 sites and obtained batches that we grew for 6 days under controlled conditions to then obtain single-cell transcriptomes and full-body volumes via X-ray microscopy. Preliminary comparisons of single cell atlases for Swedish and French *Platynereis* populations provided first insights into cell abundance changes for specific cell types and site-specific gene expression programs.

Future directions

Whole-body correlation of cellular gene expression and MorphoFeature profiles
The PlatyBrowser combines a cellular-resolution gene expression atlas with a whole-body EM volume, which can now be used for the mapping of the multimodal single-cell data. For example, we have started to map entire cellular transcriptomes to the atlas. This involves Hybridization Chain Reaction (HCR) for the most specific and consistently expressed gene per genetically-defined cell type and register the resulting confocal image to the atlas (Figure 3).

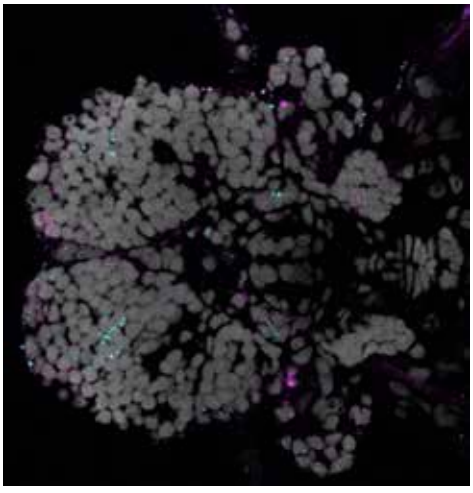

Figure 3:

Localizing cell type-specific expression by hybridization chain reaction (HCR)

Confocal images of 6dpf P. dumerilii larva stained with DAPI (depicted in gray); EMX2 (labeled with Alexa 546, depicted in cyan), Tll/nr2e1. Image by Gülce Serka and Luca Santangeli.

Ultimately, we plan to combine cellular-resolution transcriptomics with MorphoFeatures and thus enable the combined molecular and morphological characterization of differentiated cell types for an entire animal. This will be highly valuable for generating hypotheses about which sets of genes are responsible for implementing specific cellular morphologies and thus bring us closer than ever towards studying the genotype-to-phenotype transition at the cellular level.

To move from correlation to causation, we will knock-out cell type-specific transcription factors and/or effector genes and generate electron microscopy volumes for the relevant body parts. These will be analyzed manually, or systematically by applying the established set of MorphoFeatures to the newly generated volumes.

Establishing new anchor species for whole-body genotype-phenotype correlation.
We plan to extend the combined molecular-morphological approach to other, closely related or remote species, such as (1) the sister-species *Platynereis massiliensis;* (2) another nereidid annelid, *Hediste virens;* (3) a distant annelid such as *Capitella telata;* (4) the nemertean Lineus ruber, and (5) the chiton *Acanthochitona crinite*. For each of these species we will select early differentiation stages that we can obtain synchronously staged and in sufficient number, for EM fixation and for single-cell sequencing.

Hotspots of cellular variation in Platynereis dumerilii
Having collected invaluable datasets during the past two years on the TREC expedition, our efforts will turn towards in-depths analysis of these data. We have already and will continue to establish pipelines for barcoding as well as genome, single-nuclei transcriptomic, ATAC, and microbiome sequencing, combined with an analysis of morphological variation based on confocal imaging of antibody-labelled specimens, expansion microscopy, and/or medium-resolution, beamline-based X-ray microscopy. We will attempt to identify highly variable cell types with changes in abundance, in quantitative and qualitative gene expression, and most importantly, showing differential expression of different alleles in diverse populations along the coast. All of these analyses will require substantial method development and will jointly enable the identification of cellular hotspots of variation.

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Publication statistics
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Chewing and grinding

2.3 DR. JOSEPHINE BAGERITZ
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Fields of Interest
Muscle stem cell niche, cellular heterogeneity, Wnt signaling, single-cell transcriptomics, spatial transcriptomics

Summary and outlook
Our research deconstructs the dynamic crosstalk between muscle stem cells (MuSCs) and their niches during development to understand how stem cell number and cellular identity are built and regulated, and their impact on adult muscle biology, with the goal of promoting healthy aging. We use modern techniques such as AI-assisted microscopy image analysis, cell type-specific CRISPR gene editing, single-cell RNA sequencing, and spatial transcriptomics to dissect key principles and their underlying mechanisms at cellular resolution. We utilize the *Drosophila* larval muscle stem cell niche, composed of two MuSC populations: indirect flight muscle stem cells (IFM-MuSC) and direct flight muscle stem cells (DFM-MuSC), along with their progenitors and surrounding epithelial and tracheal cells. To better understand stem cell niche communication, we integrated single-cell transcriptome data and computed spatial expression maps of the *Drosophila* muscle stem cell niche. In line with spatially restricted Wnt ligand expression, Tcf downregulation experiments showed a reduction in IFM-MuSC number. To characterize spatial phenotypes, we developed an AI-based image analysis tool called Deep-MuSC Analyzer. This tool, applied to images of genetically perturbed muscle stem cell niches, suggests a stem cell population-specific interplay of signaling pathways. Moving forward, we plan to dissect the molecular mechanisms for specific MuSC regulation and their interplay with signaling pathways. Our initial findings suggest that downstream Wnt components play a critical role. This systematic analysis of Wnt components in the muscle stem cell niche will provide deeper insights into MuSC regulation during development and contribute to strategies for promoting healthy aging.

Research highlights since 2021
Over the past year, our team has made significant strides in understanding and advancing the field of single-cell transcriptomics and muscle stem cell (MuSC) biology. A notable achievement is our contribution to a book chapter titled “Analysis of Single-Cell Transcriptome Data in *Drosophila*,” authored by S. Yousefian, M.J. Musillo, and J. Bageritz. This work provides a detailed guideline for single-cell RNA sequencing data of *Drosophila*. In collaboration with colleagues from the COS Institute, we have co-authored two publications. The first, led by Nishimiya-Fujisawa et al., delves into the ancient split of germline and somatic stem cell lineages in Hydra. The second, with De Jaime-Soguero et al., explores developmental signals and their link to genome maintenance and cell fate. In both projects, we contributed our single-cell transcriptome expertise.

Currently, we are in the final stages of preparing two manuscripts for publication. The first manuscript examines the role of Wnt signaling in the muscle stem cell niche, utilizing our newly developed AI-based tool, called Deep-MuSC Analyzer (Figure 1). This tool has allowed us to dissect the intricate signaling pathways and their specific impact on the pool

size of MuSC populations. Unexpectedly, and in contrast to current beliefs, our data point to an integration of FGF and Wnt signaling to specifically regulate MuSC populations in spatially defined regions. Our data strongly support a model in which Wnt ligands from the niche seem to have no influence on MuSC pool size.

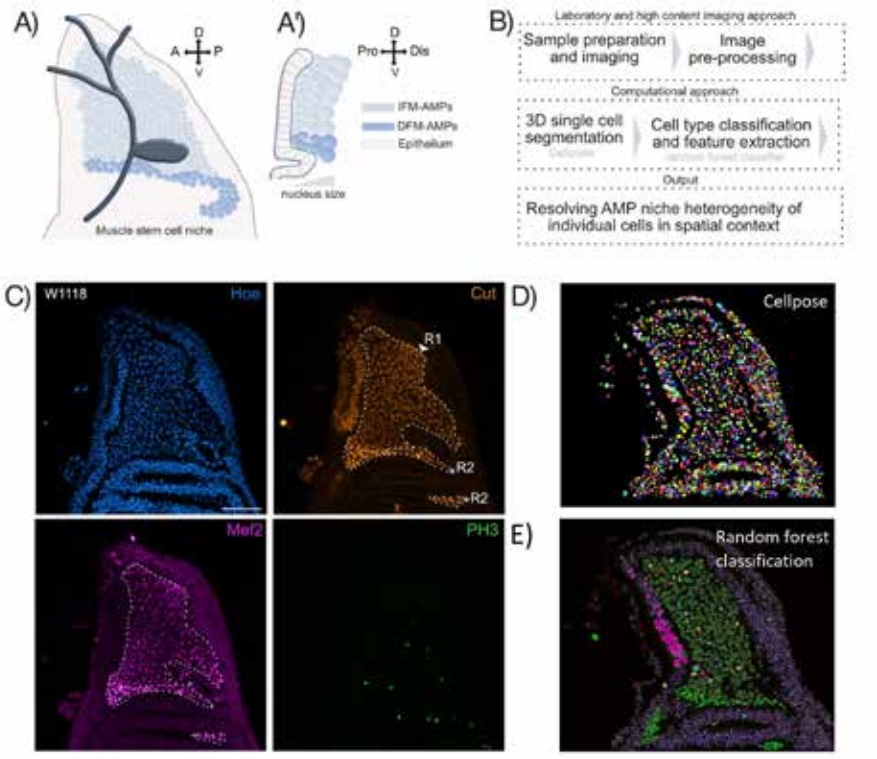

Figure 1:
Deep MuSC-Analyzer to study the developing muscle stem cell niche of *Drosophila*.

A) Scheme of the larval muscle stem cell niche of *Drosophila*. A') Orthogonal view. Indirect and direct flight muscle stem/progenitor cells (IFM and DFM-AMPs, respectively) are colored in light blue and ocean blue, respectively. The epithelial niche is shown in light grey, and the trachea is depicted in dark blue. B) Overview of the Deep-MuSC-Analyzer pipeline. C) Confocal images of the muscle stem cell niche stained with the respective markers and dyes for segmentation and cell type identification (Hoechst to label the nuclei, Cut to label IFM (Cut-low) and DFM (Cut-high), Mef2 as a universal mesodermal marker, and PH3 to identify mitotically active cells). D) 3D Cellpose segmentation result shown in one z-plane. E) Random forest classification identifies the different cell populations of the larval muscle stem cell niche of *Drosophila* (blue cells = epithelium, green = IFM-AMPs, dark green = DFM-AMPs, magenta = epithelial cut+ tissue).

In the second manuscript, we have integrated existing single-cell transcriptome data from two groups, providing a comprehensive resource for the scientific community. Our analysis highlights cell cycle state differences within the MuSC populations and has led to the identification of a previously uncharacterized MuSC/progenitor subpopulation, shedding light on the heterogeneity within the muscle stem cell niche.

Presenting our findings at international conferences received positive recognition, highlighting the impact of our research. Our findings pave the way for future functional studies to dissect the role of MuSC populations in adult muscle biology. To translate our findings to higher organisms, we have initiated a funded collaboration with experts in human and mouse muscle stem cell biology. Additionally, I was recently accepted into the +Program of the Health + Life Science Alliance Heidelberg Mannheim, which not only provides funding for our research but also supports the engineering aspect of our work. With the support of this program, the aim is to understand the basic principles of MuSC fusion and functional muscle generation, and with this gained knowledge, to synthesize artificial muscle stem cells. This not only advances our knowledge of muscle stem cells but also provides broad applications in muscle biology.

Future directions

Building on our foundational work, we will delve deeper into the impact of altered developmental pool sizes of muscle stem cells (MuSCs) on adult muscle function throughout the lifespan, including aging. Utilizing the genetic tools of *Drosophila* and our gained knowledge on spatial signaling activity in MuSC populations, we will modulate MuSC pool sizes to investigate how these changes influence muscle maintenance, regeneration, and overall function in adult organisms. By systematically varying specific

MuSC populations during development, we aim to elucidate the long-term consequences on muscle health and identify potential strategies for promoting healthy aging. In parallel, we will focus on the critical pupal stages of *Drosophila* development, where MuSCs either fuse to form functional muscle fibers or remain as satellite cells, which are essential for adult muscle regeneration. Our research will explore the cellular and molecular mechanisms that govern this decision-making process, emphasizing the roles of lipid composition of plasma membranes and cellular mRNA contents necessary for proper muscle fiber generation. This approach will not only enhance our understanding of MuSC regulation during development but also inform our ongoing efforts to engineer artificial muscle stem cells. By dissecting the intricate processes that underpin muscle stem cell fusion and satellite cell maintenance, we aim to develop innovative strategies for muscle repair and regeneration, potentially translating our findings to higher organisms and clinical applications. Through these studies, we aspire to contribute significantly to the fields of muscle biology and regenerative medicine, ultimately improving muscle health across the lifespan.

Selected publications since 2021

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Publication statistics

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2.4 PROF. DR. LÁZARO CENTANIN
CLONAL ANALYSIS OF POST-EMBRYONIC STEM CELLS

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Fields of Interest
Post-embryonic stem cells, post-embryonic organogenesis, hierarchical organisation of lineages, genetic chimeras, phenotypic variation within a genus, stem cell niche formation, coordination of growth within and among vertebrate organs

Summary and outlook
Fish, like most vertebrates, grow in size during their entire post-embryonic life (Figure 1A). Growth in fish is sustained by the activity of adult stem cells (aSCs), which generate new cells that are integrated into already functional organs. Adult stem cells in fish need to fulfil two different functions: a) the homeostatic replacement of lost cells, an activity that aSCs also carry-out in mammals, and b) the addition of new cells to increase organ size in a coordinated manner. Understanding the differences and similitudes between homeostatic and growth stem cells has been one of the main interests of my lab. We believe that growth stem cells constitute an ancient state of adults stem cells in general, and hypothesise the homeostatic stem cells like those found in mammals can revert to a growth state upon diverse stimuli.

We use *Oryzias latipes* (medaka) and other species within the same genus (Figure 1A, B) as model organisms. We focus on the neuromasts of the lateral line system and the gills are proxy to understand how cells, tissues and organs coordinate their growth to maintain organismal proportions and functionality in an animal that is constantly changing its size and therefore, its metabolic demands. Both neuromasts and gills expand by generating new functional units, and we have revealed that these are generated and maintained by stem cells of different lineages. We tackle the hierarchical organization of the different lineages by generating genetic clones, wild-type/mutant mosaics, and inter-species chimeras.

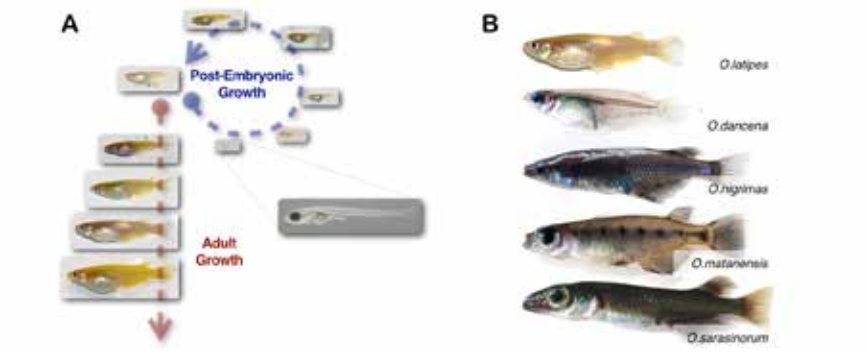

Figure 1:

Post-embryonic growth and phenotypic range among Oryzias species. A) Teleost fish as medaka grow during their entire post-embryonic life. Post-embryonic growth is fuelled by the activity of adult stem cells (Adapted from Centanin et al, 2011). B) Species in the Oryzias genus display a huge range of cellular, morphological and physiological features.

Research highlights since 2021

During the last years we have been reporting on inter-lineage relations during the establishment and expansion of stem-cell driven organogenesis. We explore how changes in one tissue affects the organisation of other neighbouring tissues and started gaining insights into the autonomous vs inter-dependent nature of lineages during organogenesis. We have revealed different cases depending on the system that was studied, which help us revealing a hierarchical organisation to ensure coordinated growth.

Phenotypic Diversity in the Genus Oryzias

The genus *Oryzias* encompasses more than 30 species distributed in three main subgroups (*latipes*, *javanicus*, *celebensis*), and cover ca. 18 million years of diversification. We have established colonies of different *Oryzias* species displaying morphological and physiological variation, and have a running collaboration with laboratories that host different *Oryzias* species. We started analysing the phenotypic range existing in the genus using the lateral line system and the gill as a model system and organ, respectively.

Lateral line: A remarkable diversity of lateral line patterns exists in adult teleost fishes, the basis of which is largely unknown. By analysing the lateral line patterns and organ numbers in 29 *Oryzias* species and strains we observed a rapid diversification of the lateral line system - i.e, number of neuromasts per cluster, number of parallel lateral lines (Figure 2). Curiously, the patterns did not match phylogenetic relationships but rather correlate with the species body size. Analysing pattern in hybrids, this suggested a polygenic control over neuromast numbers and positioning. The diversity in lateral line patterns most likely results from differences in embryonic development and post-embryonically, where simpler embryonic patterns generate less complex adult patterns and organ numbers, arguing for a linkage between the two processes.

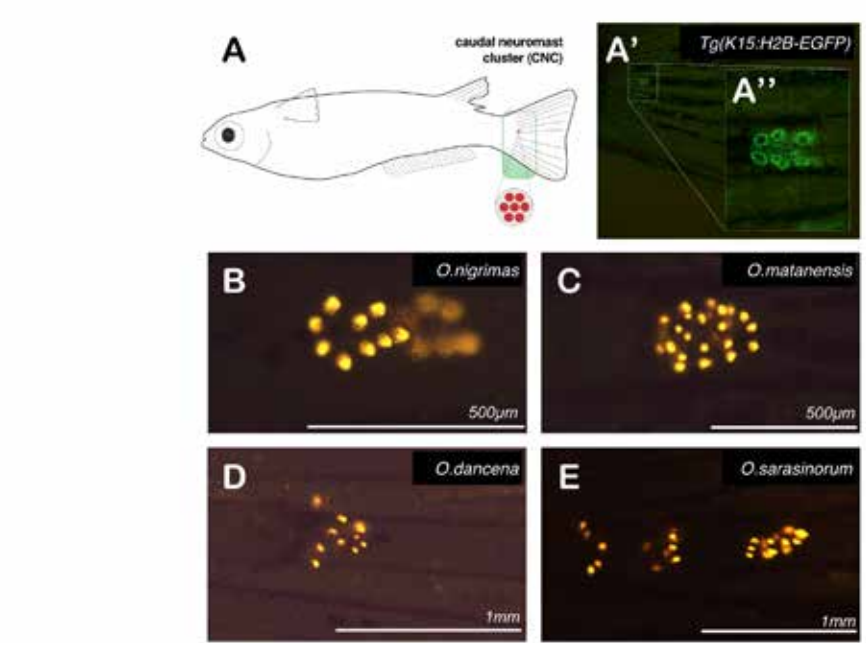

Figure 2:
The lateral line is quantitative read-out for species-specific traits. A) Neuromasts are the organs of the lateral line system, and are arranged in clusters along the surface of the fish. The caudal neuromast cluster (CNC) has a stereotypic position at the most anterior region of the caudal fin. B-E) Different species among Oryzias display a species-specific number, size and organisation of neuromasts in the CNC. (Adapted from Seleit et al, 2021)

Gills: we started a detailed analysis on gill filaments from different *Oryzias* species, considering parameters as maximal filament length, constant filament width & circumference, differences in cell type composition, and proportion of growth domains vs differentiated domains. Contrary to the case of the lateral line, we observed that these parameters do not correlate linearly with body size, but rather different species use diverse strategies to expand a respiratory epithelium that sustains their metabolic demands. For instance, some larger species display a higher number of thinner gill filaments, while other large species display a lower number of wider filaments. Using hybrids, we are starting to learn about

the intrinsic logic of the system and how these different parameters are inter-dependent or independent from each other. We have also established sc-RNA seq for the *O.latipes* and *O.dancena* gills, and we are currently focusing on differential genes as candidates to modulate the ratios of differentiated cells that characterise each species.

Chimeras & Inter-Lineage Coordination
We have been addressing the inter-lineage relation, and trying to tackle lineage hierarchies, using organs and systems that rely on multiples lineages for their morphogenesis, anatomical configuration and/or function. We used our two model organs, the neuromasts of the lateral line and the gills to explore the inter-dependance of their constituent lineages. Lateral line: within teleosts, there is substantial morphological variation among related species, and it is still unclear how tissues, organs and systems accommodate such diverse scaffolds. Using tissue-specific transgenic lines, laser ablation experiments and WT/mutant chimeras, we showed that the patterning of pLL neuromasts results from sequential organ-autonomous programs and non-autonomous instructions from adjacent tissues. As an example of the latter, we determined that the aberrant pLL pattern observed in keratin mutants depends on a mutant epithelium, since a wild type epithelium in keratin mutant fish rescues the aberrant phenotype. Our results disentangle intrinsic from extrinsic properties of a sensory system. We speculate that intrinsic programs guarantee proper organ morphogenesis, while instructive interactions from surrounding tissues facilitate the accommodation of sensory organs to the diverse teleost body plans.

Gills: our previous work has revealed the existence of up to 4 different lineages that build up each filament in a medaka gill. Since a *Oryzias* gill is composed of ca. 1000 filaments — and bigger fish can largely exceed that number — these four lineages must be coordinated in order to iteratively establish each functional unit. We have been generating chimeras following two different rationales: a) mutant / WT chimeras within the *latipes* species, and b) inter-species chimeras within the *Oryzias* genus. Both approaches have revealed an organiser role for one the lineages, while the other seems to adapt to the growth pace that the organiser sets up. We are expanding our analysis on chimeras to characterise how a single lineage from another species can phenocopy an entire functional unit in a foreign physiological environment.

Future directions
Niche Induction Dynamics: Previous work from our lab showed that neuromasts stem cells recruit their own niche from neighboring epithelial cells. We have also reported that niche induction in medaka is a polyclonal process, which implies different cells undergoing the same molecular and morphological transformation. Niche recruitment is restricted to early organogenesis, and followed by an active mitotic activity that expands the niche compartment both in medaka and zebrafish. When are particularly intrigued about the processes that put an end to the induction-competence in this and many other inducting paradigms. Putting a stop to the cell-type induction has the advantage of consolidating cell fates, and we will explore its molecular determinants using both the neuromas system as well as lens recruitment in medaka/zebrafish chimeras.

Species diversity and the identification of lineage hierarchies: A plethora of body plans exists in teleost fish, which raises immediate problems for the set-up of most tissues, organs and systems. On the one hand they must be tunable and easily adjustable to fit the diverse body plans among the clade. On the other hand, they must be reproducible within and between members of the same species. Our aim is to identify the strategies that guarantee balancing the strict internal properties of the system (reproducibility) with a plastic response to the immediate microenvironment and the general environment (adaptability). We will tackle this by the generation of inter-species chimeras and the identification of dominant lineages that can impose an alien morphological trait in the host species (Figure 3).

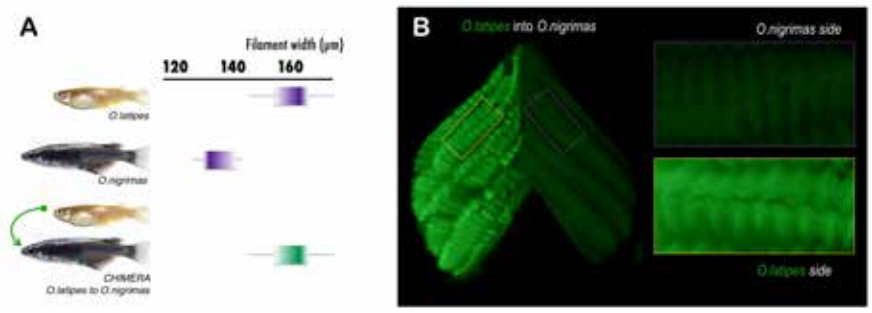

Figure 3:

Inter-species chimeras allow the identification of lineage hierarchies. A) Oryzias species cover a wide morphological space, which can be depicted in different organs. In the gills, the filaments display a species-specific width. B) The transplantation of a single lineage from *O.latipes* can instruct host lineages from *O.nigrimas* to execute an *O.latipes* morphological program.

Adaptation to different salinities: fish have ionocytes, which are specialised cells participating in osmotic regulation among other functions. Many species within the *Oryzias* group tolerate a broad range of salinities, and this adaptation includes major adjustments in the size and number of monocytes as well as expression levels of cell-type specific ion-pumps. We have already identified a number of species-specific responses to salinity that will allow us better understand the overlap between genetic programs and environmental adaptation. We are performing sc-RNAseq in different species which were grown at different conditions to characterise the molecular changes occurring in each cell type, and also implementing inter-species transplantation to disentangle the lineages involved in sensing the salinity changes and executing the osmotic response.

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Publication statistics

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A B C D E F G H

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

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Fields of Interest
We study the function, regulation and evolution of the circadian clock. specifically we focus on mechanisms of photoreception, gene expression control as well as DNA repair. We use various models including fish as well as corals in a comparative approach.

Summary and outlook
During the past 3 years, we have invested in the study of clocks and clock function in various model species. We have revealed a differential influence of day length on learning in male and female medaka and plan to use the genetic tools available for this species to explore the molecular mechanisms underlying this effect of photoperiod. Our continuing work with the blind cavefish, *Phreatichthys andruzzii* has demonstrated the presence of a blind, but strongly food-regulated clock in this species which allows us to study the mechanisms which distinguish light- from food-entrainable clocks. In addition, we have studied the differential conservation of photolyase genes in this cavefish species together with loss of photolyase function medaka lines. Our combined results have revealed overlap between DNA repair and circadian clock function for 6-4 photolyase. Furthermore, we have shown that CPD photolyase mediates light-independent repair of oxidatively damaged DNA in addition to its catalysis of the photoreactivation of UV-induced DNA damage. A systematic characterisation of a multitude of photoreceptors in *anthozoa* and *medusozoa* has set the stage for us to explore the evolution of non-visual opsins, cryptochromes and photolyases and in particular the selective pressures that have shaped photoreceptor diversity. In collaboration with the Guse group (LMU) we will use various genetic tools in the Anthozoan model, *Aiptasia*, to assign functionality to individual photoreceptors in relation to circadian as well as lunar rhythmicity.

Research highlights since 2021
Many lines of our research over the past 3 years have stemmed from our focus on particular model species that provide unique insight into particular questions.
Day length is a key environmental signal that regulates many physiological systems according to seasonal changes in the environment. However, the mechanisms whereby animals detect and respond to changes in photoperiod remain poorly understood. One of the most attractive features of the fish model, *medaka* is that it is a photoperiodic species. Thus, day length is a major determinant of its reproductive function, with the fish only breeding during long day conditions in the summer. We have developed a learning paradigm where the fish have to activate an optical sensor in order to obtain a food reward. Under long day conditions, male fish show a strong learning deficit while females perform efficiently. As a mixed sex group, females drive group learning while males prioritise mating over feeding behaviour. Instead, under short photoperiod, where mating does not take place, the males performance improves to a level similar to the females. Therefore, we have revealed that photoperiod has sex-specific effects on the learning performance of this seasonal vertebrate.

How do environmental conditions shape the evolution of light-dependent functions such as DNA repair and the circadian clock? Blind *cavefish* represent powerful models to address this question due to their evolution under perpetual darkness in some cases over the course of millions of years. Our previous work with the Somalian blind *cavefish*, *Phreatichthys andruzzii* has revealed that under artificial light dark cycle conditions, these fish show arrhythmic patterns of clock gene expression and locomotor activity and that this results from loss of function mutations in the circadian clock light input pathway. We have now demonstrated that under constant darkness, feeding these animals at the same time of day, each day is sufficient to entrain robust circadian rhythms of locomotor activity. Furthermore, delivery of food with a much lower frequency, down to one meal every 4 days, is also sufficient to entrain robust circadian rhythms. We have made similar observations with the other well studied *cavefish Astyanax mexicanus,* while lower feeding frequencies fail to entrain rhythmicity in surface-dwelling species such as *zebrafish*. These results imply that for blind *cavefish* species, food represents an extremely strong synchroniser for circadian activity. A comparison of the circadian clock mechanism in blind *cavefish* with species such as the *zebrafish* should provide important new insight into the differences between light and food entrainable clocks at the molecular level.

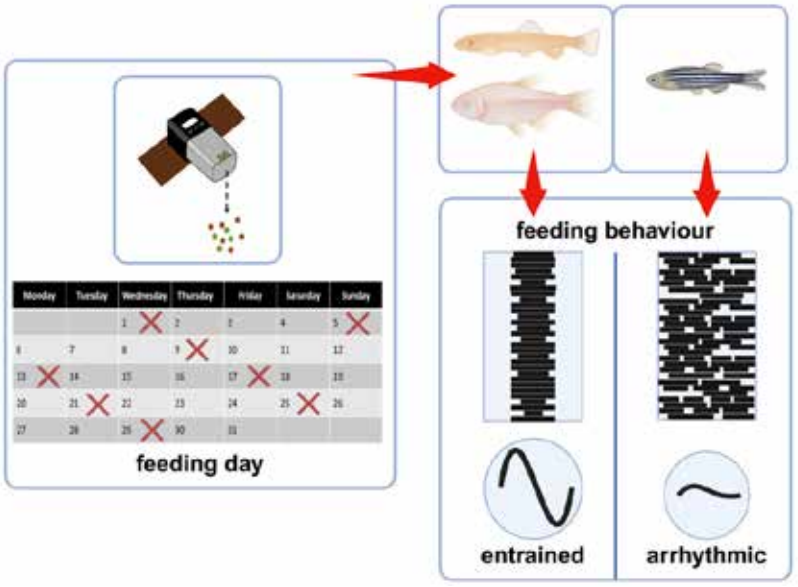

Figure 1:

Experimental proceedure used to test the effect of feeding frequency on circadian locomotor activity in zebrafish and blind cavefish. Automatic feeders were used to deliver food to the fish at the same time of day but with different frequencies, ranging from once per day to once each 4 days. Locomotor activity was then automatically measured using infrared photocells connected to a computer in order to plot actograms. One meal consumed each 4 days was sufficient to entrain robust circadian rhythms of activity in the blind cavefish but led to arhythmicity in zebrafish.

Our cavefish model has also provided insight into the evolution of photolyases, the flavoproteins which catalyse the light-dependent repair of UV damaged DNA, so-called photoreactivation. Several loss-of-function alleles have been encountered for the 6-4 and DASH photolyase genes in *P. andruzzii,* a finding which is consistent with the absence of UV radiation to induce DNA damage and the lack of visible light to drive the repair reaction. In contrast, a fully functional CPD photolyase gene has been conserved in this blind *cavefish*. Using knockout lines for each of the photolyases in medaka as well as gain of function cell lines where the photolyases have been ectopically expressed in mammalian cells, we have discovered a light-independent function for CPD photolyase in the repair of oxidatively damaged DNA. These results are consistent with the hypoxic environments of many subterranean cave systems which can lead to conditions of elevated oxidative stress. Using the *medaka* lines, we have also demonstrated that as well as serving as a DNA repair factor, 6-4 photolyase can physically interact with and modulate various clock transcription factors such as TEF, Clock and Bmal. Thus 6-4 photolyase exhibits dual functionality in the circadian clock and DNA repair.

Our study of photoreceptor genes in *Cnidaria* has revealed a more diverse photoreceptor repertoire in Anthozoa than in Medusozoa. Anthozoa retained all three opsin subclasses, which diversified into at least six subtypes. In contrast, in Medusozoa, only one class with a single subtype persists. Similarly, in Anthozoa, we documented three photolyase classes and two cryptochrome (CRY) classes, whereas CRYs are entirely absent in Medusozoa. Interestingly, we also identified one Anthozoan CRY class, which exhibited unique tandem duplications of the core functional domains. We explored the functionality of Anthozoan photoreceptors in the model species *Aiptasia*, which recapitulates key photobehaviors of corals. We show that the diverse opsin genes are differentially expressed in important life stages common to reef-building corals and *Aiptasia* and that CRY expression is light regulated. We thereby provide important clues linking coral evolution with photoreceptor diversification.

Future directions

The future direction of our work centres on the investments we have already made in developing various model systems.

We plan to explore the sex-specific effects of photoperiod on learning in medaka in more detail by initially searching for sex-specific and photoperiod dependent changes in the brain transcriptome. Our aim with this analysis will be to identify transcriptomic signatures which are sex- and photoperiod-specific and which we can then use to study the underlying molecular and neuronal mechanisms which respond to day length changes. The amenability of *medaka* to genetic analysis such as CRISPRCas9-mediated genome engineering as well as the panel of inbred lines provide unique tools to study not only photoperiod dependent functions but also how these mechanisms, and thereby learning may differ in the individuals of a wild population.

The *cavefish* represents a vital model with which we can explore a number of key issues. While the entrainment of the circadian clock by light has been extensively studied in many models, we still know comparatively little about how feeding time synchronises the clock. Comparing clock regulation by feeding time in *cavefish* and *zebrafish* should provide us with unique new insight into the fundamental differences between feeding and light-entrained clocks. The conservation of CPD photolyase function in blind *cavefish* implies that this photolyase performs an important light - independent function. One central goal will be to explore in more mechanistic detail, how CPD photolyase interacts with and thereby mediates the repair of oxidatively damaged DNA. Preliminary data also suggests links between CPD photolyase function and aging since loss of CPD photolyase function in medaka lines leads to a reduction of longevity. Therefore we will systematically examine whether loss of CPD photolyase function medaka show premature onset of senescence marker expression in cells.

In collaboration with the Guse group, now based at LMU, Munich, an important goal will be to assign functions to the multiple opsin photoreceptors. By using the *Aiptasia* model to generate loss of function opsin mutations, we will explore the contribution of individual photoreceptors to circadian clock regulation, phototactic behaviour as well as lunar-regulated reproduction. Furthermore we wish to understand the functional significance of the Anthozoan cryptochromes which carry tandem duplications of central core domains.

Selected publications since 2021

Di Rosa, V., Frigato, E., Negrini, P., Cristiano, W., López-Olmeda, JF., Rétaux. S., Sánchez-Vázquez, FJ., **Foulkes, NS.**, Bertolucci, C. (2024) Sporadic feeding regulates robust food entrainable circadian clocks in blind cavefish. iScience. 27, 110171.

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Publication statistics
https://scholar.google.de/citations?hl=en&user=XZZ7KeMAAAAJ

2.6 N. N.
ANIMAL MOLECULAR PHYSIOLOGY PROJECT LEADER DR. FRANK MÖHRLEN

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

Summary and outlook
We work on molecular aspects of sensory physiology with a focus on olfaction, nociception and motor control. We examine the function of ion channels in sensory neurons, and we try to understand how these channels contribute to transduction, sensitivity and sensory performance. Our main interest are calcium-gated chloride channels that appear to mediate neuromodulatory functions in various neurons involved in sensory information processing. We have investigated their role in olfactory transduction and in motor learning. In the field of nociception, we are interested in the interactions between the olfactory system and the trigeminal nociceptive system. Nociceptive chemical stimuli change odour perception, and olfactory co-stimulation in turn alters trigeminal nociception and e.g. the perception of head ache. We are looking for points of cross-talk between the two systems in nose and brain to understand this cross-modal signal processing

Research highlights since 2021
We identified the subcellular localization of the anoctamin channel proteins in epithelia in the nasal cavity, as well as in Purkinje neurons in the cerebellar cortex. 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. ANO2 channel expressed

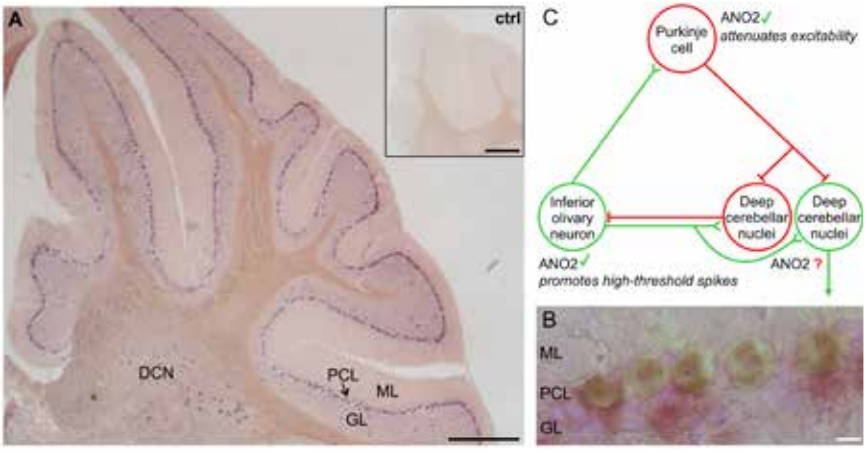

Figure 1:
ANO2 in the olivo-cerebellar circuit. (C) Schematic illustration of a cerebellar module summarizing the recent findings about the role of ANO2 in the olivocerebellar cortex. Glutamatergic neurons are shown in green and GABAergic neurons are shown in red. ANO2 has been shown to be expressed in cerebellar Purkinje cells (A, B), as well as in inferior olivary neurons where it accelerates repolarization after high-threshold calcium spikes and, thus, promotes the generation of these calcium spikes. In Purkinje cells, ANO2 attenuates excitability upon strong activation.

in Purkinje neurons modulate the inhibitory input to cerebellar Purkinje cells and limit their excitability. Using electrophysiological recordings on Purkinje cells we identified an attenuating effect of ANO2-mediated chloride currents on the instantaneous simple-spike activity during strong current injections (Figure 1). Moreover, we report a reduction of inhibitory currents from molecular-layer interneurons, lasting for several seconds. 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.

Our previous studies in the field of nociception have shown that within the nasal epithelia, the excitability of trigeminal fibers was found to be unaffected by activity in the olfactory system or ANO2 directly. 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. Using the expression of c-Fos in mouse SpVc neurons, we quantified network activity upon exposure to the noxious stimulus mustard oil. We found that co-stimulation with rose or lavender odor profoundly reduced induced network activity (Figure 2). Behavioral experiments revealed a reduced level of nocifensive behavior and a reduced facial expression of perceived pain intensity in the presence of odorants. These results demonstrate the analgesic potential of odor stimulation for the trigeminal system and provide an explanation for the palliative effect of odors e.g. in the treatment of headache.

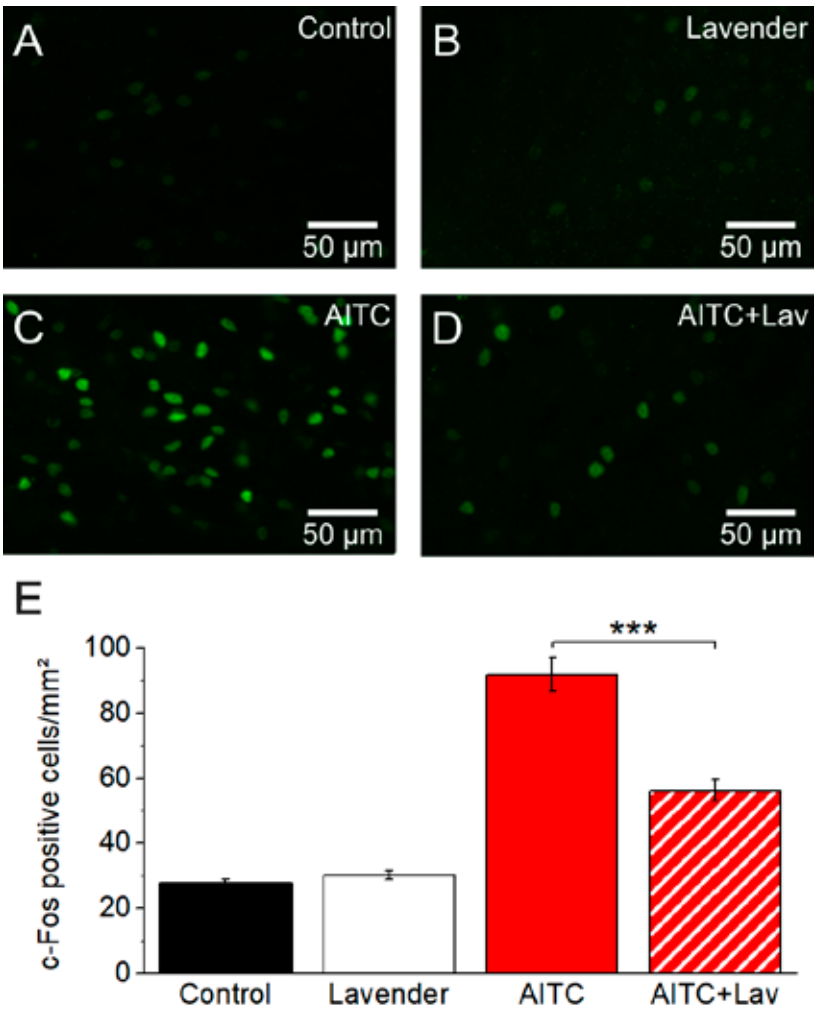

Figure 2:

Lavender oil inhibits nociceptive processing in the brainstem SpVc. (A-D) c-Fos immunosignals, a marker for neural activity, obtained from SpVc without (control) and with the indicated stimuli. (E) The diluted lavender oil did not raise c-Fos density significantly *(white bar)*, indicating its lack of trigeminal potency. Exposure to AITC, however, induced intense c-Fos expression *(red bar)*, which was reduced to ~55% c-Fos positive cells, indicating a strongly reduced lavender odor inhibited AITC-induced neural activity.

Future Directions

Our future interest lies in the role that ion channels play in the processing of olfactory and nociceptive signals. 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. In a study in humans, further pathophysiological aspects of this interaction will also be investigated. Nasal obstruction plays a central role in pathophysiological disorders of olfactory and trigeminal perception and remains to be among the most frequent nasal complaints in Otorhinolaryngology. Chronic rhinosinusitis, a nasal inflammatory disease, is often associated with nasal obstruction and other associated sinonasal symptoms such as olfactory loss, trigeminal pain, or mucosal swelling. The aim of this study is to investigate the various factors that determine the diagnosis of nasal obstruction, to further investigate the predictors for post-operative improvement in nasal obstruction and to determine the underlying physiological processes. In a multi-center partnership (Dresden, Basel, Heidelberg), extensive assessment of nasal obstruction, trigeminal function, olfaction, and psychologic condition and quality of life will be performed.

Publication statistics

https://scholar.google.de/citations?user=1ev-4agAAAAJ&hl=de


80
60

2.7 DR. KASPER VAN GELDEREN
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Fields of Interest
Plant cell biology, light and temperature perception, phytochrome B photobodies, light signalling, nuclear bodies, structural biology, developmental plasticity.

Summary and outlook
Plants display both amazing phenotypic robustness and phenotypic plasticity. This enables them to adapt and survive in stressful environments where light quality or temperature constantly changes. Phytochrome B (phyB) is a plant photoreceptor that detects red (670 nm) and far-red (730 nm) light and also acts as a temperature sensor. Plants detect far-red light to perceive their neighbors and compete effectively for available space and light by eliciting shade avoidance responses. High temperature triggers a similar response in order to compensate for a reduced carbon fixation. The complexity of these responses is regulated in phyB photobodies, which are subnuclear (300 nm) liquid-liquid phase separated (LLPS) bodies that form in red or white light, and disappear in darkness or in high far-red light conditions (Figure 1), which is accelerated in high ambient temperatures. PhyB undergoes phase separation from the nucleoplasm and assembles nuclear photobodies, a process promoted by multivalent protein-protein interactions. Photobodies are hypothesized to be sites of signal transduction, light-regulated protein degradation and/or transcription. The light signaling and cell biology lab investigates how phyB photobodies form and contribute to light signalling, by a combination of live and high-resolution imaging, biochemistry and development. The lab is funded by a DFG Emmy Noether grant on this topic. We are collaborating with groups on cryo-ET and *in silico* structural studies to get a complete overview of the macro and ultrastructure of the phyB photobody. Additionally, the lab investigates how far-red light leads to differences in root development via shoot-to-root transport of transcription factors.

Research highlights since 2021
The Light Signaling and Cell Biology lab.
phyB exists in two states, the active far-red absorbing form, and the inactive, red light absorbing form. Prolonged exposure to darkness, increased temperature, or irradiation with far-red (FR) light causes phyB inactivation and changes the size and localization of photobodies (Figure 1). phyB photobodies form through specific and multivalent protein-protein interactions, which aid the separation of a phase within the nucleus. It has been recognized that phyB photobodies do not form at random in the nucleus, but have some form of nucleation site. How phyB photobodies form and where they nucleate is unclear though. Dr. Kasper van Gelderen is funded by the Emmy Noether program to investigate this question.

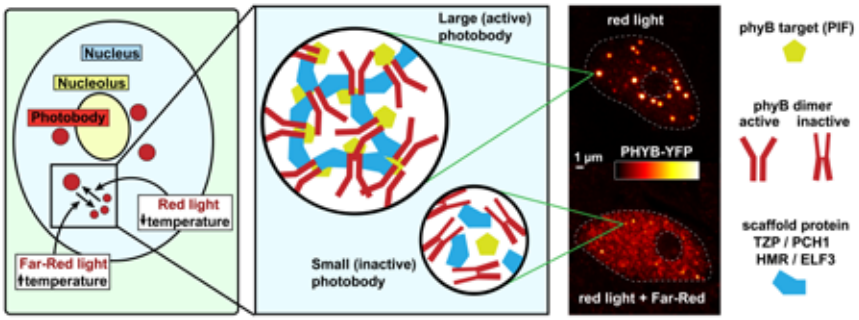

Figure 1:

phyB photobody dynamics likely depend on multivalent protein-protein interactions. Red light can induce formation of phyB photobodies, while far-red light and higher temperature can induce the loss of photobodies or the formation of smaller photobodies. Photobodies are composed of Phytochrome dimers, either active or inactive. Cofactors of the phyB photobody such as PCH1, play an important role in photobody formation, which is not fully understood. Targets of Phytochromes (e.g. PIFs) also localize to photobodies and their degradation is regulated there.

Live imaging of phyB photobodies

The van Gelderen lab wants to gain a deep understanding of the dynamic and compositional changes of phyB photobodies in a spatial manner. Therefore, we are performing live imaging of phyB photobodies using light stimuli (flexible LEDs) at the confocal microscope. In these experiments we have discovered that phyB photobodies disappear within 10 minutes of far-red (FR) light treatment (Figure 2), while the constitutively active variant of phyB, YHB, does not. Interestingly, the N-terminal domain knockout phyB-YFP^{dN89} does not make photobodies in red (R) light, but rather in FR light, while the N-terminal phosphorylation mutant Y104E has a similar behaviour. This result is extremely interesting, since it has been shown that the phyB-YFP^{dN89} mutant has a very low amount of active phyB, questioning the logic that phyB photobodies always contain active phyB. We are extending the live imaging method into a long-term switching experiment (Figure 2, lower panel), where we can follow the behaviour of photobodies over 1.5 hours, which enables us to test the limits of this system. This also shows us that N-terminal mutant phyB photobodies are less stable than wild type phyB-YFP photobodies.
We have developed the competency to do live-imaging with a precise control of temperature using the VAHEAT device (Interherence gmbh). Using this method, we have found that there is a very fast temperature response of phyB photobodies, where in one example, there is a quick disappearance of photobodies followed by a reappearance. This could represent a temporary falling apart of the phyB photobody due to the detrimental effects of higher temperature on phase separation.

Composition of phyB photobodies

To deepen our understanding of the changing protein composition of phyB photobodies, we are using proximity labelling to map (changes in) the phyB photobody interactome. Proximity labelling is a powerful protein-protein interaction technique that allows the discovery of not only stable, but also transient interactions. We have constructed a ***phyB-9 pphybB:phyB-mCitrine-turboID*** line that displays normal photobody behavior for this experiment. To test the candidates from proximity labelling, we have set up a mesophyll protoplast system to perform multicolour colocalization studies and visualize the interactions using FRET-FLIM. This will show us how the composition of the photobody changes dynamically, and also will enable us to see the interactions in the phyB photobody ***in vivo***.

Structure of phyB photobodies

We are exploring the ultrastructure of phyB photobodies nuclear bodies using electron microscopy on protoplast samples prepared by high pressure freezing and Tokuyasu fixation with subsequent immunogold labelling (Electron Microscopy Core Facility, Heidelberg). This has shown us that phyB photobodies are roughly 300 nm in size and have a shell/core structure. PhyB labels on the outside of the phyB photobody, indicating that there is a core of protein which is not phyB and that could play important structural or regulatory roles. To take this further, we are working together with the group of Dr. Stefan Pfeffer (ZMBH Heidelberg) on Cryo-Electron Tomography. Cryo-ET is a technique which allows the imaging of nano-scale biological structures in an unprecedented 3D manner, which can reveal how individual protein complexes, such as the large (10 nm) phyB dimer, are grouped and ordered in the phyB photobody.

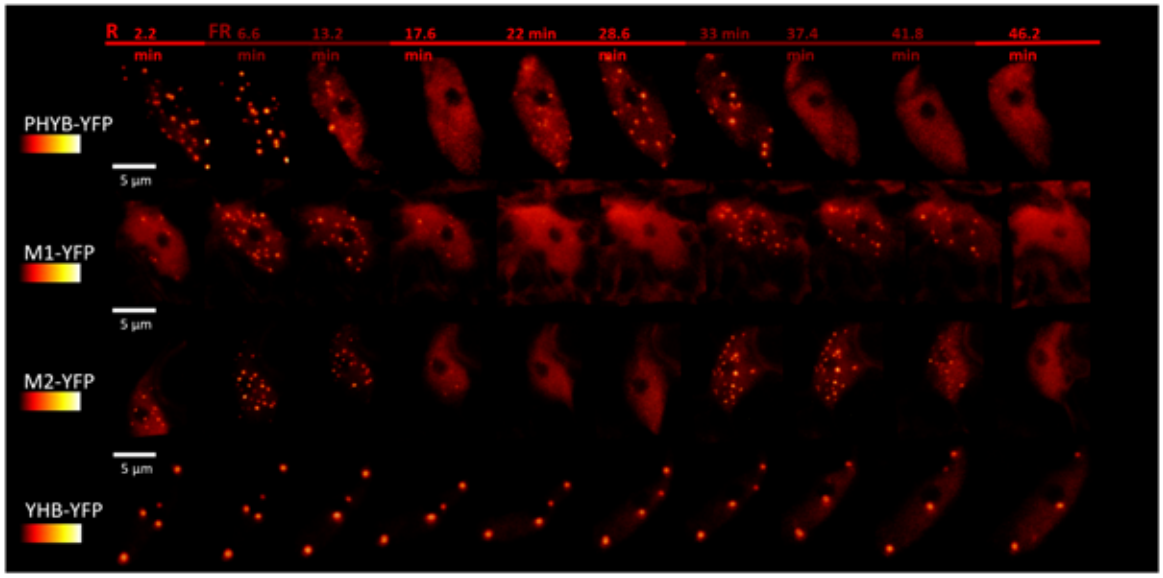

Figure 2:

phyB photobody live imaging shows a reversal in photobody formation in phyB mutant lines. Timelapse confocal images during a long R/FR switching experiment of cotyledon epidermis cell nuclei of wild type phyB-YFP, two phyB mutants (M1 and M2), and constitutively active phyB -YFP (YHB).

Disrupting phyB photobodies

We are developing new molecular tools to disrupt the phyB photobodies with a high-throughput chemical screen directed against nuclear body formation. The aim of this chemical screen is to discover compounds that can specifically target phyB photobodies using the *phyB-9 pphyB:phyB-YFP* line. These compounds can then be used to manipulate photobodies specifically, without changing environmental conditions, and serve a function as cell-biological tools for live imaging, or to perturb signalling and development locally. For this chemical screen we are working with the Chemical Biology Core Facility of the EMBL in Heidelberg and are screening 96-well plates with a specialized fluorescence microscope (ACQUIFER).

Future directions

In vitro nuclear extract droplets in oil.

phyB photobodies are formed by some manner of phase separation. To properly study this process the van Gelderen and Ruggieri (Heidelberg CIID) laboratories have been funded by the 'Health and life science alliance Heidelberg Mannheim' to develop a nuclear extract in oil *in vitro* droplet system. We aim to develop this *in vitro* cell free system of nuclear Arabidopsis extracts to study the behaviour and biophysics of phyB photobodies. This method will allow us to add precise amounts of protein, remove or add RNA and DNA and allow the use of molecular crowders, solvents, or salt gradients to study phase separation and the biophysical properties of the phyB photobody.

COP1 photobodies and phyB.

COP1 is an E3 ubiquitin ligase and a central regulator of light signaling that also forms photobodies. COP1 is known to degrade transcription factors that promote light-induced growth with notable examples being HY5 and PIF3, but it also promotes the turnover of phyB. COP1 is one of the earliest proteins in plants to be identified in nuclear bodies or aggregates, and COP1 can be present in phyB photobodies, but not in every condition. Despite the extensive studies done to decipher the functional roles of COP1 and phyB, there is very little knowledge on the aspect of COP1 photobody formation. Therefore, a DAAD funded PhD student is investigating what the overlap and mutual dependence of COP1 and phyB photobodies are, and particularly how COP1 photobodies form. To this end she is looking at COP1 photobody formation and composition in R and FR light and low and high ambient temperature (Figure 3).

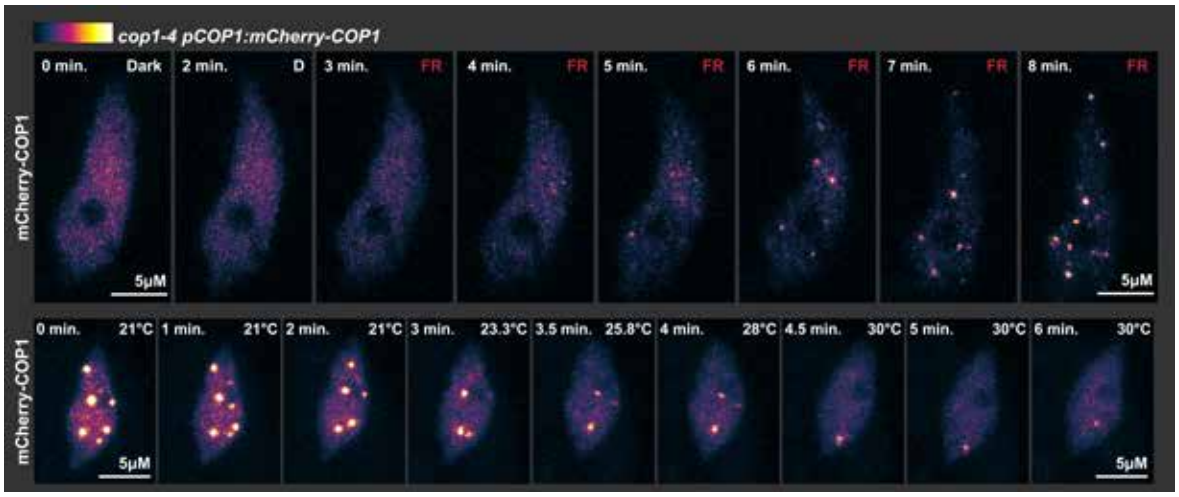

Figure 3:
Live imaging of COP1 nuclear bodies demonstrates fast light and temperature-induced dynamics. Leaf epidermis cells of native promoter, complementing, mCherry-COP1 imaged by confocal microscopy with far-red light treatment (upper panels), and temperature treatment (lower panels).

Light-mediated root development and HY5.
When plants perceive FR light they elongate their shoot organs to enhance light capture, however they also reduce their root growth, potentially optimizing resource utilization. This reduced root growth is achieved by the downregulation of lateral root emergence via the transcription factor HY5. In FR, HY5 is stabilized by phyA and phyB mediated inhibition of COP1 and HY5 is then transported from shoot to root to downregulate lateral root growth locally. However, the mechanism of HY5 shoot-root transport is not clear and a DAAD-funded PhD student in our lab is investigating the mechanism of HY5 shoot to root transport by following shoot-induced HY5 in its journey to the root and investigating a splice variant of HY5 with potentially increased mobility.

Selected publications since 2021

van Geldern, K., van der Velde, K., Kang, C.-K., Hollander, J., Petropoulos, O., Akyuz, T., Pierik, R. (2023). Gibberellin transport affects (lateral) root growth through HY5 during Far-Red light enrichment. BioRxiv doi:10.1101/2023.04.21.537844 (OA)

Garcia-González, J., and **van Geldern, K.** (2021) Bundling up the Role of the Actin Cytoskeleton in Primary Root Growth. Front. Plant Sci. (2021) doi: 10.3389/fpls.2021.777119 (OA)

Glanc, M., & **van Geldern, K.***, Hörmayer, L., Tan, S., Naramoto, S., Zhang, X., Domjan, D., Vcelarová, L., Hauschild, R., Johnson, A., de Koning, E., van Dop, M., Rademacher, E., Janson, S, Wei, X., Molnar, G., Fendrych, M., De Rybel, B., Offringa, R., and Friml, J. (2021) AGC kinases and MAB4/MEL proteins maintain PIN polarity by limiting lateral diffusion in plant cells. Current Biology 31: 449-451. doi: 10.1016/j.cub.2021.02.028 (OA)
*Authors contributed equally

van Geldern, K., Kang, C., Paalman, R., Keuskamp, D., Hayes, S., and Pierik, R. (2018) Far-Red Light Detection in the Shoot Regulates Lateral Root Development through the HY5 Transcription Factor. The Plant Cell 30: 101–116. doi: 10.1105/tpc.17.00771 (OA)

van Geldern, K., Kang, C., and Pierik, R. (2018) Light Signaling, Root Development, and Plasticity. Plant Physiology 176: 1049–1060. doi: 10.1104/pp.17.01079 (OA)

Publication statistics
https://scholar.google.com/citations?user=Wm1USqMAAAAJ&hl=en

2.8 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
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 neighbouring cells and display a high degree of developmental plasticity. Our group uses cambium-driven radial plant growth and the related formation of vascular tissues as a paradigm for revealing fundamental concepts of multicellularity and organismal growth (Figure 1). The last years were highly productive in this regard and resulted in major contributions to the field: We revealed molecular signatures of cambium-related cells with single cell resolution, revealed mechanisms by which fluctuating water availability influences xylem formation, and identified and characterized novel cell fate regulators with regard to their effect on chromatin structure. In addition, computational modelling of cellular dynamics targeting regulatory networks and mechanical aspects of radial plant growth developed into a central part of our work. Through these achievements, we continued to follow our mission of establishing a comprehensive view on a growth process with a central role in shaping terrestrial ecosystems and the global carbon cycle.

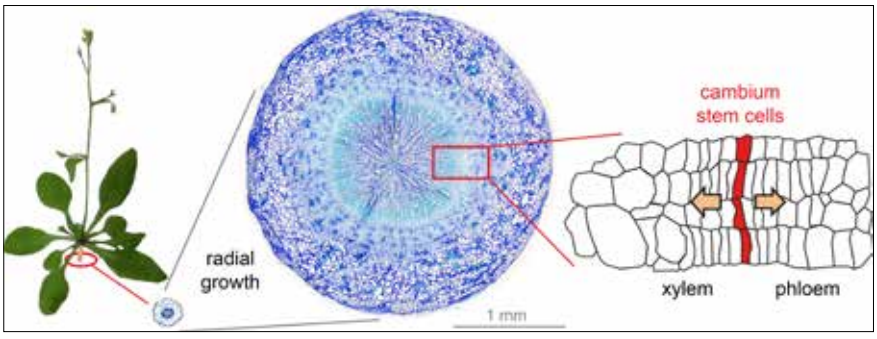


Figure 1:
Arabidopsis organs grow radially driven by cambium stem cells. Shown is a comparison between hypocotyl cross sections collected from two and six weeks-old plants. The hypocotyl connects the root and the shoot system and the production of the vascular tissues xylem (wood) and phloem (bast) by cambium stem cells is essential for fulfilling this function.

Research highlights since 2021
Wood formation is fundamental for the remarkable growth of plant bodies by continuously providing cells for long-distance transport of water and nutrients. Vessel elements, the water transporting units within woody tissues, are generated from a cylindrical domain of cambium stem cells producing different vascular cell types in a bidirectional manner. To get a detailed view on cambium stem cells regulation and on the trajectory of CSC-derived cells with high spatial resolution, Dongbo Shi, postdoc in the group, revealed the individual transcriptomes of several thousands of nuclei isolated from radially growing Arabidopsis or-

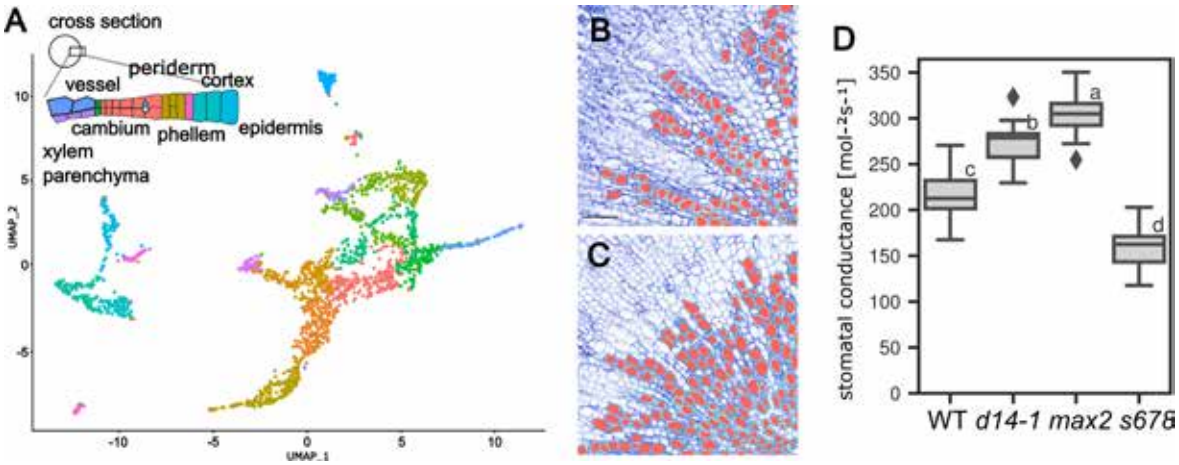


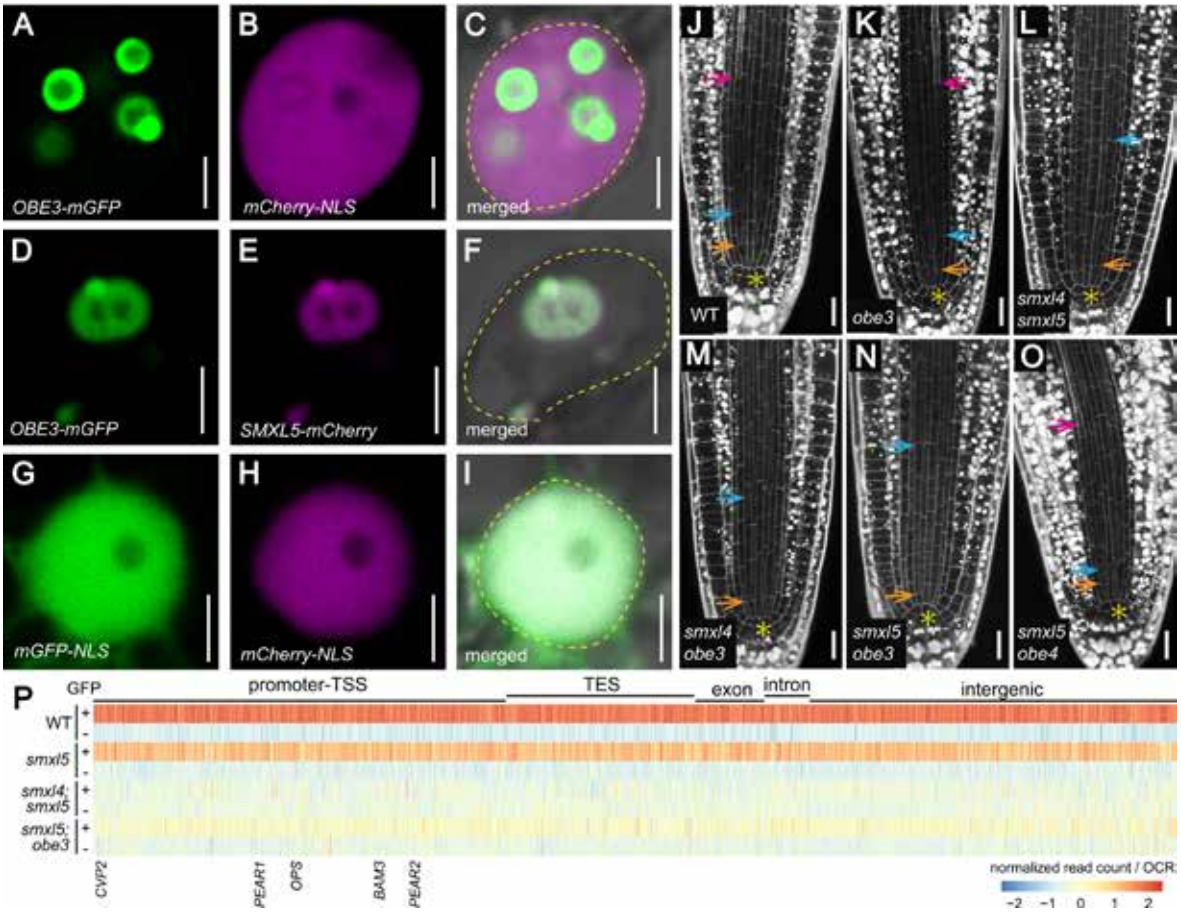
Figure 2:

The SL signalling pathway modulates vessel formation and water transpiration. A UMAP plot of 10x Chromium snRNA-seq analysis using 2,061 nuclei organised in 18 clusters obtained through unsupervised clustering analysis. A scheme of major hypocotyl cell types is shown on the top left corner. B, C Cross-sections from wild type and the SL receptor mutant d14. Vessels are marked in red. D Transpiration in wild type and the SL signalling mutants d14 and max2 and the smxl6;7;8 triple mutant defective for the proteolytic targets of the SL signalling pathway.

gans (Zhao et al., 2023, Figure 2A). When analysing these transcriptomes, he discovered that the activity of the hormonal strigolactone (SL) signalling pathway is very different in the various cambium-related cell types. Confirming a role of the pathway in the formation of vascular cell types, our postdoc Jiao Zhao and PhD student Kiara Kaeufer found that SL signalling negatively regulates vessel element formation and thereby plant water usage (Zhao et al., 2023, Figure 2B, C). One important tool for our analysis was a novel genetically encoded ratiometric SL signalling sensor, established by our postdocs Changzheng Song and Jiao Zhao, that enables the examination of SL signalling distribution at cellular resolution in intact plants (Song et al., 2022). Highlighting the importance of vascular tissue composition for the overall plant water balance, alteration of vessel element formation had a direct impact on transpiration rates through leaf stomata (Zhao et al., 2023, Figure 2C). Our results demonstrated the importance of structural alignment of water transporting tissues to unstable water regimes.

In addition to probing xylem development, we also continued our work on phloem specification. The phloem tissue mediates long-distance transport of energy metabolites along plant bodies and is, like the xylem, characterized by an exceptional degree of cellular specialization. In a study driven by the PhD students Eva-Sophie Wallner, Nina Tonn and Laura Luzzietti, we revealed that the ubiquitously expressed PHD-finger protein OBERON 3 (OBE3) forms a central module with the phloem-specific SUPPRESSOR OF MAX2 1-LIKE 5 (SMXL5) protein (Figure 3A-I) driving the phloem developmental program (Wallner et al., 2023, Figure 3J-O). By protein interaction studies and phloem-specific ATAC-seq analyses, the students showed that OBE3 and SMXL5 proteins form a complex in nuclei of phloem stem cells where they promote a phloem-specific chromatin profile (Figure 3P). Our findings highlighted how a combination of ubiquitous and local regulators generate specificity of developmental decisions in plants.

One important constraint in investigating cambium regulation is that cambium dynamics elude direct experimental access due to obstacles in live-cell imaging. To address this issue, our PhD student Ivan Lebovka in collaboration with Ruth Großeholz (COS, Heidelberg) and Roeland Merks (Leiden University, Netherlands), developed a cell-based computational model visualizing cambium activity and integrating the function of central cambium regulators (Lebovka et al., 2023). Performing iterative comparisons of plant and model anatomies, Ivan concluded that a minimal framework of regulators is sufficient for instructing tissue organization. Overall, the team paved the way for a computational simulation study of our PhD student Xiaomin Liu which was conducted in a collaboration with Mathias Höfler and Karen Alim (Technical University of Munich, Germany). In this work, the team coupled anatomical analyses with a cell-based vertex model to analyse the role of mechanical stress in radial plant growth at the cell and tissue scale (Höfler et al., 2024). Simulations revealed a distinct stress pattern with circumferential stresses in cambium



stem cells which coincided with the orientation of cortical microtubules. Our work underlined the significance of mechanical forces in tissue organization through self-emerging stress patterns during the growth of plant organs. Overall, our computational models are versatile tools for investigating cambium regulation, highlight the role of intercellular communication within the cambium, and show that a limited number of factors are sufficient to create radial growth by bidirectional tissue production.

Figure 3:

Interaction of OBE3 and SMXL5 in phloem development A–I OBE3 and SMXL5 proteins co-localize in plant nuclei. Fluorescent signals and bright-field images of epidermal plant nuclei transiently co-expressing different fusion proteins (G–I). The dashed yellow line indicates the outlines of nuclei in merged images (C, F, I). J–O Phloem development is specifically impaired in smxl4;smxl5, obe3;smxl4 and obe3;smxl5 double mutants. Yellow asterisks mark the quiescent centre. Pink arrows indicates sieve elements. Orange and blue arrows mark the first and second periclinal division, respectively, in the developing phloem cell lineage. P Phloem-specific chromatin structure shown by the relative abundance of ATAC-seq reads mapped to open chromatin regions (OCRs) usually found in phloem cells (+) but not in non-phloem cells (-) of wild-type (WT). Read counts are displayed according to the colour code shown at the bottom.

Future directions

The role of SMXL proteins in vascular tissue formation has developed into a central topic in the group in the last years. This started with the discovery that SMXL3, SMXL4, and SMXL5 proteins are central regulators of phloem development and continued with the finding that SMXL6, SMXL7, and SMXL8 proteins promote the formation of vessel elements in a strigolactone- and drought-dependent manner. Obvious questions to be addressed in this context are which genomic regions are targeted by the different SMXL proteins, what is the nature and dynamics of SMXL-containing protein complexes and what is their cell type-specific action.

Another important direction in the next two to three years will be the establishment of a second model system beside *Arabidopsis thaliana* in the lab. Here, we chose *Brachypodium distachyon*, a representative of monocotyledonous species which usually do not develop a cambium but establish intercalary meristems driving stem elongation. How the function and production within intercalary meristems is organized and how intercalary meristems in monocotyledonous species differ from the cambium in dicotyledonous species will be an exciting new topic to be tackled.

Beside these directions, probing the relevance of our lab-based discoveries in ecological or agricultural contexts will be a more adventurous and completely new aspect within our research. Here, especially the involvement of strigolactone signalling in mediating structural adaptations to drought stress will be a fruitful path. All together, we believe that our portfolio of future directions will generate exciting insights into a whole set of fundamental biological phenomena in the next years to come.

Selected publications since 2021

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Song, C., Zhao, J., Guichard, M., Shi, D., Grossmann, G., Schmitt, C., Jouannet, V., **Greb, T.** (2022) Strigo-D2 – a bio-sensor for monitoring spatio-temporal strigolactone signaling patterns in intact plants. Plant Phys 188(1):97–110

Publication statistics
https://scholar.google.de/citations?user=I48EQt8AAAAJ&hl=de

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Fields of Interest
Fructan metabolism in chicory,
new breeding techniques (NBT), greenhouse gas production from plants, role of plants in climate change

Summary and outlook

Many higher plants use fructans as storage carbohydrates. They fulfill diverse physiological functions in the plant, but are also a valuable resource for humans with a wide range of applications. In collaboration with partners from breeding and industry, we are investigating the molecular physiology of fructan metabolism in chicory, a plant, which is used for industrial inulin production, but is also an important model for fructan metabolism. The aim of this project is to understand the regulation of inulin metabolism as comprehensively as possible and thus create a basis for the targeted breeding of chicory to improve inulin content and stability.

Another research focus is on the production and consumption of nitrous oxide and methane by vegetation. Both gases act as greenhouse gases and play a decisive role, because although they occur in much lower concentrations than carbon dioxide, they are far more potent. The contribution of plants to the global budget of the greenhouse gases nitrous oxide and methane has so far been largely ignored and is being investigated in an interdisciplinary research project.

Research highlights since 2021

Role of fructans in chicory

Chicory roots exhibit significant inulin degradation at the end of the growing season due to low temperatures at night. This loss of inulin is attributed to the activity of fructan exohydrolases. The enzyme occurs in three isoforms, 1-FEH1, 1-FEH2a and 1-FEH2b. While the relative contributions of the different 1-FEH isoforms are not yet clear, previous breeding studies indicate a prominent role of 1-FEH2b. We investigated the different expression patterns for the transcripts of 1-FEH1, 1-FEH2a and 1-FEH2b, respectively, under cold treatment. Furthermore, we could show that the promoters of 1-FEH1 and 1-FEH2b are activated by the cold-inducible transcription factors CiNAC5 and CiDREB1/2, respectively. There are also indications for different responses to environmental factors of the promoters of the closely related genes 1-FEH2a and 1-FEH2b. These are caused by a single base variation in their DRE element core motif of the promoter. While in the 1-FEH2b promoter the core motif mediates effective binding and activation by CiDREB2, the corresponding sequence in the 1-FEH2a promoter renders this gene insensitive to CiDREB2. Figure 1 shows our current model of FEH gene regulation mediated by different transcription factors. These mechanistic insights into the stress response are valuable for future genome engineering strategies.

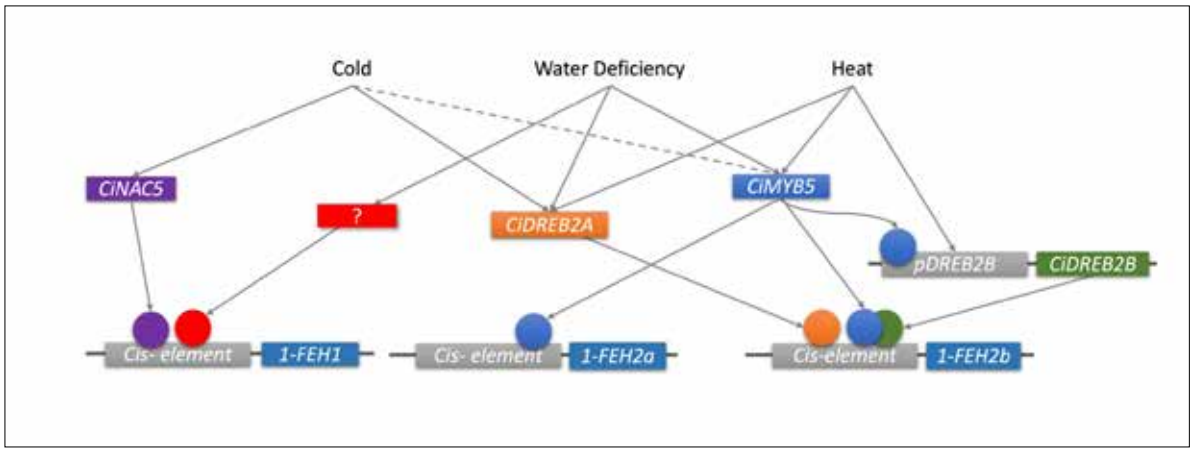

Figure 1:
Model of transcriptional regulation network for the control of chicory 1-FEH genes (Zhong, 2023)

Important greenhouse gases from plants

The mechanisms for methane (CH_4) formation by plants are largely unknown. We have established a new approach that makes CH_4 formation by plants clearly detectable. By applying ^{13}C-labeled dimethyl sulfoxide (DMSO) to the leaves of tobacco plants (*Nicotiana tabacum*) and Chinese silver grass (*Miscanthus sinensis*), the effect of light and dark conditions on CH_4 formation was investigated.

Our results indicate that CH_4 formation is clearly dependent on light conditions and plant species. Furthermore, DMSO could be a potential precursor of vegetative CH4. This novel isotopic approach has great potential to investigate physiological and environmental factors influencing CH_4 emissions from plants with high temporal resolution.

Having previously also measured N_2O emission rates of 32 plant species from 22 different families under controlled laboratory conditions and determined the first isotopic values of N_2O emitted by plants, the tools are now available for methane and nitrous oxide to address studies on the contribution of plants to the global budget of these gases.

Future directions

The findings on which transcription factors play a crucial role in stress-mediated regulatory aspects of fructan metabolism provide fundamentally new insights and open up new avenues for biotechnological approaches. In this context, we are establishing new breeding techniques (NBT) with *in vitro* cultivated chicory plants to verify the results in planta.

In the future, we want to further uncover the fundamental role of vegetation in the production and consumption of methane and nitrous oxide and extend it from the laboratory to field conditions. This interdisciplinary research together with earth sciences, geography and environmental physics has the potential to help control greenhouse gas emissions and promote the development of global strategies to mitigate climate change.

Selected publications since 2021

Schroll, M., Lenhart, K., **Greiner, S.**, Keppler, F. (2022) Making plant methane formation visible—insights from application of 13C-labeled dimethyl sulfoxide. Plant-Environment Interactions 3 (3) 104-117

Publication statistics

https://scholar.google.de/citations?user=q63FAhoAAAAJ&hl=de

2.9 PROF. DR. RÜDIGER HELL
PLANT MOLECULAR BIOLOGY

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Fields of Interest
Molecular biology and physiology, regulation of metabolism, metabolite sensing, nutrition, environmental stress, protein modification, proteostasis

Summary and outlook
The Department of Molecular Biology of Plants investigates the role of metabolism in plants in the context of growth control and responses to changing environments. The metabolism of sulfur with the unique cysteine synthase protein complex is in the centre of research, being involved proved in general growth regulation as a nutrient, translational and metabolic control based on the sulfur-containing amino acids and as defense and redox mediator due to sulfur-based functions in glutathione and specialized metabolites. These topics are complemented by the research group of Dr. Markus Wirtz who investigates cellular acclimation processes to stress governed by protein turnover and damage control via acetylation of protein N-termini.

Projects related to sulfur metabolism were nutrient sensing and acclimation of organ growth by the target of rapamycin, changes of sulfur status in response to heavy metals and selenium and drought acclimation via cysteine-dependent ABA synthesis in guard cells. Together with the Wirtz group the role of N-terminal acetylation was investigated with respect to biotic stress and a novel nonAc-degron identified (Miklánkova et al., 2022). The third research direction in the department is represented by the Metabolomics Core Technology Platform that provides scientific services for the entire Heidelberg Life Science Campus. The support of investigation of metabolic processes in tumor cells is the focus of a cooperation contract with the German Cancer Research Center (DKFZ Heidelberg). The BMBF consortium SMART-CARE (Medical Faculty, DKFZ, Appl. Univ. Mannheim, MCTP) aims to establish metabolomics, proteomics and bioinformatics for clinical translation (Gegner et al., 2022).

Research highlights since 2021
Plants are able to adjust their growth to environmental constraints. In case of soil sulfate deficiency, they arrest shoot growth but maintain root growth to find new mineral sources. We showed that the Target of Rapamycin (TOR) kinase is differentially regulated in shoot and root of Arabidopsis, resulting in a massive shift of the root-to-shoot ratio upon prolonged starvation. The underlying processes of this developmental acclimation are induction of autophagy in the shoot and export of sucrose induction by sucrose transporter and SWEET facilitator genes in the vasculature. In the roots, however, TOR remains activated due to the delivery of sucrose, suppressing autophagy but promoting translation and meristem activity (Dong et al., 2022). Work with a cooperation partner revealed that TOR in tomato is not only linked to sulfur but also to iron starvation, possibly mediated by changes in citric acid levels (Coppa et al., 2023)

Earlier work on the partitioning of sulfur between protein translation and glutathione synthesis, i.e. flux distribution depending on undisturbed or stress determined growth, had led to the observation of differential responses of redox state in the cytosol and plastids (Speiser et al., 2018). These findings were now followed up by triggering changes in redox state by growing plants in the presence of selenite and selenate (Khan et al., 2022). Both sulfate analogs enter the root and can subsequently be reduced to selenide by the sulfate assimilation pathway to become part of Se-cysteine and Se-methionine, leading to eventual intoxicating of the cells. Arabidopsis plants deposited selenite but not selenate mainly in the shoot, and at the same time triggered enhanced uptake of sulfate. Using redox sensitive roGFP for live cell imaging, we found that selenite first caused the oxidation of the plastid-localized glutathione pool and had little impact on the redox state of the cytosolic glutathione pool. Selenate application caused the opposite, i.e. more oxidation of the cytosolic compared to the plastid glutathione pool. Thus, selenium-species dependent differences in partitioning contributed to whole plant toxicity, presumably due to subcellular compartment-specific impacts on the glutathione redox buffer.

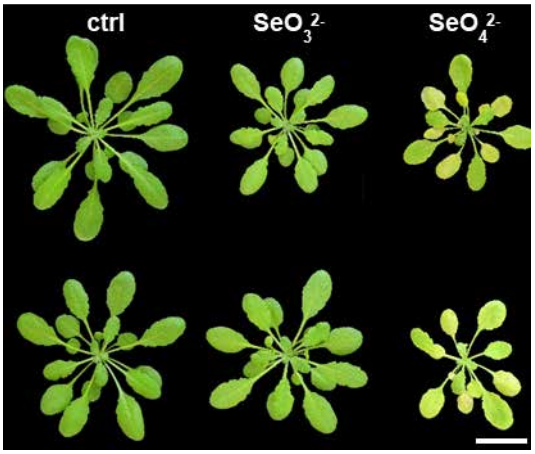

Figure 1A:
Top view of six-week-old hydroponically grown Arabidopsis plants that were challenged for seven days with 50 µM selenite or selenate.

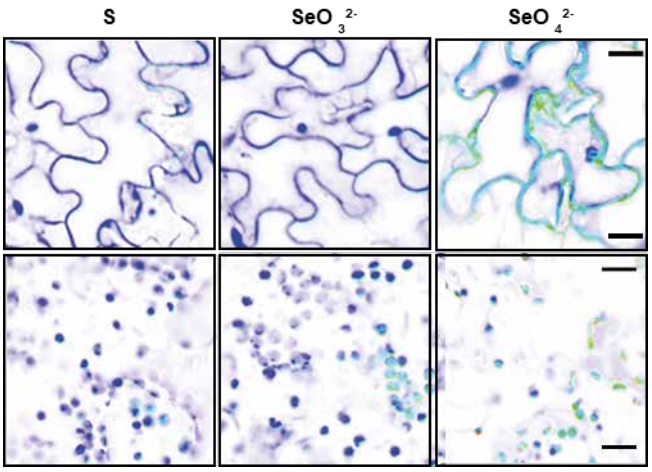

Figure 1B:
Grx1-roGFP2 signal ratio in the cytosol (upper row) or plastids (lower row) of leaves from hydroponically grown Arabidopsis plants treated with selenite, selenate or without additional selenium supply (only sulfate) for 120 hours.

Molecular mechanisms of drought stress resistance have become a major part of the departmental activities, combining projects from sulfur metabolism and N-terminal protein acetylation. Our published work has established xylem-transported sulfate as a new long-distance signal that allows the stomata to perceive the soil water status (Batool et al., 2018). We showed that sulfate promotes the synthesis of cysteine by triggering *de novo* ABA production in guard cells and that in principle this process is sufficient to promote stomatal closure. In addition, we found that high light stress-induced stomatal closure also depends on the ability to synthesize cysteine and ABA *de novo*. Recent work shows that

the activation of the ABA biosynthetic pathway in guard cells depends on activation of the absisic aldehyde oxidase AAO3 by trans-sulfuration of its cofactor and *de novo* synthesis of cysteine. Using a plastid cysteine synthase complex that has been engineered to be constitutively associated and enzymatically active we can now generate Arabidopsis plants with more closed stomata displaying enhanced drought resistance without growth penalty. These findings define the plastid cysteine synthase complex in guard cells as a sensor for water deficit that mediates guard cell autonomous ABA synthesis.

Improving plant performance during stress via enhanced glutathione levels through de-regulated cysteine formation has a long history in molecular plant physiology but has not been very successful. This is presumably because of the association of the two biosynthetic enzymes in the regulatory cysteine synthase complex (CSC). This complex is unique in general metabolism since it is not functioning in substrate channeling but sensing of two metabolites of the pathway. Indeed, overexpression of the rate-limiting enzyme serine acetyltransferase (SAT) in the plastids of transgenic tobacco caused phenotypic aberrations, including set-up of the photosynthetic apparatus. However, when we expressed a mutated enzymatically inactive form of SAT that still can form the CSC these effects were abolished. This is the first finding that points to the plastid CSC itself as a trigger for controlling sulfur assimilation and photosynthetic capacity in leaves (Wirtz et al., 2023).

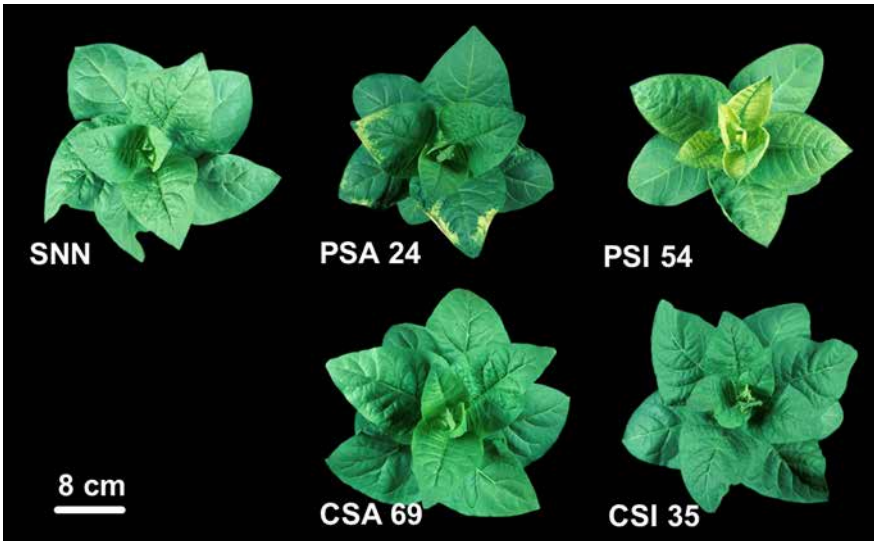

Figure 2:
Expression of an enzymatically active (PSA, CSA) or inactive (PSI, CSI) AtSAT3 subunit of the cysteine synthase complex from Arabidopsis in chloroplasts (PSA, CSI) but not in the cytosol (CSA, CSI) of transgenic tobacco plants causes chlorosis in young leaves.

Future directions

The elucidation of cysteine synthesis in subcellular compartments and guard cells and the dissection of the CSC mechanisms point to much further reaching implications for the regulation of sulfur metabolism. Given the fact that sulfur is an essential macronutrient with functions in numerous cellular and organismic processes, the focus on the CSC will possibly lead to answer fundamental questions of growth control under environmental stress conditions, here mainly water limitation as stress factor.

The investigations regarding the role of sulfate as long-distance signal that is converted to a metabolite in guard cells to trigger de novo cysteine synthesis for ABA formation is of highest relevance for drought stress physiology and far from being understood. Recent evidence from our team also relates high light stress-triggered stomata closure to the plastid CSC but with signal transduction by OPDA. Thus, the plastid CSC, at least in guard cells, appears to emerge as a central hub for the integration of stress signals towards promotion of cell-specific ABA synthesis.

This line of research will be continued with detailed analysis of the control of ABA biosynthetic pathway, by inclusion of more external and long distance stress signals (e.g. CLE25), and structural composition and regulation of the plastid CSC to obtain a full and mechanistic picture of the signal transduction pathways involved. The project is accompanied by

engineering of CSC components, based on the successful cooperation with F. Zhao and S. Sun, Nanjing Agric. University, starting with arsenic resistance in rice (Sun et al., 2021). The discovery of a mutation in one of the CSC subunits that causes constitutive association and activation of the plastid CSC at otherwise unchanged properties was the starting point of developing such CSCs in Arabidopsis. This tool on one side enables the targeted manipulation of cysteine (and glutathione) levels in a cell-compartment specific manner which is highly useful in basic research on regulation of cysteine metabolism. On the other side, such engineered plants allow studies on drought and heavy metal stress resistance and potentially transfer to crop plants.

Selected publications since 2021

Coppa, E., Vigani, G., Aref, R., Savatin, D., Bigini, V., **Hell, R.** and Astolfi, S., (2023). Differential modulation of Target of Rapamycin (TOR) activity under single and combined iron and sulfur deficiency in tomato plants. Plant J. 115, 127-138.

Wirtz, M., Leemhuis, W., **Hell, R.** (2023). Dynamic association of the plastid localized cysteine synthase complex is vital for efficient cysteine production, photosynthesis, and grana thylakoid formation in transgenic tobacco. J. Exp. Bot. 74, 3379-3394.

Dong, Y., Aref, R., Forieri, I., Schiel, D., Leemhuis, W., Meyer, C., **Hell, R.** and Wirtz, M. (2022). The plant TOR kinase tunes autophagy and meristem activity for nutrient stress-induced developmental plasticity. Plant Cell 34, 3814-3829.

Gegner, H. M., Mechtel, N., Heidenreich, E., Wirth, A., Garcia Cortizo, F., Bennewitz, K., Fleming, T. H., Andresen, C., Freichel, M., Teleman, A. A., Kroll, J., **Hell, R.** and Poschet, G. (2022). Deep metabolic profiling assessment of tissue extraction protocols for three model organisms. Front. Chem. 10, 869732.

Miklánková, P., Linster, E., Boyer, J.B., Weidenhausen, J., Mueller, J., Armbruster, L., Lapouge, K., De La Torre, C., Bienvenut, W., Sticht, C., Mann, M., Meinnel, T., Sinning, I., Giglione, C., **Hell, R.** and Wirtz, M. (2022). HYPK promotes the activity of the Nα-acetyltransferase A complex to determine proteostasis of nonAc-X2/N-degron-containing proteins. Sci. Adv. 8, eabn6153.

Publication statistics

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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, abscisic acid biosynthesis

Summary and outlook
The composition of cellular proteomes rapidly adjusts in response to internal and external stimuli. Protein modifications add yet another layer of plasticity to this intricately balanced and dynamic system. N-terminal acetylation is among the most common protein modifications in higher eukaryotes. In this report term, we have achieved substantial progress in identifying and characterizing the N-acetylation machinery in plants. We uncovered the existence of a previously undescribed N-degron that is shielded by the activity of the N-acetyltransferase A (NatA) complex. Furthermore, we revealed the regulatory function of HYPK on the ribosome-anchored NatA complex. Our findings explain the substantial destabilization of the plant proteome in NatA activity-impaired mutants. Next, we aim to understand the relevance of N-terminal acetylation for protein aggregation and autophagy-mediated protein clearance.

Research highlights since 2021
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^{α}-acetyltransferase A (NatA) complex, which is tethered to the ribosome and accounts for the cotranslational acetylation of 40% of the proteome. The core NatA complex is essential in plants and comprises the ribosome-anchoring subunit NAA15 and the catalytically active NAA10. ABA application depletes the abundance of the core NatA, which causes substantial tolerance to soil drying and increases the tolerance towards diverse pathogens.
Motivated by these findings and reports on NatA-mediated destabilization of yeast proteins, we analyzed the proteome stability in core NatA-depleted plants. Surprisingly, we found that depletion of NatA activity leads to a 4-fold increase in global protein turnover via the ubiquitin-proteasome system in Arabidopsis. A targeted analysis of NatA substrate stability uncovered that NTA absence triggers protein destabilization via a previously undescribed and widely conserved nonAc/N-degron (Linster et al., 2022).
In humans, the core NatA interacts with two additional proteins, NAA50 and HYPK, affecting core NatA activity *in vitro*. We identified the plant homolog of HYPK (Huntingtin Yeast-interacting Partner K, Figure 1) as a critical but non-essential regulator of NatA activity.

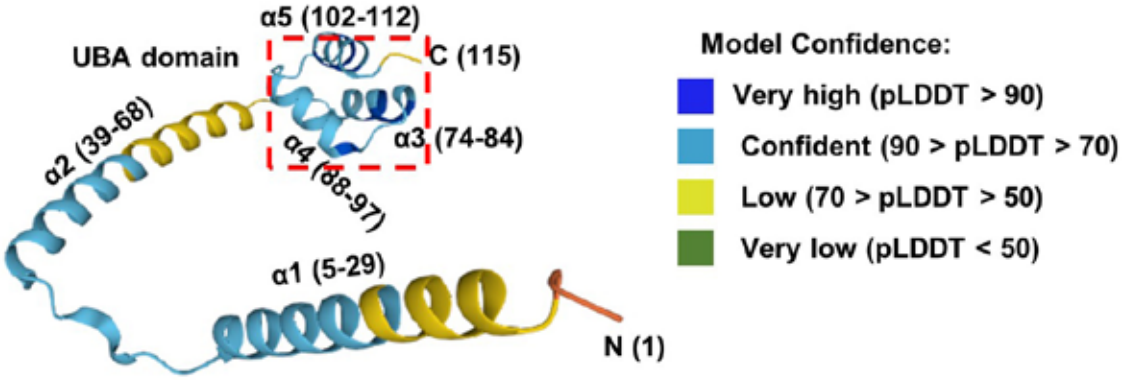

Figure 1:
Prediction of AtHYPK structure by AlphaFold. The red box indicates the UBA domain.

The absence of HYPK also results in global proteome destabilization in plants and can be rescued by ectopic expression of human HYPK, strongly suggesting that the HYPK function is conserved in multicellular eukaryotes (Miklánková et al., 2022). Next, we disentangled the role of the highly conserved UBA domain and helix α1 of HYPK for NatA activity at the ribosome. We found that helix α1 must be correctly positioned at the NatA complex by the UBA domain to fulfill its activity-enhancing function and that the interaction of the core NatA with the UBA domain stabilizes NAA15 in planta in an organ-specific manner (Gong et al., 2024).

In sharp contrast, NAA50 interacts with the ribosome-anchoring subunit NAA15 but does not impair NatA activity or global proteome stability. Unlike NatA activity-impaired mutants, NAA50-depleted plants display no drought tolerance and are not impaired in seed yield. However, they are substantially resistant to diverse pathogens, establishing a new candidate for marker-assisted breeding of high-yield and pathogen-resistant varieties (Armbruster et al., 2024).

Future directions

NatA-depleted mutants display up to 4-fold higher protein translation rates to cope with the massive destabilization of NatA substrates. In yeast, acetylation by NatA promotes the folding of the nascent chain extruding from the ribosome. Because of the high translation and its potential impact on folding, we aim to test, if the absence of the NatA complex also causes enhanced protein aggregation in plants. Such protein aggregates are toxic in eukaryotic cells and cause neurodegenerative diseases like Chorea Huntington in humans. Protein aggregates are cleared in plants and humans by selective autophagy. We will thus characterize the contribution of autophagy to the substantially enhanced protein turnover in plants depleted of the core NatA or HYPK, which human homolog interacts with the huntingtin protein.

The phytohormone abscisic acid (ABA) is the central regulator of the drought stress tolerance of plants. We aim to understand the substantial tolerance of NatA-depleted plants to soil drying by crossing NatA-depleted plants with mutants impaired in the synthesis or sensing of ABA and will test if the enhanced protein turnover is partially caused by the constitutive ABA response observed in NatA-depleted plants.

Selected publications since 2021

Armbruster, L., Pozoga, M., Wu, Z., Eirich, J., Thulasi Devendrakumar, K., De La Torre, C., Miklankova, P., Huber, M., Bradic, F., Poschet, G., Weidenhausen, J., Merker, S., Ruppert, T., Sticht, C., Sinning, I., Finkenmeier, I., Li, X., Hell, R., **Wirtz, M.** (2024) N^{α}-acetyltransferase NAA50 mediates plant immunity independent of the N^{α}-acetyltransferase A complex. Plant Physiology, 195, 3097-3118

Gong, X., Boyer, J.-B., Gierlich, S., Pozoga, M., Weidenhausen, J., Sinning, I., Meinnel, T., Giglione, D., Wang, Y., Hell, R., **Wirtz, M.** (2024) HYPK controls stability and catalytic activity of the N-terminal acetyltransferase A in *Arabidopsis thaliana*. Cell Reports 43, 113768

Dong, Y., Aref, R., Forieri, I., Schiel, D., Leemhuis, W., Meyer, C., Hell, R., **Wirtz, M.** (2022) The plant TOR kinase tunes autophagy and meristem activity for nutrient stress-induced developmental plasticity. Plant Cell 34, 3814-3829

Linster, E., Forero Ruiz, F.L., Miklankova, P., Ruppert, T., Mueller, J., Armbruster, L., Gong, X., Serino, G., Mann, M., Hell, R., **Wirtz, M.** (2022) Cotranslational N-degron masking by acetylation promotes proteome stability in plants. Nature Communications 13, 810

Miklánková, P., Linster, E., Boyer, J.B., Weidenhausen, J., Mueller, J., Armbruster, L., Lapouge, K., De La Torre, C., Bienvenut, W., Sticht, C., Mann, M., Meinnel, T., Sinning, I., Giglione, C., Hell, R., **Wirtz, M.** (2022) HYPK promotes the activity of the N^{α}-acetyltransferase A complex to determine proteostasis of nonAc-X^{2}/N-degron-containing proteins. Science Advances 8, eabn6153

Publication statistics

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

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Fields of Interest
Molecular evolution of development, Wnt and TGF-ß signalling, transcriptional networks; cnidarian biology including regeneration and stem cell biology; evolution of the germline and the nervous system (neuronal cell types including the cnidarian characteristic nematocytes), gradients and axis formation.

Summary and outlook
To understand the origin and evolution of key regulators of animal development, we have focused almost exclusively on cnidarians, fascinating creatures at the base of metazoan evolution. They have a gastrula-like diploblastic body plan and a simple nervous system. Our genomic work on *Hydra, Nematostella, Aiptasia* and *Aurelia* revealed an unexpected genomic complexity of these ancient organisms and showed that the genetic repertoire needed to build the bilaterian body plan was already present in the common cnidarian-bilaterian ancestor. We uncovered the existence of the blastoporal Wnt signalling centre in cnidarian embryos (*Nematostella*), corresponding to the hypostomal region of adult animals, which is crucial for gastrulation and the formation of their body axis and which has an organizer like function. Our studies have also shown that the molecular vectors for dorso-ventral and left-right body axis in bilaterians, Bmp and Nodal signalling, respectively, are already present in cnidarians. Cnidarians also have an almost unlimited capacity for regeneration, which attracted Abraham Trembley (1744), and which was a major theme in our research on *Hydra*. The fact that cnidarians, the undisputed sister group of bilaterians, already possess an almost complete molecular toolkit of the more complex animals has revived research on these simple animals, which have played an important role in establishing fundamental concepts in developmental biology since the beginnings of zoological research.

Research highlights since 2021

The evolution of signalling pathways.
A major focus of our research is Wnt signalling, an animal-specific signalling pathway. Wnts are involved in all stages of animal life, including early development (gastrulation), regeneration and disease. Understanding the evolutionary origins of Wnt signalling will shed light on general mechanisms of this pathway and the evolution of fundamental developmental processes. In collaboration with Michaela Holzem and Michael Boutros, we have reviewed open questions regarding the origin and evolution of the Wnt signalling pathway and its molecular components (Holzem et al, 2023). Recent advances in computational biology and the availability of genome sequences, particularly for basal metazoans and closely related pre-metazoan species, are enabling a better understanding of the evolutionary origin of Wnt signalling. In our work, we provide an overview of the canonical and non-canonical Wnt signalling pathways and components. Recent data on the origin of each stage of the Wnt pathway, starting with the evolution of Wnt protein ligands, followed by Wnt ligand secretion and trafficking, allow us to reconstruct the ancestral original Wnt pathway with its pre-metazoan and novel metazoan-specific components.

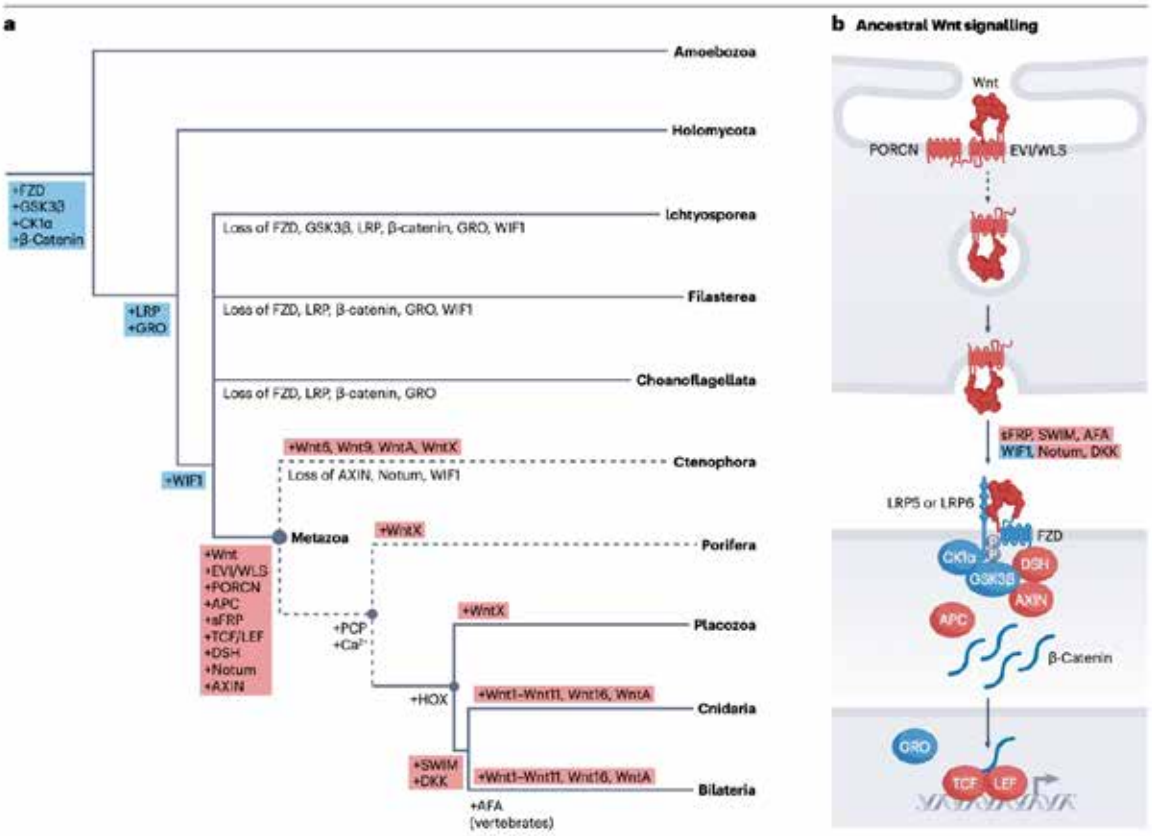

Figure 1:

The evolutionary origin of Wnt signalling. (a) Origin of selected Wnt signalling components in different clades of pre-metazoans (b) Reconstruction of the original Wnt signalling pathway with pre-metazoan (blue) and metazoan-specific (red) components (Holzem et al 2024, Nature Rev. Genetics).

Did animal evolution begin with a predatory lifestyle?

Gastrulation in animal embryogenesis controls axis and germ layer formation and is regulated by Wnt signalling. To explain the evolutionary origin of this fundamental process, Ernst Haeckel (1834 - 1919) postulated that the gastrula with its blastopore mouth was the starting point of animal evolution, a theoretical concept that has been repeatedly challenged. When we analysed *Aiptasia* embryos - to complete the life cycle of this emerging model for coral reef research - we made the surprising discovery that it is not the ingestion of endosymbionts (dinoflagellates) but the capture of live food that is the bottleneck in the settlement of *Aiptasia* planula larvae (Maegele et al, 2023). Already at the end of gastrulation, larvae begin to feed actively on crustacean nauplii (Figure 1). This ‚predatory gastrula' not only closes the life cycle of *Aiptasia*, but also highlights the functional nature of this larval stage as proposed in Haeckel's Gastraea theory. This predatory lifestyle has important implications for the evolution of early metazoans. The presence of cnidarian stinging cells used for prey capture is mirrored by extrusive organelles in protist eukaryotes, ctenophores and planarians, and suggests that predation played a crucial role in the early evolution of metazoans from unicellular eukaryotes, although further analysis of cell types in more taxa is needed. We suggest that predation may have been the major driving force behind early metazoan evolution and the development of organised nervous systems.

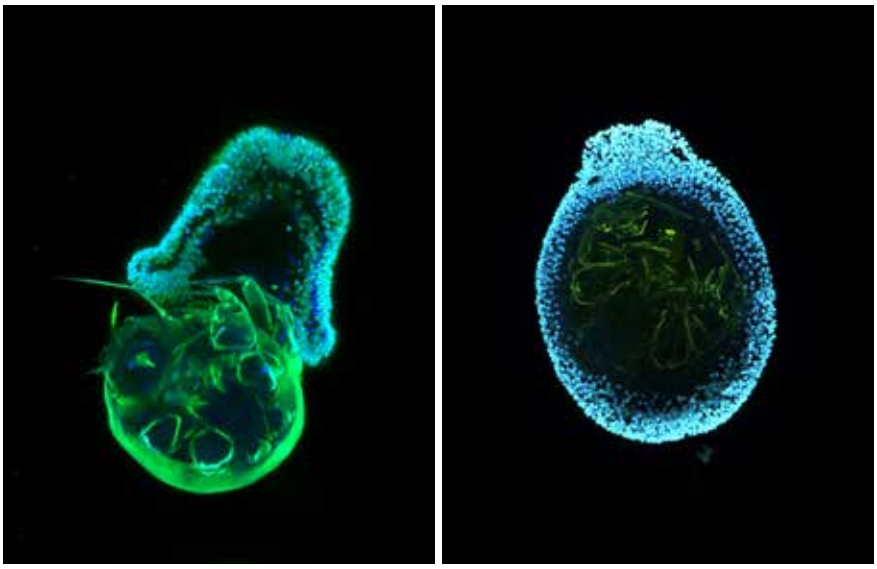

Figure 2:

Food capture by an *Aiptasia* gastrula-like larva point to early evolutionary events in metazoans (Maegele et al 2023, PNAS).

Regeneration: from injury to patterning.

Our previous proteomic and transcriptomic work on *Hydra* regeneration revealed an initial injury response and subsequent signalling-driven patterning of the regenerating tissue. While MAPKs played a key role in the initial injury response, a cascade of Wnts was involved in patterning the regenerating tissue. When we analysed these processes in more detail in the PhD work of Anja Tursch, we found that MAPKs were activated independently of the wound site and in response to calcium and ROS signalling (Tursch et al, 2022). Surprisingly, Wnt3 and Wnt9/10c acted downstream of MAPK signalling in a location-independent manner and were able to partially rescue regeneration in tissues treated with MAPK inhibitors. Foot regeneration could be reversed to head formation by pharmacologically increasing ß-catenin signalling or by the application of recombinant Wnts. Based on these data, we proposed a model in which a ß-catenin-based stable gradient of head-forming capacity along the primary body axis determines the fate of regenerating tissues through differential integration of an indiscriminate injury response. Given the high degree of evolutionary conservation of MAPKs and Wnts, we propose that this mechanism is deeply embedded in our genome.

Figure 3:

Regenerating lost body structures in *Hydra*. Activation of MAPKs upon injury after head removal shows p-Erk (green) and p-p38 (red) (left). Regenerating gastric piece of a *Hydra* forms “head” structures on both extremities upon ectopic stabilization of ß-catenin during early regeneration (Tursch et al 2022, PNAS).

The nervous system of Hydra.

Hydra is a paradigm of a simple nerve net. Like many cnidarians, *Hydra* is characterised by a large number of different neuronal subpopulations expressing different neuropeptides, organised into a nerve network that extends throughout the body column. This net-

work is required to control spontaneous behaviour: elimination of neurons results in polyps that are immobile and unable to capture and ingest prey. We have re-examined the structure of the *Hydra* nerve network by immunostaining fixed polyps with an antibody initially raised against a synthetic peptide of the cytoplasmic region of *Hydra* cadherin. This novel antibody, which surprisingly stained all *Hydra* nerve cells, revealed two distinct nerve networks, one in the ectoderm and one in the endoderm (Keramidioti et al, 2023). Both neural networks do not contact each other, a surprising result confirmed by serial block face SEM. Furthermore, both neuronal subnetworks consist of bundles of parallel and overlapping neurites. Results from transgenic lines show that neurite bundles contain different neuronal circuits and therefore neurites in bundles require circuit-specific recognition. The constant turnover of existing neuron-neuron and neuron-epithelial cell interactions is currently unknown and is being investigated in several other *Hydra* and neurobiology labs.

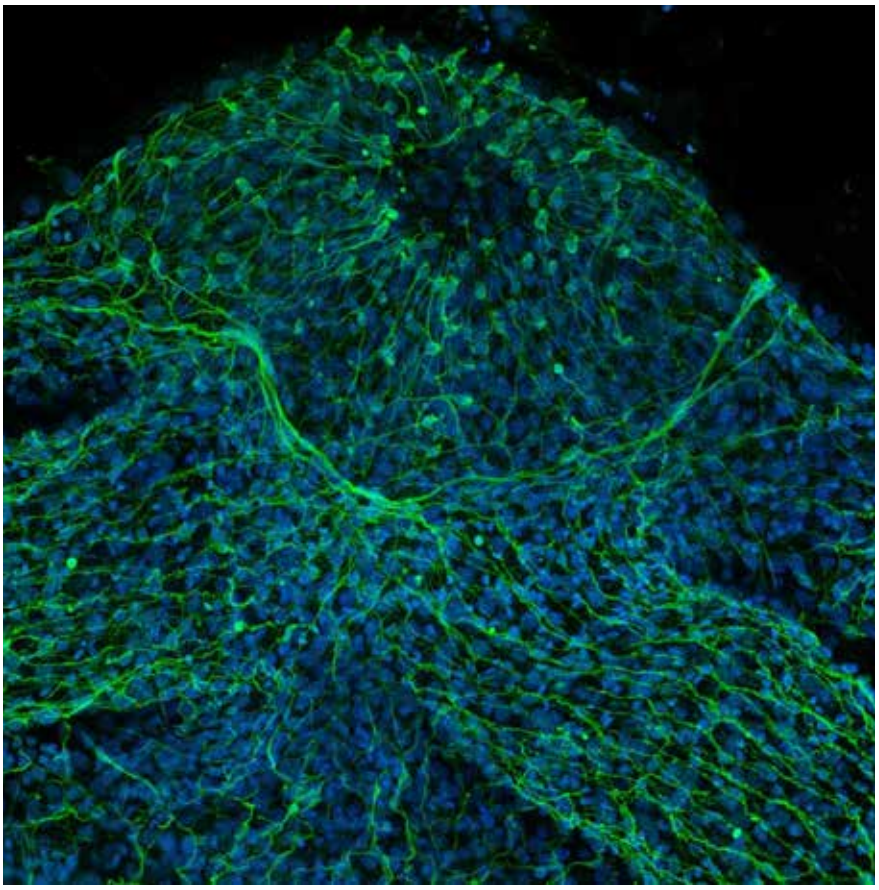

Figure 4:
Image of the oral Hydra nerve net stained with our pan-neuronal antibody (Keramidioti et al 2004); nuclei are labelled with DAPI (blue). From Holstein (2023) Evolution: Neuronal control of an archaic mouth. Current Biology 33, R1304-R1306.

Future directions

Current work within the SFB1324 addresses the hierarchy of Wnts (Wnt code) in the *Hydra* head organizer and their interplay with physical cues during regeneration and *de novo* pattern formation. To understand the activation of the Wnt-network (*Hydra* head organizer) in steady state animals and during regeneration we analyze the activation range of Wnts with an emphasis on antagonistic Wnts and epigenetic aspects of Wnt and ß-catenin signaling. We will combine these studies with an analysis of the transcriptomic and proteomic landscape of the emerging head organizer. To understand how biophysical (mechanical) and biochemical (Wnt signaling) cues interact, we are currently studying in a cooperative work with Motomu Tanaka (Biophysics) and Anna Marciniak-Czochra (Mathematics) the

symmetry break in regenerates and in reaggregates under conditions of *de novo* pattern formation and regeneration. A 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 2021

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Holstein, T.W. (2022). The role of cnidarian developmental biology in unraveling axis formation and Wnt signaling. Dev Biol. https://doi.org/10.1016/j.ydbio.2022.04.005

2.11 PROF. DR. GÁSPÁR JÉKELY
EVOLUTIONARY NEUROBIOLOGY

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Fields of Interest
Marine zooplankton, connectomics, neuromodulation, neuronal circuits, origin of nervous systems, evo-devo, phylogeny, neuropeptides and their GPCRs, ciliary coordination, phototaxis, eye evolution, sensory cells, mechanosensation, ciliated larvae, corals, biophysics of microswimmers

Summary and outlook
Our team works on the systems neurobiology of small aquatic larvae with a focus on how these organisms coordinate whole-body actions and sense and interact with their environment. We combine experimental biology and connectomics to understand the function and diversity in marine invertebrate nervous systems and the evolution of marine larval brains and behaviours (Figure 1).

Figure 1:
SEM image of a *Platynereis* juvenile.

By volume electron microscopy (vEM), we map entire animal bodies at nanometer resolution and obtain wiring diagrams of full nervous and effector systems. Through genetic approaches, live imaging and behavioural studies we integrate across scales of organisation, linking molecular function through neurons, circuits and behaviour to ecology. Our integrated research program uniquely allows us to study how marine organisms interact with their environment and decipher the molecular basis of environmental adaptations, for example, to temperature changes. Since animals and nervous systems first evolved in the ocean, understanding nervous systems and behaviours across marine life forms is essential for charting the evolution of animal intelligence. To answer the fundamental question of how nervous systems control behaviour in different animals, it is essential to map neuronal

connections and decipher how specific activity patterns arise from synaptic connectivity. Another essential step is to find out how neuronal activity is modulated by a wide variety of neuromodulators, including neuropeptides and monoamines, under different conditions. To investigate these questions at the systems level, our team is studying nervous systems of transparent organisms from zooplankton, including the marine bristle worm *Platynereis dumerilii* and other aquatic invertebrates.

Research highlights since 2021

Over the past years, my laboratory has been developing the annelid *Platynereis dumerilii* as a new model for circuit neuroscience and zooplankton behaviour. We used connectomics, genetics, behavioural analysis and neuronal activity imaging to study sensory-motor responses and neuromodulation in *Platynereis* larvae. This work enabled us to link structure and function in whole-body circuits that underpin behaviour.

A key resource to understand the neuronal control of behaviour has been a whole-body synaptic connectome reconstruction based on a volume EM dataset of a *Platynereis* larva (Verasztó et al. 2024). We reconstructed and annotated over 9,000 neuronal and non-neuronal cells and classified differentiated cells into 202 neuronal and 92 non-neuronal cell types. In the connectome, we analyse modularity, multisensory integration, left-right and intersegmental connectivity and motor circuits for ciliated cells, glands, pigment cells and muscles. We could also link over the years several behaviours, including visual phototaxis, startle response, pressure sensation, ciliomotor control and UV avoidance to specific circuits in the connectome (Figure 2).

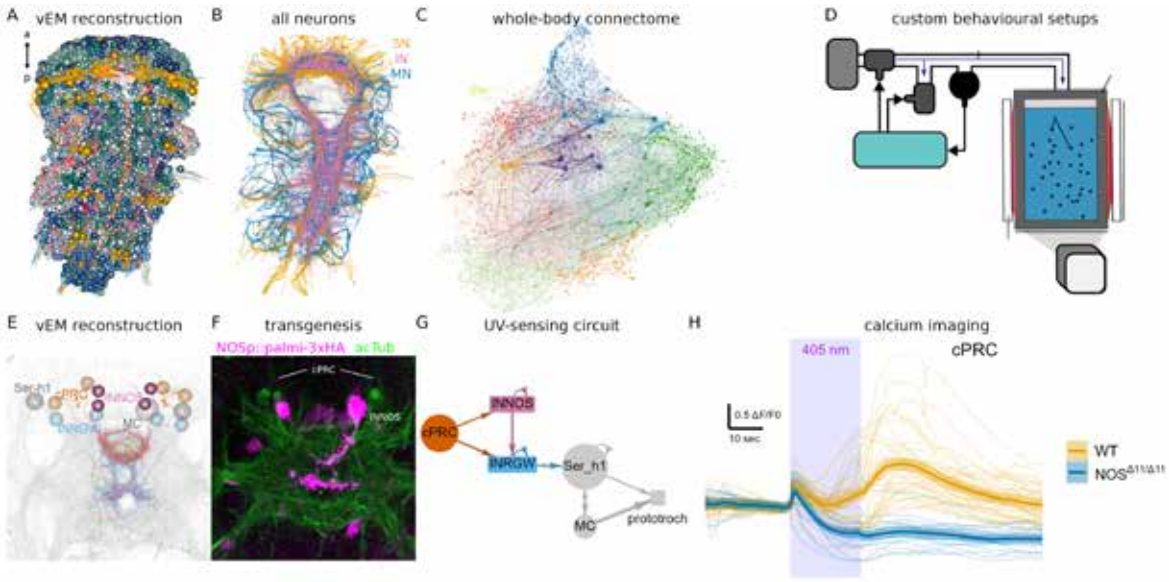

Figure 2:

Whole-body vEM and circuit analysis in the Platynereis larva. (A) All cells in a whole-body EM volume. (B) Neurite processes of all neurons in the larva coloured by neuron type (ventral view). SN, sensory neuron; IN, interneuron; MN, motoneuron. (C) Full connectome graph of the three-day-old larva synapse wiring diagram. (D) The custom-built pressure chamber used to analyse barotactic behaviour. (E) Volume rendering of all neurons in the UV-avoidance circuit. (F) Expression of a NOS reporter. (G) Wiring diagram of the UV-avoidance circuit. (H) Calcium dynamics in the UV-photoreceptor cells in wild type larvae and CRISPR-knockout larvae for the nitric-oxide synthase (NOS) gene.

In parallel, we have continued our work on the evolution of neuromodulatory systems across animals. The synaptic connectome is a scaffold, which can be reconfigured due to the action of multiple neuromodulators. Every circuit is multiply modulated and behavioural flexibility can only be understood by considering how these chemical signalling layers interact with the connectome (the “multi-layered connectome”). To comprehensively reconstruct neuromodulatory networks we have been working on neuropeptidomes and peptide GPCRs across animals. Recently, we completed a large-scale neuropeptide deorphanisation project in the sea anemone *Nematostella vectensis* (Thiel et al. 2023). We screened 64 peptides derived from 33 precursors (many of them newly identified by mass spec) against 161 GPCRs and identified 31 receptors specifically activated by 1 of 14 peptides. Mapping GPCR and neuropeptide expression to single-cell sequencing data revealed how cnidarian tissues are extensively connected by multilayer peptidergic networks.

To experimentally characterise how neuromodulators shape circuit activity and behaviour, we focused on nitric oxide (NO), a gaseous diffusible neuromodulator (Jokura et al, 2023) and its function in UV-avoidance behaviour. We found that nitric oxide synthase (NOS), the enzyme producing NO, is expressed in interneurons postsynaptic to the UV-sensing brain ciliary photoreceptors. UV stimulation of the photoreceptors triggers NO production in the postsynaptic interneurons and NO signals retrogradely to induce sustained post-stimulus activation of the photoreceptor cells. This late-phase activation then leads to the inhibition of serotonergic ciliomotor neurons thereby inducing downward swimming. In NOS mutants, retrograde signalling, circuit output and UV avoidance are defective. We also identified the receptor of NO expressed in the ciliary photoreceptor, an unconventional guanylyl cyclase with a NIT domain (NIT-GC). Morpholino-mediated knockdown of NIT-GC also eliminated retrograde NO signalling. We characterised the activity of all cells in the circuit both in wild type and mutant/morphant larvae and developed a mathematical model that integrates the molecular and circuit layers and captures circuit dynamics.
Behavioural flexibility can also result from multisensory integration or at the level of multimodal sensory neurons. As an example, recently we found that the UV-sensing ciliary photoreceptor cells also respond to changes in hydrostatic pressure (Bezares-Calderón et al., 2023). Hydrostatic pressure is a dominant environmental cue for marine organisms but the mechanisms of responding to pressure changes by zooplankton have remained elusive. By calcium imaging, we could show that the ciliary photoreceptors respond to pressure changes in a graded manner, with an activation pattern that is different from the UV response of the same cells. The differential activation of the same sensory cells by either pressure or UV leads to different downstream circuit dynamics and different behaviour. Increases in pressure induce rapid, graded and adapting upward swimming due to faster ciliary beating. UV triggers downward (head down) swimming to avoid the surface layer. Overall, by leveraging the connectome, genetic mutants and whole-circuit imaging, we could show how the activation of the ciliary photoreceptors and their downstream circuit drives two distinct behaviours.

Future directions
One of the future directions in the lab is the comparative connectomics of marine invertebrate larvae, enabled by the extraordinary developments in vEM and computational image analysis.

We will collect whole-body volumes across taxa and compare cell types and circuits for mechanosensory, chemosensory and other systems. We will work with collaborators with species-specific expertise and access to rare specimens (ZooCell Marie Curie Network). Analogous neural systems occur across large evolutionary distances in diverse metazoans. For example, dedicated cilio-motor circuits are present in larvae across several phyla. These can now be compared to the reference circuit from *Platynereis* that we have previously fully reconstructed and functionally analysed.

Similarly, we aim to compare visuo-motor systems across aquatic species in an ERC AdG project (2022-2026). We will combine vEM with behavioural assays and biophysical approaches. The goal is to chart the functional diversity of simple eyes and provide a new framework for understanding the evolution of animal vision (Figure 3).
We have learned by reconstructing the entire *Platynereis* larval connectome how such a resource can transform our understanding of an organism and stimulate targeted experimental approaches. The ultimate aim of our comparative approach is to infer patterns and transitions in nervous system evolution and to reveal general principles of circuit organisation. Comparative connectomics is anticipated to transform neuroscience as comparative genomics transformed genetics.

We will continue to develop tools for *Platynereis* systems neuroscience. We will develop whole-brain calcium imaging, targeted genome editing and acquire a developmental connectome series of *Platynereis*. Such a connectomic time-series would allow synapse-level insights into how neural circuits grow and rewire and how it correlates with the changes in

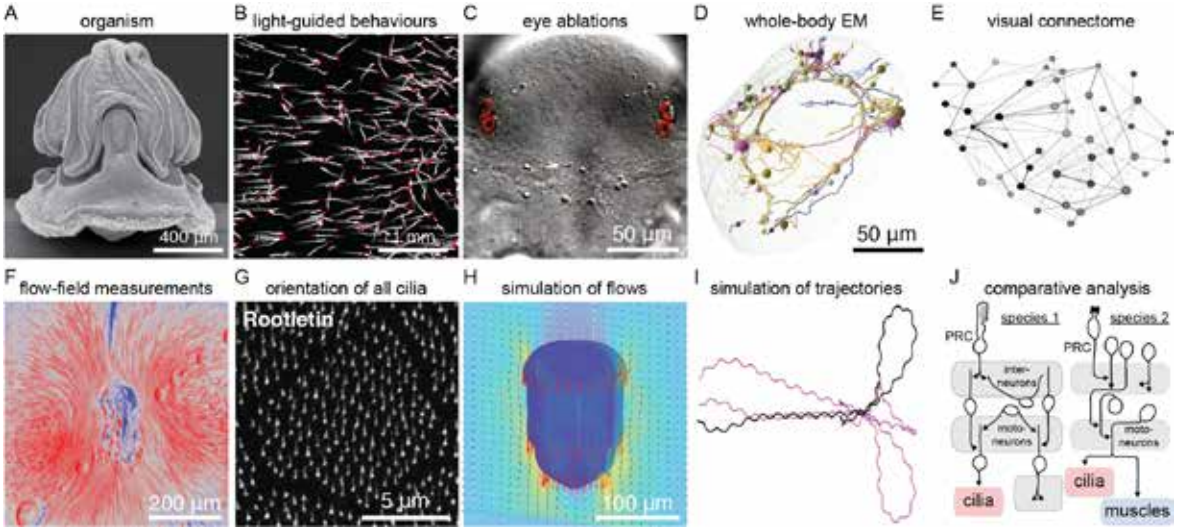

Figure 3:

Visual circuits and photobehaviour in marine larvae. (A) A tornaria larva with eyespots and ciliary bands. (B) Schistosoma miracidia phototaxis. (C) Annelid pigmented eyes. (D) vEM reconstruction of an annelid trochophore larva. (E) A fictitious connectome graph. (F) Cilia-generated flow field around a tethered annelid larva. (G) Super-resolution image of a rootletin immunostaining in Macrostomum lignano. Image: Juliette Azimzadeh. (H) Computer simulation of cilia-generated flows. (I) Phototactic trajectories as modelled in the PLATYSWIM package. (J) Schematic of hypothetical visual circuits.

behaviour. We will also study neuropeptide modulators by analysing already available mutants or by combining bath applications with whole-brain imaging. In parallel, we will work with established collaborators to study the biophysics of *Platynereis* larvae and other ciliated microswimmers. The aim is to integrate genes and neurons with whole-body behaviour.

Selected publications since 2021

Verasztó, C., Jasek, S., Gühmann, M., Bezares-Calderón, L.A., Williams, E.A., Shahidi, R., and **Jékely, G.** (2024). Whole-body connectome of a segmented annelid larva. bioRxiv. 10.1101/2024.03.17.585258. (Elife reviewed preprint)

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Publication statistics

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Fields of Interest
Molecular evolution in cnidarian model organisms (*Hydra* and *Nematostella*), extracellular matrix, Wnt signaling, body axis patterning, organelle morphogenesis, proteomics, imaging.

Summary and outlook
Our research is focused on evolutionary and developmental aspects of Wnt signaling and ECM in cnidarians, a sister group to bilateria (Fig. 1). Our model organisms include *Hydra* and the sea anemone *Nematostella*. In the last years, we identified ECM factors involved in Wnt-regulated pattern formation and orchestrate larva-to-polyp transition. In addition, we have identified novel factors that regulate the development of the cnidarian stinging organelle (cnidocyst). Our future research will be focused on selected ECM factors in the context of regeneration and body axis patterning. In addition, we will study the role of Trp ion channels in cnidocyst function.

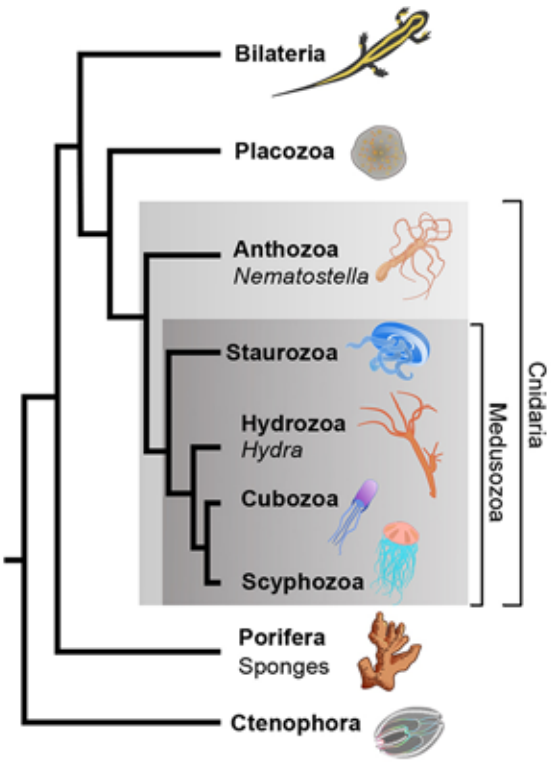

Figure 1:
Cnidaria are early diverging animals that form a sister group to bilaterians. They comprise the anthozoa (sea anemones, corals) and the medusozoa (hydrozoans, jellyfishes).

Research highlights since 2021

We have established the complete matrisome of the *Nematostella vectensis* across it's major life stages by using quantitative mass spectrometry of decellularized mesoglea (ECM). The integration of the matrisome with single cell transcriptome atlases revealed that Nematostella's complex ECM is predominantly produced by gastrodermal cells, confirming the homology of the cnidarian endoderm with bilaterian mesoderm. The transition from larva to polyp is characterized by massive epithelial remodeling signified by an upregulation of metalloproteases and basement membrane components. The enrichment of Wnt/PCP pathway factors during this process further indicates directed cell rearrangements as the main driver of the polyp's axial elongation. Our study identifies conserved matrisomal networks that coordinate transitions in *Nematostella's* life history. These data were established in cooperation with Aissam Ikmi's group from the EMBL and are in preparation for submission

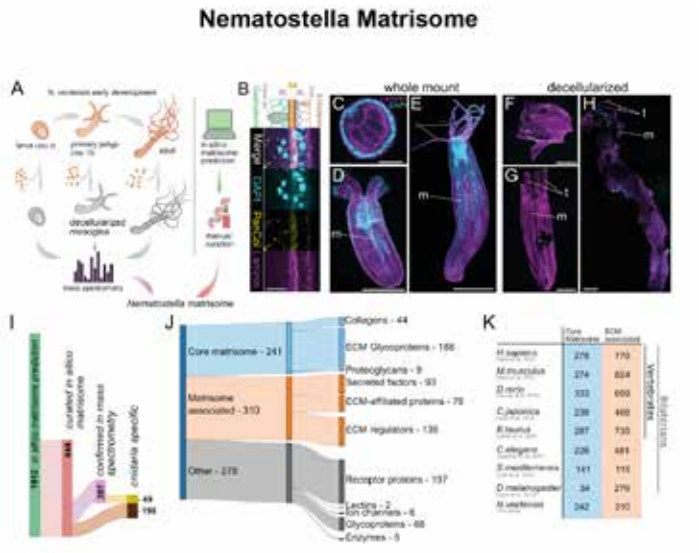

Figure 2:
Analysis of the Nematostella matrisome. (A-H) Mesoglea from larvae, primary polyps and adults was decellularized and analyzed by mass spectrometry and laminin staining. (I-K) In parallel, an in silico matrisome was predicted yielding 844 proteins that can be categorized into core, associated and other matrisome proteins. The complexity of Nematostella's core matrisome equals that of vertebrates. From: Bergheim et al., in preparation.

We identified a myosin II homolog in *Hydra,* which is involved in the complex morphogenesis of the cnidarian stinging organelle and have shown that it is essential for the formation of the tubule expelled during cnidocyst discharge. Myosin II forms a contractile collar initiating vesicle membrane protrusion and stabilizing tubule growth. We have additionally shown that a lectin (NOWA) is responsible for initiating the tubule invagination process by a molecular lectin-carbohydrate zipper. These findings have been published in Garg et al., 2023.

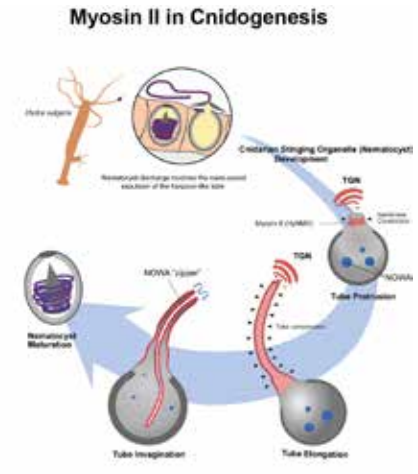

Figure 3:
Role of Myosin II in cnidocyst development.

In cooperation with the groups of Thomas Holstein and Motomu Tanaka, we have performed elasticity mapping of the ECM in *Hydra*, which revealed distinctive patterns along the body axis. A proteomic analysis of the ECM showed that these patterns correlate with the distribution of metalloproteases. Activation of the Wnt/b-catenin pathway alters ECM elasticity, suggesting a mechanism whereby high protease activity under control of Wnt/b-catenin signaling causes remodeling and softening of the ECM. These findings have been published in Veschgini et al., 2023.

Future directions

Within the frame of the WNT CRC (SFB1324), we want to expand our research on the relation between ECM and Wnt-related morphogenesis in cnidarians by performing a large siRNA screen in Hydra targeting ECM factors and examining the impact on head regeneration and axis formation. In particular, we are interested in how the directionality of Wnt activity gradients is influenced by the ECM by using life imaging in Wnt reporter lines. This will also involve the in vivo function of a Wnt-specific protease we have characterized previously as being essential for head organizer restriction (Ziegler et al., 2021).

Our research on cnidocysts will be focused on discharge control by Trp family ion channels that we have identified to be part of the mechanosensory cnidocil complex, which is homologous to vertebrate hair cells. We will decipher the molecular composition of this sensory apparatus in *Hydra* by proteomics, immune-EM and serial section reconstructions of tentacle tissue. Furthermore, we will examine the function of selected novel components of the mechanosensory complex by targeted siRNA KDs and transgenic lines. In this context, we have also started a cooperation to analyze the mechanism by which clownfish inhibit the discharge of cnidocysts in symbiotic sea anemones.

Selected publications since 2021

Garg, N., Stibler, U.K., Eismann, B., Mercker, M., Bergheim, B.G., Linn, A., Tuchscherer, P., Engel, U., Redl, S., Marciniak-Czochra, A., Holstein, T.W., Hess, M.W., and **Özbek, S.** (2023). Non-muscle myosin II drives critical steps of nematocyst morphogenesis. iScience 26(3): 106291.

Maegele, I., Rupp, S., **Özbek, S.**, Guse, A., Hambleton, E.A., and Holstein, T.W. (2023) A predatory gastrula leads to symbiosis-independent settlement in *Aiptasia*. Proc Natl Acad Sci U S A. 2023 Oct 3;120(40):e2311872120. doi: 10.1073/pnas.2311872120. Epub 2023 Sep 25. PMID: 37748072

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

Publication statistics

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Fields of Interest
Evolutionary biology spanning a wide range of dimensions in space and time, bridging disciplines such as taxonomy, systematics, micro- and macroevolution, environmental science, field research and collection-based approaches. We are combining interdisciplinary research with economical science, geo-sciences, and political sciences.

Summary and outlook
Over the past four years, we have successfully transitioned to an integrated approach, combining molecular-evolutionary, systematic, and taxonomic research with high spatio-temporal resolution environmental *in-situ* and *ex-situ* experiments and datasets. Our department's evolutionary and biodiversity research spans biological variation from molecules to landscapes, with a focus on complex organismal biology.
The Brassicaceae family remains one of our key model systems for addressing fundamental questions of plant evolution and adaptation, encompassing millions of years of evolutionary history. Our database portal, *BrassiBase*, serves the scientific community with knowledge and information. We presented a new classification system for the entire Brassicaceae family and a reference phylogeny, marking significant milestones. We will continue to explore the genomic basis of adaptation and speciation.
An exemplary multidisciplinary and eco-evolutionary research project focuses on the Atacama Desert CRC1211 – Earth, Evolution at the Dry Limits (Figure 1). Our work on the epiarenic *Tillandsia* vegetation provided detailed insights into biotic and abiotic interactions at population and landscape scales. This project plays a major role in CRC1211, contributing to its successful transition into the third and final funding period (2024-2028).

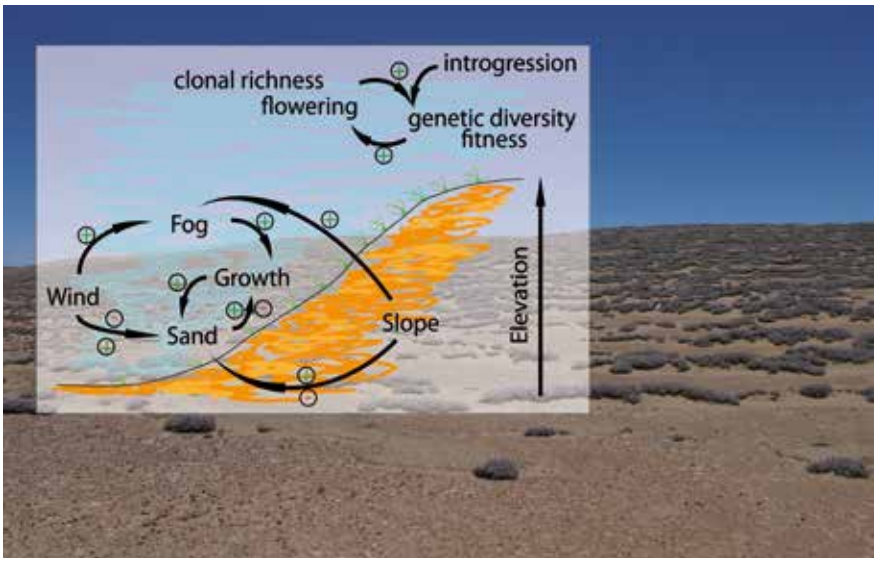

Figure 1:
Tillandsia fog oasis in the Atacama Desert and biotic and abiotic interactions.

One of our most ambitious challenges is the renewal of the Botanic Garden. This effort underscores commitment of the Heidelberg University to preserving organismic-biological collections for long-term research and teaching. It highlights our responsibility to protect biological diversity and acknowledges that current environmental protection efforts are insufficient. Our ultimate mission is to contribute to improving this situation.

Research highlights since 2021

Our research insights into evolutionary principles of the Brassicaceae family have illuminated also the evolutionary success of non-sexually propagating species. For instance, in *Cardamine bulbifera*, the transition to vegetative propagation is hypothesized as a key innovation enabling rapid distribution expansion in postglacial woodland vegetation throughout Europe. This transition, along with ecological differentiation, may have been triggered by introgressive gene flow from its sister species in East European refuge areas (Ru et al. 2022). We are currently advancing this study system to understand the adaptive potential of high polyploid genomes lacking sexual reproduction through comparative and functional genomics.

Another significant study system is the cold-adapted, sexually reproducing genus *Cochlearia*. With accelerating global warming, understanding plant adaptation to environmental change is increasingly urgent. We revealed the complex evolutionary history of *Cochlearia* (Brassicaceae), a Pleistocene relic that originated from a drought-adapted Mediterranean sister genus during the Miocene. During the Pleistocene, *Cochlearia* rapidly diversified and adapted to circum-Arctic regions and other cold habitats, driven by the repeated glaciation cycles (Wolf et al. 2021). This sudden ecological shift involved a complex, reticulate polyploid evolution.

Our Cochlearia research, developed further in collaboration with Levi Yant (Nottingham, ERC collaboration), considers polyploidy – a result of whole-genome duplication (WGD) – as a major evolutionary driver. However, WGDs are highly disruptive mutations, and their fitness consequences are not fully understood. We investigated whether WGDs result in greater genomic structural variant (SV) diversity and how they influence evolutionary dynamics. We found both negative and positive interactions between WGDs and SVs. Masking of recessive mutations due to WGDs leads to the accumulation of deleterious SVs across ploidal levels (from diploids to octoploids), potentially reducing the adaptive potential of polyploid populations. Conversely, ploidy-specific SVs show signals of local adaptation in polyploids more frequently than in diploids (Hämälä et al. 2024).

A reliable phylogenetic-systematic context is essential for most projects. For Brassicaceae, we recently presented a new classification system (German et al. 2023). The underlying genomic data from a large gene-capture experiment were shared with a global initiative involving 279 researchers from 138 organizations. This research contributed to the most comprehensive “tree of life” for flowering plants, which we co-authored (see Figure 2, Zuntini et al. 2024), providing new insights into their origin and relationships. Flowering plants, constituting around 90% of all known land plants, emerged over 140 million years ago, and their dominance over other plants remains a key research question. The new data will aid in identifying new species, refining plant classification, discovering new medicinal compounds, and conserving plants amidst climate change and biodiversity loss.

Several of our projects focus on plant-environment interactions, addressing extinction threats due to environmental change and human impact. Projects within CRC1211 investigate plant robustness and adaptation in the hyperarid Atacama Desert, at the limits of vascular plant life. Epiarenic (sand-growing) *Tillandsia* vegetation in the Chilean-Peruvian Atacama Desert exemplifies extreme adaptation in plant-poor ecosystems. These plants cover thousands of square kilometers and represent a major carbon sink in the hyperarid core. The most abundant diploid species are *T. landbeckii* in Chile and *T. purpurea* in Peru. Spatio-temporal distribution overlaps have led to potentially adaptive gene flow between species, forming present-day gene pools. Species distribution modeling suggests

that since the Last Glacial Maximum (LGM), both species have shifted their ranges, forming suture zones from Peru to northern Chile. We demonstrated inter-species gene flow crossing ploidy levels, highlighting a strategy for rapid adaptation to environmental changes and providing *Tillandsia* populations with an efficient mechanism for conserving new genotypes through clonal propagation.

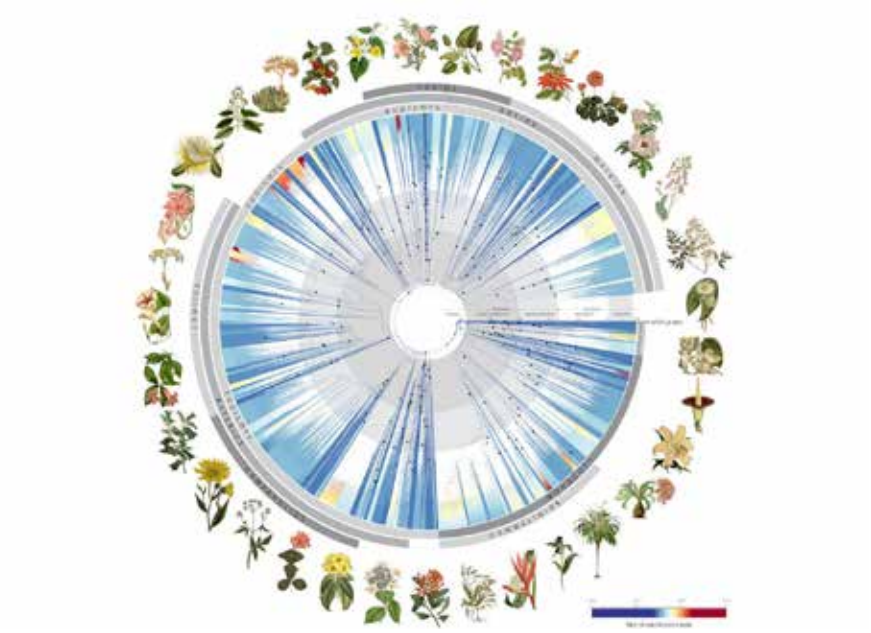

Figure 2:
Tree of life of the angiosperms, A.R. Zuntini, T. Carruthers et al.: Phylogenomics and the rise of the angiosperms, Nature 629: 843-850 (published online 24 April 2024).

Future directions

Our future research will continue to advance along three main directions. First, we are committed to comparative evolutionary genomics in the Arabideae and Cochlearieae tribes. This research will enhance our understanding of parallel and convergent evolution. Adaptation to new environments over evolutionary timescales is crucial for understanding how life persists amid changing conditions, a topic of immediate relevance today. The study of adaptation is often hindered by a lack of replication, making it difficult to generalize findings. Convergent evolution, however, provides nature's own replicate experiments, allowing us to overcome this limitation. We are developing tools and concepts to study the fate of orthologous and paralogous gene copies in diploids and polyploids, and to explore their potential in reconstructing evolutionary histories.

On a landscape scale, we investigate the evolutionary dynamics of adaptation. Water, essential for all life, is a defining feature of habitable Earth. The evolution of life in extremely water-limited environments, which cover significant portions of the Earth, remains poorly understood. The Atacama Desert (CRC1211) offers a unique natural laboratory for this research. We collaborate with interdisciplinary groups to study unique ecosystems such as fog oases and the bromeliad family. As CRC1211 enters its third phase, we aim to develop a mechanistic and computational model of the ecology and population dynamics of *Tillandsia landbeckii*. Additionally, we will continue and expand projects on large-scale evolutionary differentiation and adaptation in Europe, such as the study of the orchid genus *Platanthera*, which integrates evolutionary genomics with field pollinator observations and scent analyses.

Our third research pillar is exemplified by AgroBioDiv (Figure 3), an interdisciplinary, participatory project that blends biology and political science to foster biodiversity within larger landscapes. The project aimed to develop integrated strategies and political instruments for increasing biodiversity in Baden-Württemberg through organic agriculture for long-term sustainability. Although the project concluded in 2024, we continue to build on its successful collaborations and expand our network. We have secured substantial funding to

Figure 3:

The future of agricultural systems – ecological transition within a sustainable landscape? M.A. Koch et al., Reducing pesticides without organic certification? Potentials and limits of an intermediate form of agricultural production. Cogent Food & Agriculture 9: 2202892.

establish a consortium to study the political process related to the negotiation of the Nature Restoration Law in the EU Council, assess public opinion on food security and nature protection, review EU-level measures to increase wild flora biodiversity, and conceptualize how nature restoration can impact individual nutrition. This consortium will strengthen interdisciplinary research on wild plant biodiversity and the transformation of the agri-food system in the EU.

Selected publications since 2021

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Publication statistics

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Fields of Interest
Role of gene regulatory networks in animal development focusing on the mechanisms of Hox protein action in muscle and nervous systems, the establishment, maintenance and plasticity of neural networks, the control of stem cell homeostasis in the male germline system

Summary and outlook
Our research has significantly advanced the understanding of how specific transcription factors regulate development, neuronal wiring, and cellular differentiation. We have investigated the distinct roles of Hox proteins, the genetic codes governing muscle and motoneuron connections, and the interactions between transcription factors and chromatin. We have identified the importance of cell specific combinations of homeodomain transcription factors in controlling precise programs translated by cells into highly context dependent outcomes. Moving forward, we now employ multiomics approaches to unravel the complex regulatory networks and interactions that drive precise developmental processes. By integrating single cell transcriptomics and epigenomics combined with proteomics, we aim to achieve a comprehensive understanding of transcription factor specificity and chromatin dynamics. This integrated approach will provide deeper insights into the fundamental mechanisms underlying development and differentiation, enhancing our knowledge of the intricate regulatory systems that govern cellular processes. Through these efforts, we seek to uncover new paradigms in gene regulation and cellular differentiation, advancing the field of developmental biology.

Research highlights since 2021
Over recent years, our research has delved into various aspects of developmental biology, focusing on the intricate mechanisms underlying morphogenesis, neuronal wiring, cell differentiation, and metabolic responses to mitochondrial dysfunction. This body of work spans from the molecular intricacies of Hox protein binding specificities to the systemic impacts of mitochondrial function on cell proliferation.
Hox proteins, despite their similar binding specificities *in vitro*, govern distinct morphologies *in vivo*. This apparent paradox is illuminated by our findings on Hox low-affinity binding sites. Notably, anterior Hox proteins exhibit greater promiscuity than their posterior counterparts, posing the question of how they achieve specificity. Our study of the AP2x enhancer, which is activated by the Hox transcription factor Deformed (Dfd) in the maxillary head segment, reveals that although it lacks canonical Dfd-Exd sites, it contains several predicted low-affinity sites. These sites are robustly bound by Dfd-Exd complexes, and their conversion into optimal binding sites only modestly increases binding strength. These minor variations in affinity alter the enhancer‘s sensitivity to different Dfd levels, consequently affecting AP-2 expression and maxillary morphogenesis. Our findings suggest that Hox-regulated morphogenesis results from the co-evolution of Hox binding affinity and dosage, ensuring precise target gene regulation.

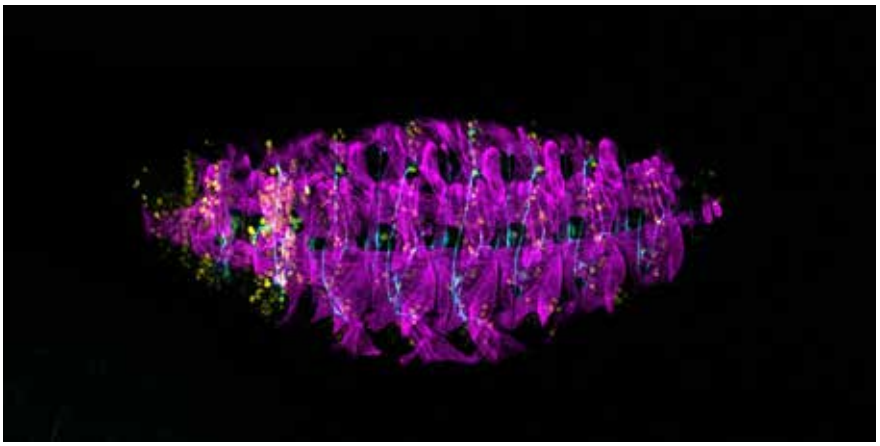

Figure 1:
Drosophila embryo with muscles shown in pink, axonal projections in light blue and the Hox protein Antennapedia in yellow.

In the realm of neuronal circuit formation, our investigation into Drosophila embryonic motoneurons has shed light on the genetic determinants of synaptic specificity. Using single-cell genomics, imaging, and genetic analyses, we have demonstrated that a cell-specific combination of homeodomain transcription factors and downstream immunoglobulin domain proteins is crucial for establishing specific connections between motoneurons and target muscles. Our genetic evidence indicates that five homeodomain transcription factors and four immunoglobulins play significant roles in neuromuscular wiring. Disrupting these transcription factors leads to synaptic wiring defects, partly replicated by modulating their immunoglobulin targets. This suggests that the expression of homeodomain transcription factors and immunoglobulins is directly linked and critical for the proper formation of neuronal circuits.

Addressing the regulation of early lineage-specific master regulators, we have explored the mechanisms by which these factors are silenced to permit differentiation. Our work in the Drosophila mesoderm has identified the Hox transcription factor Ultrabithorax (Ubx) as pivotal for repressing the master regulator Twist. Through CRISPR-Cas9-mediated loss-of-function and overexpression studies, we have shown that Ubx significantly influences *twist* transcription. Mechanistic analyses reveal that Ubx binds to the twist promoter in conjunction with the NK-homeodomain protein Tinman, recruiting the Polycomb DNA binding protein Pleiohomeotic for silencing. This interaction is essential for coordinated muscle differentiation, highlighting Ubx‘s critical role in Twist repression.
In a different line of research tackling the question how the metabolism affects development, we have investigated the cellular responses to mitochondrial dysfunction, particularly how defects in the electron transport chain (ETC) modulate signaling pathways. Our research demonstrates that Notch signaling, combined with ETC attenuation via COX7a knockdown, induces substantial over-proliferation. This tumor-like growth is driven by a transcriptional response mediated by the eIF2α-kinase PERK and ATF4, which activates genes involved in metabolism, nutrient transport, and mitochondrial chaperoning. Interestingly, this stress adaptation enhances progenitor cell fitness, making them more susceptible to Notch-induced proliferation. The over-proliferation is not due to direct transcriptional cooperation between Notch and ATF4 but is partly mediated by pH changes from Warburg metabolism induced by ETC attenuation. Our findings suggest that the PERK-ATF4 pathway monitors ETC function and can be exploited by growth-promoting signals, leading to oncogenic activity.

In sum these studies provide significant insights into the molecular and genetic mechanisms driving development and differentiation. They highlight the nuanced interplay between transcription factors, enhancer binding affinities, and cellular metabolic states in shaping developmental outcomes. Through this multifaceted approach, we aim to unravel the complexities of developmental biology, offering new perspectives and potential therapeutic targets for developmental disorders and diseases linked to these fundamental processes.

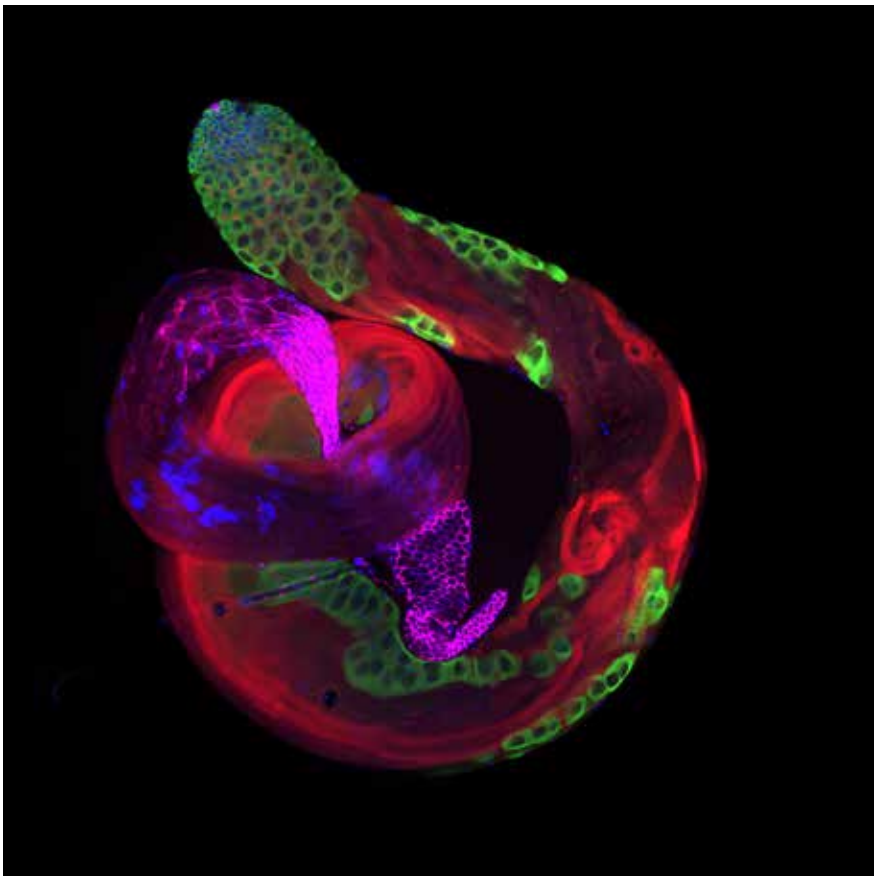

Figure 2:
Drosophila adult testis with germ cells shown in green, somatic cells and spermatids in red, hub cells and terminal epithelial cells in pink.

Future directions

Our future research will build on the recent discoveries and further explore the mechanisms of transcription factor specificity and interaction. We have shown that the anterior Hox protein Deformed (Dfd) activates its target gene AP-2 exclusively via a Dfd-specific enhancer, distinguishing it from the closely related Hox protein Sex combs reduced (Scr). This specificity raises important questions about how such precision is encoded in these and other Hox proteins. To address this, we will generate Dfd-Scr chimeras at their endogenous genomic loci and assess their DNA-binding preferences and protein-protein interaction capabilities on a genome-wide scale. Using the regulatory interaction between Dfd and AP-2 as a case study, this research will greatly enhance our understanding of the specificity that closely related Hox proteins exhibit *in vivo*. It will also shed light on how analogous groups of transcription factors precisely regulate target genes in the cellular context of the organism.

In another line of investigation, we discovered that specific combinations of homeodomain transcription factors in individual muscles control their precise connections with motoneurons. However, the mechanisms by which these TFs control downstream effector molecules to achieve specific muscle properties remain unclear. To address this, we will use advanced genomic and genetic techniques with single-cell precision in the Drosophila embryonic muscle and nervous system. Our results will elucidate for example how cell-specific „homeocodes“ govern muscle subroutines, including synaptic specificity, and clarify the cellular and nuclear heterogeneity within Drosophila multinucleated muscle. This study complements our efforts in the motoneuronal system, enabling comparative and integrative analysis of regulatory processes in interconnected tissues.
Building on our findings that the Hox transcription factor Ultrabithorax (Ubx) interacts with components of the chromatin remodeling Brahma complex in a lineage-restricted manner,

and recognizing the dual role of Hox TFs in binding to open and closed chromatin, we will explore the hypothesis that Hox TFs act as pioneer factors for chromatin. We hypothesize that Hox chromatin pioneering depends on binding to low-affinity DNA motifs on nucleosomes, fine-tuning generic lineage programs to region-specific requirements through interactions with lineage-restricted pioneer TFs. This research could reveal new dimensions of chromatin dynamics and the role of Hox proteins in regulating complex developmental processes.
Collectively, these studies aim to deepen our understanding of transcription factor specificity, chromatin interactions, and the regulatory mechanisms driving precise developmental outcomes. Through this comprehensive approach, we hope to uncover new paradigms in gene regulation and cellular differentiation.

Selected publications since 2021

Pinto, P.B., Domsch, K., Gao, X., Wölk, M., Carnesecchi, J., **Lohmann, I.** (2022). Specificity of the Hox member Deformed is determined by transcription factor levels and binding site affinities. Nat Commun. 13(1):5037. doi: 10.1038/s41467-022-32408-8.

Velten, J., Gao, X., Van Nierop, Y., Sanchez, P., domsch, K., Agarwal, R., Bognar, L., Paulsen, M., Velten, L., **Lohmann, I.** (2022). Single-cell RNA sequencing of motoneurons identifies regulators of synaptic wiring in Drosophila embryos. Mol Syst Biol. 18(3):e10255. doi: 10.15252/msb.202110255.

Carnesecchi, J., Boumpas, P., van Nierop, Y. Sanchez, P., domsch, K., Pinto, H.D., Borges Pinto, P., **Lohmann, I.** (2022). The Hox transcription factor Ultrabithorax binds RNA and regulates co-transcriptional splicing through an interplay with RNA polymerase II. Nucleic Acids Res. 50(2):763-783. doi: 10.1093/nar/gkab1250.

Domsch, K., Schröder, J., Janeschik, M., Schaub, C., **Lohmann, I.** (2021). The Hox Transcription Factor Ubx Ensures Somatic Myogenesis by Suppressing the Mesodermal Master Regulator Twist. Cell Rep. 34(1):108577. doi: 10.1016/j.celrep.2020.

Sorge, S., Theelke, J., Yildirim, K., Hertenstein, H., McMullen, E., Müller, S., Altbürger, C., Schirmeier, S., **Lohmann, I.** (2020). ATF4-Induced Warburg Metabolism Drives Over-Proliferation in Drosophila. Cell Rep. 31(7):107659. doi: 10.1016/j.celrep.2020.107659.

Publication statistics

https://scholar.google.com/citations?hl=en&user=kObcMSoAAAAJ

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Fields of Interest
Mesoderm and muscle development,
muscle dedifferentiation and trans-differentiation,
Hox regulatory network,
Drosophila

Summary and outlook
Muscles develop twice during the *Drosophila* life cycle in part to adapt to the requirements of the organism. As for each stage, Hox genes are essential to guide the tissue in the chosen path, by activating and repressing factors. Since Hox factors will promote differentiation, one understudied question is: How are the Hox factors distributed during metamorphosis, a stage which remodels and redesigns the existing musculature and requires naïve undifferentiated myoblast. We have evidence that the Hox protein Ultrabithorax (Ubx) is removed from naïve myoblasts to allow proliferation. In the same regards, we speculate that Ubx might be removed during muscle dedifferentiation to promote the development of naïve myoblasts. Our current investigations are how Ubx is removed and why not other Hox proteins, like abd-A.

Research Highlights since 2021
As an independent group leader at the University of Erlangen-Nürnberg I have investigated the binding specificity of the Hox factor Ubx in the mesodermal tissue compared to the neuronal. The results established the theory that the evolutionary “old” Hox genes evolved their binding sites to adapt new opportunities within the “newly” emerged mesoderm. Highlighting the possibility that Hox factors can modify their binding sites to the given situation and that low affinity is important for new regulatory networks (Folkendt et al., 2021). After moving to the Lohmann lab in 2021 as a project leader I was involved finishing established projects that on the one hand investigated the regulatory network of Hox genes on a single cell basis in the neuronal tissue (Velten et al., 2022). On the other hand tackled the existing low affinity trade-of-model by showing that high affinity Deformed binding sites having a specific tissue related function (Pinto et al., 2022).

To establish my own ideas, I have taken my expertise in *Drosophila* muscle development and the Ubx regulatory network to investigate the requirements of the Hox input to newly emerging structures like the adult musculature during metamorphosis. Here, I will focus on two aspects: 1) Ubx function in the abdominal adult muscle precursors (naïve and quiescent myoblasts, essential for the establishment of the adult abdominal musculature) and 2) Ubx function during muscle trans- & dedifferentiation in comparison to abd-A (posterior Hox gene). In the duration of a Bachelor thesis in 2023, the investigations of Ubx function in adult muscle precursors indicated that Ubx removal is essential for the proliferation and the naïve character of these cells to establish a pool of myoblast for adult myogenesis, since the maintenance of Ubx leads to thin and unorganised abdominal muscles (Figure 1).

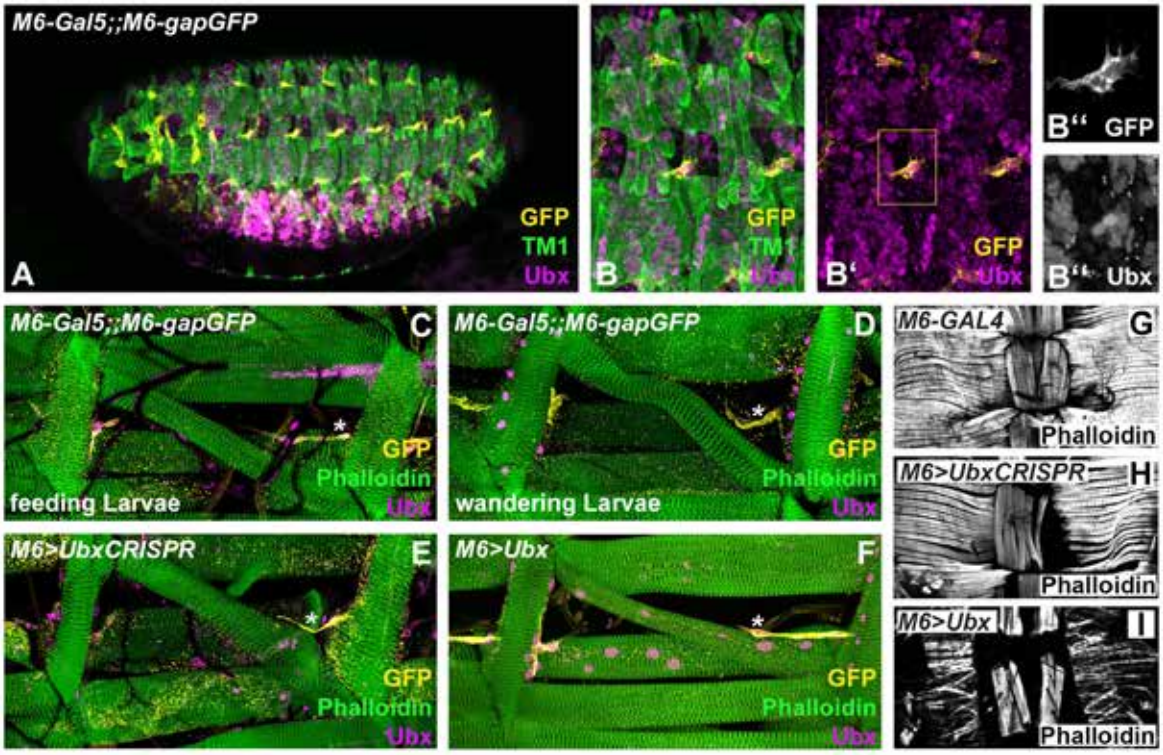

Figure 1:

Figure: A-B co-staining adults muscle precursors (GFP) with Ubx (magenta) and Tropomyosin (muscle structure protein, TM1, green). C-F investigations of Ubx expression during different larval stages and Ubx perturbations. adults muscle precursors are labelled with star. G-I Abdominal adult muscle at different Ubx perturbations experiments.

Future directions

In the future I would like to further investigate the Ubx function during metamorphosis in the two described aspects: 1) in adult muscle precursors and 2) during trans- and dedifferentiation. Muscle fragmentation and dedifferentiation is happening in the larval alary muscle, which connect the larval heart to other tissues. These muscles will fragment into single myoblast and dedifferentiate into the ventral longitudinal heart-associated muscles. Only the first three pairs of alary muscles, which are expressing Ubx during embryonic stages and after metamorphosis, will dedifferentiate. The other four pairs that express abd-A will only remodel to adult alary muscles. Having that established framework, I would like to investigate if and how Ubx is removed from the alary muscles. What is the difference between Ubx and abd-A in respect to protein structure, binding ability and regulatory network? Finally, I will tackle the well accepted assumption that Hox genes are expressed throughout the live time of an animal.

As a member of the Ingrid Lohmann group, I will further support the group by supervising project (Bachelor and Master thesis) related to the investigations of Prof Dr. Ingrid Lohmann and share my *Drosophila* muscle related knowledge.

Selected publications since 2021

Pinto, P.B., **Domsch, K.,** Lohmann, I. (2024) Hox function and specificity - A tissue centric view. Semin Cell Dev Biol. 152-153:35-43. doi: 10.1016/j.semcdb.2022.11.011.

Pinto, P.B., **Domsch, K.,** Gao, X., Wölk, M., Carnesecchi, J., Lohmann, I. (2022). Specificity of the Hox member Deformed is determined by transcription factor levels and binding site affinities. Nat Commun. 13(1):5037. doi: 10.1038/s41467-022-32408-8.

Rose, M., **Domsch, K.,** Bartle-Schultheis, J., Reim, I., Schaub, C. (2022). Twist regulates Yorkie activity to guide lineage reprogramming of syncytial alary muscles. Cell Rep. 38(4):110295. doi: 10.1016/j.celrep.2022.110295.

Folkendt, L., Lohmann, I., **Domsch, K.** (2021). An Evolutionary Perspective on Hox Binding Site Preferences in Two Different Tissues. J Dev Biol. 9(4):57. doi: 10.3390/jdb9040057.

Domsch, K., Schröder, J., Janeschik, M., Schaub, C., Lohmann, I. (2021). The Hox Transcription Factor Ubx Ensures Somatic Myogenesis by Suppressing the Mesodermal Master Regulator Twist. Cell Rep. 34(1):108577. doi: 10.1016/j.celrep.2020.108577.

Publication statistics

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2.14 PROF. DR. JAN LOHMANN
STEM CELL BIOLOGY

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Fields of Interest
I am interested in various aspects of plant stem cell regulation and its plasticity, as well as how context dependent gene function drives development.

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 cells are embedded in specialized structures called meristems, which provide an 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 network is modulated by the environment. In recent years, my lab has focused on elucidating the mechanisms underlying WUS specificity in diverse developmental contexts, its broad DNA and RNA binding capacity, as well as its cell-to-cell mobility. Another important line of research focused on the role of the environment for stem cell activity using wounding and nutrient signaling as relevant models. Studying how stem cell activity and morphogenesis are connected, we have discovered a novel pathway linking ribosomal stress to cell size and morphogenetic activity. Finally, within an ERC synergy project, we have developed and applied tools to dissect context dependent genetic networks underlying tissue development.

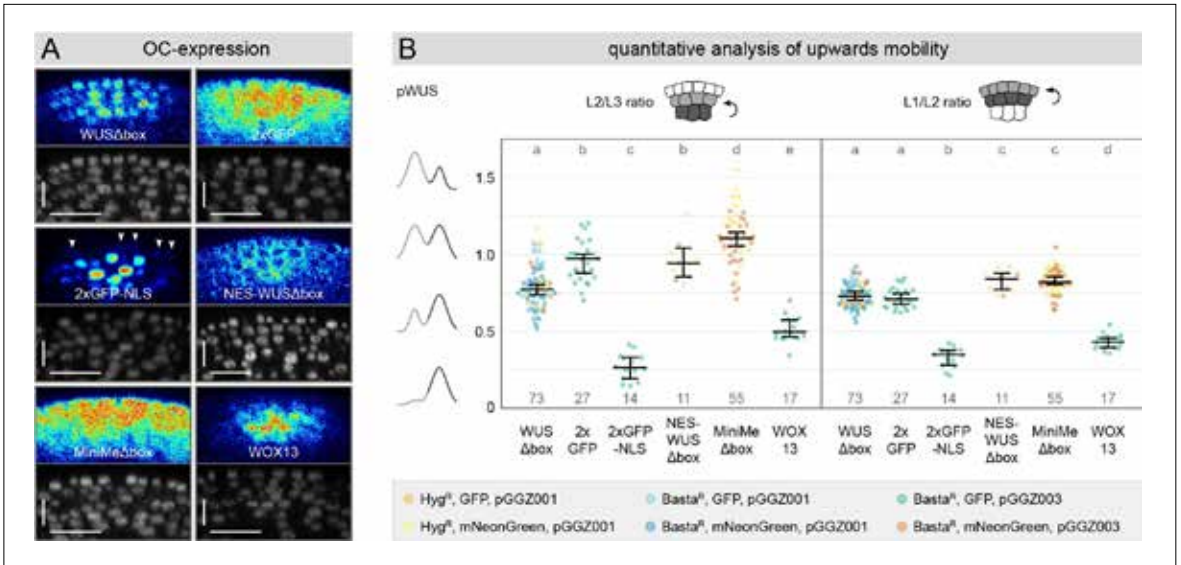

Figure 1:
Left: Imaging results showing mobility of diverse WUS alleles and controls driven from the WUS promoter. Right: Layer ratios of fluorescence derived from live imaging data by computational quantification.

Research highlights since 2021

Non-cell autonomous induction of stem cell fate is a shared feature across multicellular organisms, however the underlying mechanisms diverge substantially between the kingdoms of life. In plants, cell to cell mobility of transcription factors has emerged as a key paradigm and for the shoot apical meristem (SAM) the translocation of the WUS homeodomain transcription factor from niche cells to stem cells is essential. In the past years we systematically investigated the function of diverse WUS alleles and applied multispectral live cell imaging coupled to computational analysis and mechanistic mathematical modelling to show that WUS protein mobility is the result of balance between active transport and retention in niche cells and likely independent of the stem cell signal CLAVATA3 (CLV3). Importantly, we found that diffusion across cell layers of the meristem is not symmetrical, suggesting that there is unexpected complexity in cellular connections (Fuchs et al. BioRxiv 2024).

One additional and particularly important aspect are the signals between transient amplifying cells in the periphery and the stem cells in the center of the SAM. Since it has recently emerged that small gaseous compounds may play diverse roles in this process, we decided to analyze potential functions of Nitric Oxide (NO). Despite the importance of NO as signaling molecule in both plant and animal development, the regulatory mechanisms downstream of NO were still poorly understood. In the past years, we were able to show that NO is involved in Arabidopsis shoot stem cell control via modifying expression and activity of ARGONAUTE 4 (AGO4), a core component of the RNA-directed DNA Methylation (RdDM) pathway. We found that NO biosynthesis enzymes are specifically expressed in the periphery of the meristem and that interfering with their activity led to reduced meristems. Transcriptome analysis of meristems with enhanced and reduced NO levels identified AGO4 as a potential downstream target of NO signaling. Consistent with an important function we found that mutations in AGO4 and related AGO genes, as well as components of the RdDM pathway, which acts downstream of AGO4, cause meristematic defects, and reduce responses of the stem cell system to NO signaling. Importantly, we found that the stem cell inducing WUSCHEL transcription factor directly interacts with AGO4 in a NO dependent manner, explaining how these two signaling systems may converge to modify DNA methylation patterns. Our results revealed that NO signaling plays an important role in controlling plant stem cell homeostasis via the regulation of de novo DNA methylation. (Zeng et al. Nature Communications 2023).

Another important avenue of investigation is the interaction of the apical stem cell system with the environment, since plants continuously need to adjust their developmental program including organ initiation and growth. While many of the key factors involved in homeostasis of the SAM have been extensively studied under artificial constant growth conditions, only little is known how variations in the environment affect the underlying regulatory network. To shed light on the responses of the SAM to ambient temperature, my lab has combined 3D live imaging of fluorescent reporter lines that allowed us to monitor the activity of two key regulators of stem cell homeostasis in the SAM namely *CLV3* and *WUS*, with computational image analysis to derive morphological and cellular parameters of the SAM. Whereas *CLV3* expression marks the stem cell population, *WUS* promoter activity is confined to the organizing center (OC), the niche cells adjacent to the stem cells, hence allowing us to record on the two central cell populations of the SAM. Applying an integrated computational analysis of our data we found that variations in ambient temperature not only led to specific changes in spatial expression patterns of key regulators of SAM homeostasis, but also correlated with modifications in overall cellular organization and shoot meristem morphology (Wenzl et al. Cells and Development 2023).

This developmental plasticity requires that a diversity of signaling pathways acts in concert to modulate stem cell activity. My lab has shown that the TOR kinase network integrates metabolic- and light signals and controls expression of WUS, however, the mechanism linking TOR activity with the WUS promoter remained unresolved. In the past years we found that TOR regulates the accumulation of *trans*-zeatin, the cytokinin species mainly

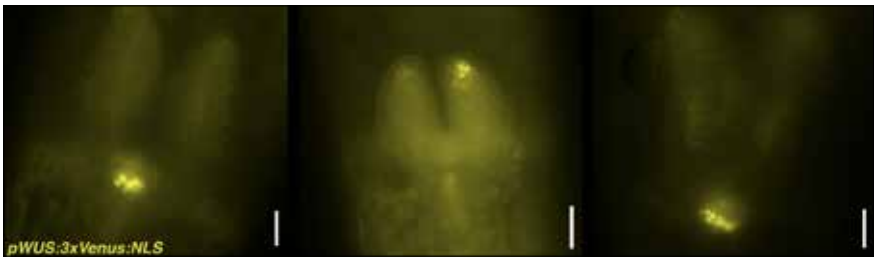

Figure 2:

Activity of the WUS promoter in young seedlings. Left: untreated control; Middle: seedling after 8 hours of treatment with TOR kinase inhibitor; Right: seedling after 8 hours of treatment with TOR kinase inhibitor and cytokinin.

responsible for shoot development. Importantly, we identified translational repression of RNAs encoding cytokinin degrading CYTOKININ OXIDASES/DEHYDROGENASE enzymes by TOR as an underlying mechanism (Janocha et al. BioRxiv 2024).

Future directions

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

1. Mechanisms of stem cell induction and maintenance by WUS

We have recently realized that WUS physically interacts with a large number of diverse transcription factors. In line with this, we have initiated an project to identify WUS interactors *in vivo* with cellular resolution. To this end, we have designed two systems based on split BioID, which can be activated by a chemical inducer. Deploying these in niche or stem cells will allow us to record the WUS interactome in these specific populations.

In addition, we have found that WUS is able to bind to RNA and we have been able to record the first full transcriptome WUS binding set. Building on this and published results that suggest a potential mechanism for homeodomain-RNA interactions, we are aiming to identify the RNAs bound by WUS in the stem cell system, their functional role, as well as the mechanisms underlying this activity.

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

We are currently following three projects in this field: The first centers on DOF transcription factors, which have been shown to be essential cell type specification switches. We have found that the expression of a number of these factors is controlled by auxin and WUS and that many of them interact with WUS. We are creating a library of higher order DOF mutants by CRISPR-Cas9 to identify the relevant players from this gene family. In the second project, we found that the wounding hormone Jasmonic Acid controls stem elongation, but that WUS protects the stem cells from its influence. We will now investigate the mechanisms underlying this activity with a focus on genes of the TIFY family of JA signaling inhibitors. In the third project, we focus on the role of WUS in controlling meristem identity and the transition from vegetative growth to flowering.

3. Decoding context dependent genetic networks in vivo

We have now created transgenic lines to perturb more than 100 genes and have created the largest single cell data set describing the immediate effects of the loss of TOR function. We will now extend our analyses to our Cas-9 library to be able to create a deep atlas of context dependent genetic functions.

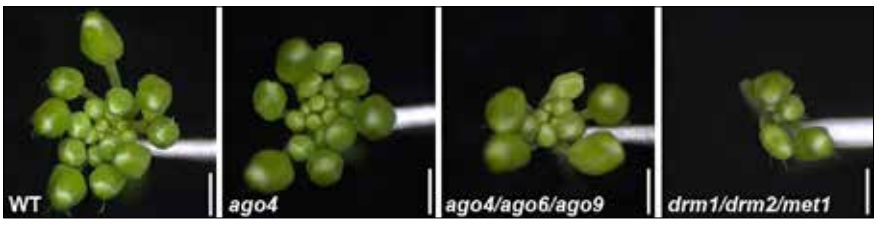

Figure 3:

Inflorescence phenotypes of genes involved in the RNA dependent DNA Methylation pathway.

Selected publications since 2021

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Stitz, M., Kuster, D., Reinert, M., Schepetilnikov, M., Berthet, B., Reyes-Hernández, J., Janocha, D., Artins, A., Boix, M., Henriques, R., Pfeiffer, A., **Lohmann, J. U.**, Gaquerel, E., and Maizel, A. (2023). TOR acts as metabolic gatekeeper for auxin-dependent lateral root initiation in *Arabidopsis thaliana*. EMBO Journal, 5;15.

Liu, Q., Liang, Z., Feng, D., Jiang, S., Wang, Y., Du, Z., Li, R., Hu, G., Zhang, P., Ma, Y., **Lohmann, J. U.***, and Gu, X. (2021). Transcriptional landscape of rice roots at the single-cell resolution. Mol Plant. 14, 384-394. *co-corresponding author

Publication statistics

https://scholar.google.com/citations?user=BkbnIxkAAAAJ&hl=en

2.15 PROF. DR. ALEXIS MAIZEL
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Fields of Interest
My lab investigates plant morphogenesis, focusing on lateral root formation in *Arabidopsis*. We study auxin signalling, mechanical forces, cellular dynamics, and gene regulation. We employ advanced imaging techniques to elucidate molecular and cellular mechanisms underlying plant organ development and growth.

Summary and outlook
My research focuses on understanding the fundamental mechanisms of plant development, particularly root development in *Arabidopsis thaliana*. The main emphasis is on lateral root formation, a prime example of post-embryonic organogenesis that enhances plants' ability to forage for nutrients and stabilize their anchoring.
Our work integrates studies of hormone signalling (especially auxin), mechanical forces, cellular dynamics, and gene regulation. Key discoveries include:

1. Quantitative analysis of cell growth and asymmetric divisions during early lateral root formation.
2. The role of microtubule-based perception of mechanical conflicts in lateral root morphogenesis.
3. Integration of tissue mechanics and auxin transport in pericycle and endodermis remodelling during lateral root initiation.
4. TOR kinase's function as a metabolic gatekeeper for auxin-dependent lateral root initiation.

Our current and future research focuses on three main questions:
How do abutting lateral root founder cells coordinately polarize and swell?
How is the direction of organ axes set during post-embryonic growth?
How do cell growth and division contribute to the emergence of cell identities?

Research highlights since 2021
Research in my laboratory consistently focuses on understanding the fundamental mechanisms of plant development, particularly root development in *Arabidopsis*. We approach this from multiple angles, integrating studies of hormone signalling (especially auxin), mechanical forces, cellular dynamics, and gene regulation. Our work is increasingly going toward understanding the interplay between different regulatory systems - for example, how auxin signalling interfaces with mechanical forces or how metabolic regulation via TOR impacts developmental processes. This holistic approach yields insights into the complex, multi-layered control of plant development. We are also committed to developing and applying advanced techniques, particularly in imaging and genetic engineering. This technical innovation supports and enables our biological discoveries.
The main research highlights since 2021 are detailed below.

1. Quantitative Analysis of Lateral Root Initiation and Development:
My lab has made significant contributions to understanding the mechanisms of lateral root formation in *Arabidopsis thaliana*. In 2021, building on tools developed in 2020 in the

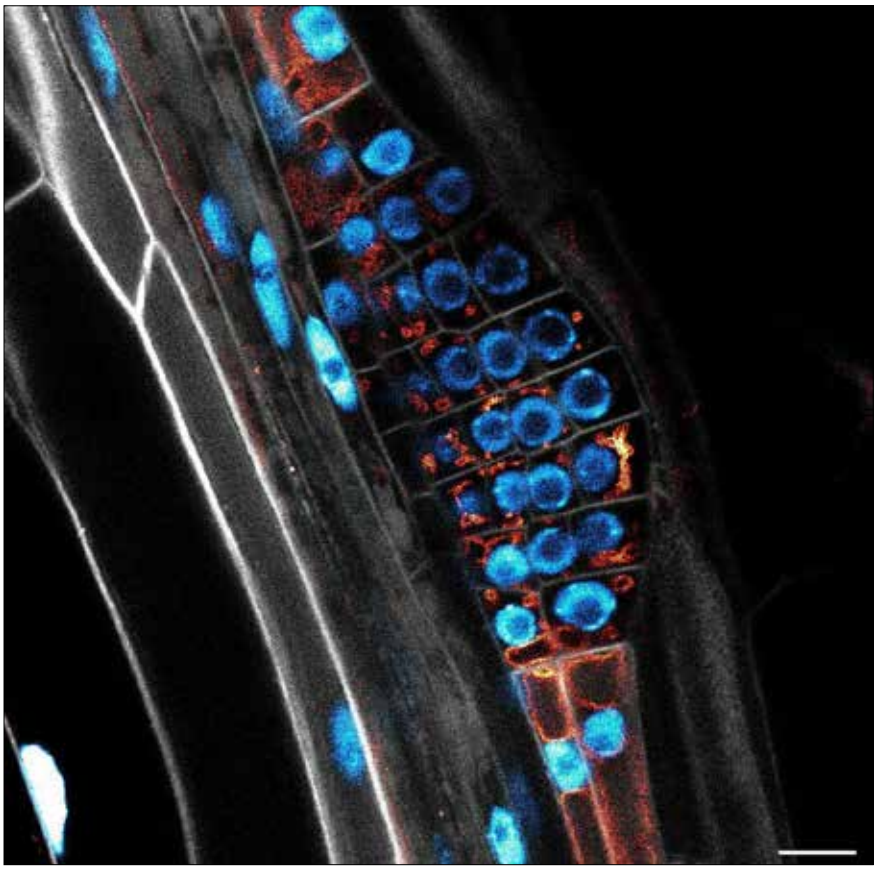

Figure 1:
Confocal section of an Arabidopsis lateral root primordium at Stage IV expressing four fluorescent markers expressed from the same transgene using GreenGate v2.0. The markers reveal the cell contour of all cells (plasma membrane, grey), the cell nuclei in the lateral root primordium (in blue) as well as a vacuolar membrane marker in the xylem pole pericycle cells and the lateral root primordium (in orange). Scale bar is 10µm. Image by M. Piepers

context of the DFG FOR2581, we quantitatively analysed the pattern of cell growth and asymmetric cell divisions in the early stages of lateral root formation. This work showed that cells integrate growth and division to precisely partition their volume upon division during the first two stages of lateral root formation (Figure 1).

Associated publication: Schütz et al. 2021

2. Integration of Hormonal Signalling, Cytoskeleton and Mechanical Forces:
A major theme in our recent work has been the role of mechanical forces in plant organ development. In 2022, we published a study demonstrating that microtubule-based perception of mechanical conflicts controls lateral root morphogenesis. In this work, we showed that microtubule arrays facing lateral root founder cells display a higher order than arrays on the opposite side of the same cell, and this asymmetry is required for endodermal remodelling and lateral root initiation. We identified that MICROTUBULE ASSOCIATED PROTEIN 70-5 (MAP70-5) is necessary to establish this spatially defined microtubule organization and endodermis remodelling and thus contributes to lateral root morphogenesis. We propose that MAP70-5 and cortical microtubule arrays in the endodermis integrate the mechanical signals generated by lateral root outgrowth, facilitating the channelling of organogenesis.
Our work is also increasingly taking a biophysical turn. We recently published a study in Biophysical Journal on auxin-mediated stress relaxation in pericycle and endoderm remodelling during lateral root initiation. In this work, we propose a model that integrates tissue mechanics and auxin transport, revealing a connection between the auxin-induced relaxation of mechanical stress in the pericycle and auxin signalling in the endodermis. We show that the endodermis initially limits the growth of pericycle cells, resulting in a modest initial expansion. However, the associated stress relaxation is sufficient to redirect auxin to the overlying endodermis, which then actively accommodates the growth, allowing for the

subsequent development of the lateral root. Our model uncovers that increased pericycle turgor and decreased endodermal resistance licence expansion of the pericycle and how the topology of the endodermis influences the formation of the new root. These findings highlight the interconnected relationship between mechanics and auxin flow during lateral root initiation, emphasizing the vital role of the endodermis in shaping root development through mechanotransduction and auxin signalling.

Associated publications: Stöckle et al. 2022, Ramos et al. 2024

3. Integration of Development and Metabolism:
Auxin continues to be a central focus of our research. In 2023, we published a study revealing that TOR (Target of Rapamycin) acts as a metabolic gatekeeper for auxin-dependent lateral root initiation. We combined metabolic profiling with cell-specific interference to show that LRs switch to glycolysis and consume carbohydrates. Interfering with TOR kinase blocks lateral root initiation. TOR inhibition marginally affects the auxin-induced transcriptional response of the pericycle but attenuates the translation of ARF19, ARF7, and LBD16. TOR is thus a central gatekeeper for root branching that integrates local auxin-dependent pathways with systemic metabolic signals, modulating the translation of auxin-induced genes.

Associated publication: Stitz et al. 2023

4. New Techniques:
My lab continues to leverage and develop cutting-edge methods. In 2023, we improved the highly popular modular cloning system GreenGate by streamlining the generation of new modules, expanding the repertoire of useable modules, and enabling the possibility of assembling complex transcriptional units in an iterative and efficient manner. This technical advancement will facilitate more sophisticated genetic studies in plants.

Associated publication: Piepers et al. 2023

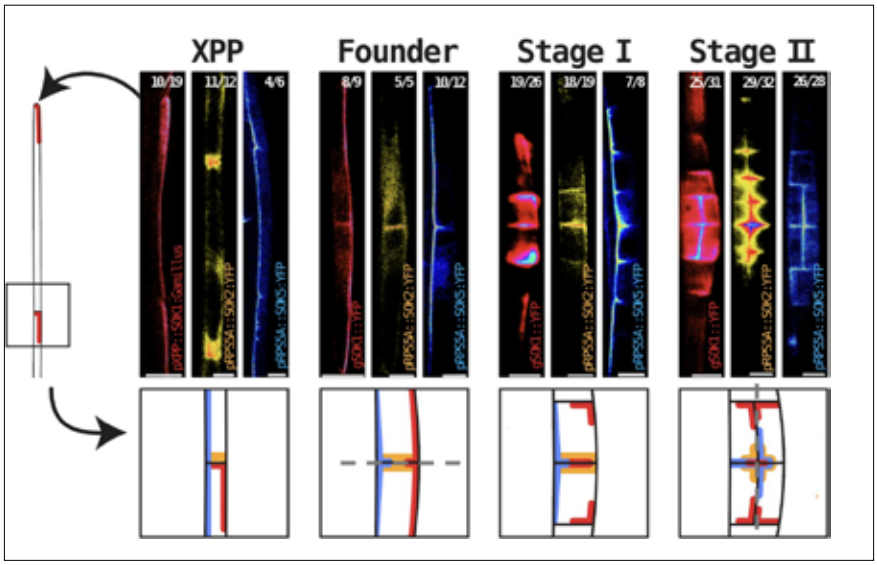

Figure 2:
Localisation of fluorescently-tagged SOK1(red), SOK2 (yellow) and SOK5 (blue) in XPP, founder cells, Stage I and Stage II primordia obtained by confocal microscopy. The diagram below represents the consensus localisation. The grey dashed lines indicate the two axes of symmetry generated at the founder cell stage and Stage II. Numbers indicate the frequency of the observed localisation observed in independent biological replicates. Scale bars 10µm.

Future directions
Our current and future focus is distributed along three questions that, combined, will improve our understanding of how cell polarity, growth, and division articulate to specify distinct identities in the developing lateral root.

1. How do abutting lateral root founder cells coordinately polarize and swell?
The common cell interface between founder cells acts as an organizing center for lateral root morphogenesis. Despite its importance, the molecular composition of this interface re-

mains poorly understood. We are investigating the role of signaling lipids and cytoskeleton regulators in founder cell polarization, using proteomic approaches to identify components localizing to this polarizing interface.
We previously demonstrated that auxin-dependent cell wall remodeling and cytoskeleton reorganization are crucial for founder cell radial swelling. We are now exploring how founder cells are initially triggered to expand radially, focusing on auxin-induced modification of apoplastic pH. We combine tissue-specific, inducible mutant analysis with precise pH monitoring to unravel the mechanism of founder cell radial swelling.

2. How is the direction of organ axes during post-embryonic growth set?
Post-embryonic formation of lateral organs requires respecification of growth and cell division along orthogonal axes. In Arabidopsis, the SOSEKI (SOK) protein family integrates global polarity cues. Our work shows that SOK polarization is set early during lateral root formation, with two symmetry-breaking events defining diverging polarity fields (Figure 2). Growth-driven changes in SOK polarization ultimately align with the new lateral root axes. SOK proteins form polymers in cell corners and interact with the cytoskeleton. Our results show that reduced SOK expression leads to impaired root development, slower cell cycle progression, and altered cell aspect ratios. We currently explore a model where SOSEKIs form mechanosensitive polymers coupling cell geometry to division.

3. How do cell growth and division contribute to the emergence of cell identities?
The transition from a single-layered to a two-layered lateral root primordium is a crucial symmetry-breaking event, requiring a shift in division plane orientation. PLETHORA transcription factors PLT3,5,7 orchestrate this transition (Figure 3). We've identified new PLT3,5,7 targets related to cell division orientation control and are investigating their regulation and mode of action.
Using single-cell RNA-seq, we're studying how the lack of this symmetry-breaking division affects cell developmental trajectories in lateral root primordia. This research will provide insights into the molecular mechanisms of symmetry breaking and its impact on cell identity specification.

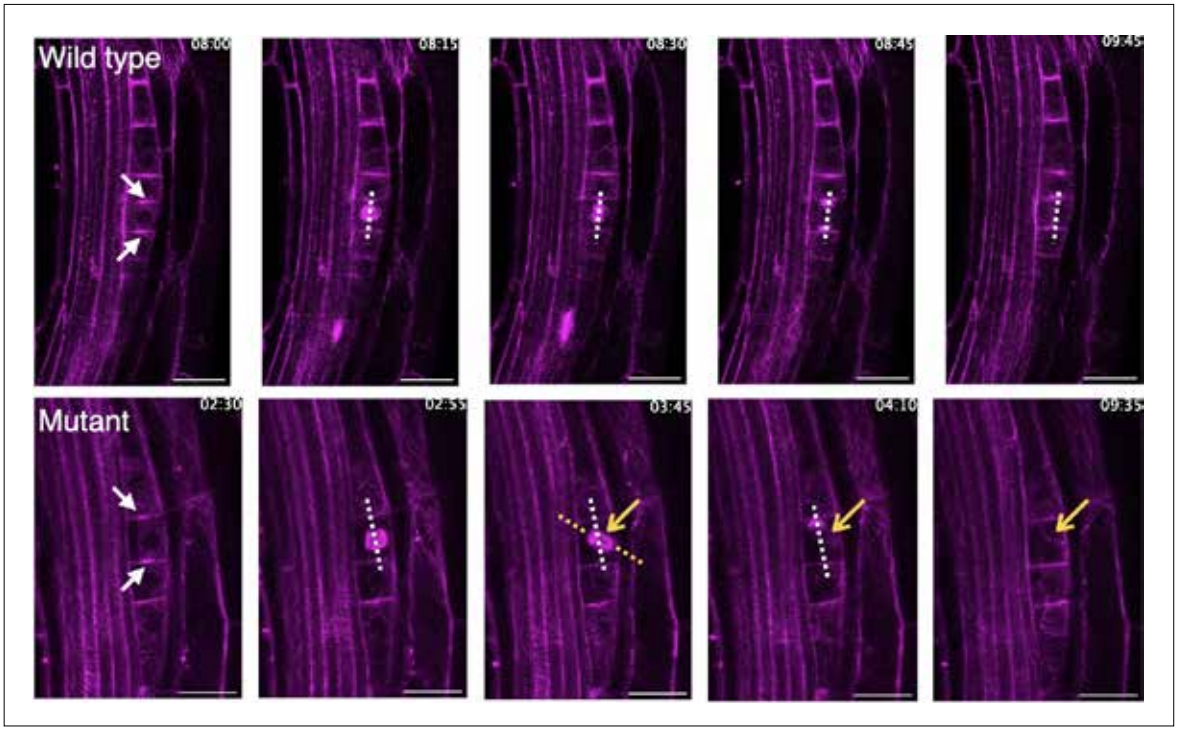

Figure 3:

Dynamics of microtubules in lateral root primordia transitioning from Stage I to II in wild type and the plt357 triple mutant (Mutant) visualised by live confocal microscopy. The white arrows and dashed line indicate the position of the pre-prophase band (PPB), marking the future division plane. In the mutant, the orientation of the cell plate (yellow dashed line) deviates from one of the PPB and leads to an aberrant division plane (yellow arrow). Scale bars 20µm.

Selected publications since 2021

Ramos, J.R.D., Reyes-Hernández, B.J., Alim, K., and **Maizel, A.** (2024). Auxin-mediated stress relaxation in pericycle and endoderm remodelling drive lateral root initiation. Biophys J. 10.1016/j.bpj.2024.06.017.

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Schütz, L.M., Louveaux, M., Vilches Barro, A., Bouziri, S., Cerrone, L., Wolny, A., Kreshuk, A., Hamprecht, F.A., and **Maizel, A.** (2021). Integration of Cell Growth and Asymmetric Division during Lateral Root Initiation in *Arabidopsis thaliana.* Plant Cell Physiol. _62_, 1269--1279. 10.1093/pcp/pcab038.

Publication statistics

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Fields of Interest
Cell cycle regulation, mitotic checkpoints, centrosome biology, ciliogenesis, stem cells, organoids

Summary and outlook
Microtubule organising centres (MTOCs), such as the spindle pole body (SPB) in budding yeast or centrosomes in mammalian cells, play a crucial role in the spatial and temporal arrangement of microtubules, which are essential for mitotic spindle formation and alignment. Additionally, specialised structures within centrosomes, known as centrioles, are directly involved in forming the primary, nonmotile cilium (Figure 1). Cilia modulate signalling pathways critical for embryonic development and tissue homeostasis. Defects causing spindle or cilia dysfunction are associated with tumour development or human diseases collectively referred to as ciliopathies, in which the function of many organs, including brain, eyes, kidney and pancreas among others, are affected. Our research group focuses on understanding primary cilia biogenesis and function and how alterations in microtubule organisation influence cell division.

Our past work helped to uncover novel players of the spindle position checkpoint, a key surveillance mechanism that contributes to error-free chromosome segregation during mitosis. Furthermore, we established novel regulators of centrosome asymmetry, cilia biogenesis and cilia length. Based on these findings, our future work will focus on the mechanisms underlying cell cycle progression and centriole maturation as well as pathways influencing ciliogenesis.

Research highlights since 2021
Control of mitotic progression
The spindle position checkpoint (SPOC) is a key mitotic surveillance mechanism in *Saccharomyces cerevisiae*. It prevents cells from completing mitosis when the mitotic spindle misaligns with respect to the mother-to-daughter cell polarity axis. In SPOC-deficient cells, defects leading to spindle misalignment are not detected. Consequently, these cells proceed through several rounds of mitosis without correctly segregating chromosomes and eventually undergo cell death.
Previously, we have shown that one of the most downstream components of the SPOC, the kinase Kin4, blocks mitotic exit through down-regulation of the mitotic exit network (MEN), a signalling pathway required for the M- to G1-phase transition. Using Kin4 as a bait in high-throughput genetic screens, we have identified more than 20 novel SPOC components. Among these, we found the kinase Mck1, a glycogen-synthase-kinase-3 (GSK-3) homolog in yeast, and many components of the conserved chromatin-remodelling complex, SWR1-C. We established that Mck1 works independently of Kin4 to maintain the SPOC in an active state. This function of Mck1 requires the phosphorylation and degradation of the mitotic cyclin-dependent kinase (M-Cdk) inhibitor Cdc6 (Rathi et al., 2022).

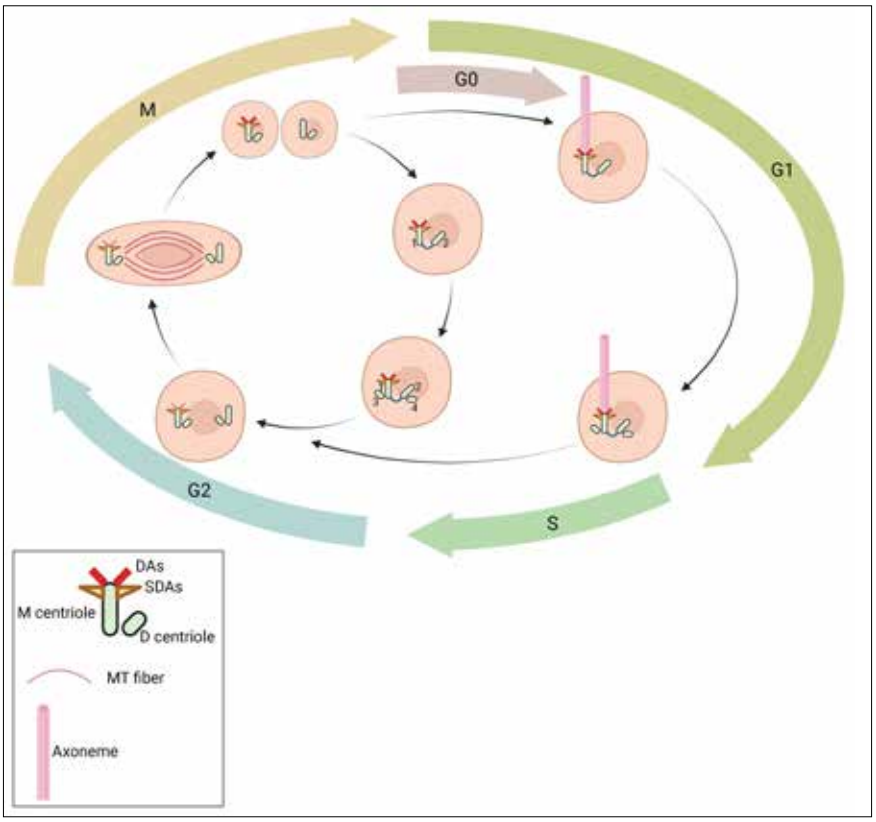

Figure 1:
The centriolar cycle. Ciliogenesis regulation during the cell cycle involves controlled centrosome duplication, with one daughter cell inheriting a mature centrosome and forming a primary cilium in G0/G1. Cilium dynamics are governed by kinases like Aurora A, PLK1, and NEK2. M, mother; D, daughter. DAs, distal appendages; SDAs, subdistal appendages.

Stabilisation or overproduction of Cdc6 in mitosis leads to mitotic exit in cells with misaligned spindles, highlighting the importance of maintaining lower levels of M-Cdk inhibitors for checkpoint function (Rathi et al., 2022). Moreover, we established that SWR1-C plays a key role in SPOC as an essential complex that prevents mitotic slippage (i.e. the slow exit from mitosis) following SPOC activation (Caydasi et al., 2023). This mitotic slippage required the Cdc14-early anaphase release pathway, the SAGA (Spt-Ada-Gcn5 acetyltransferase) histone acetyltransferase complex, proteasome components and the M-Cdk inhibitor Sic1 (Caydasi et al., 2023). Based on these analyses, we propose that a robust SPOC-induced cell cycle arrest requires: (a) an initial activation step that relies on both Kin4-dependent and Kin4-independent pathways to block the MEN-signalling cascade, and (b) maintenance mechanisms involving Mck1 and SWR1-C, which are essential for sustaining mitotic arrest, partly through the regulation of M-Cdk inhibitors.

Centriole integrity, cilia biogenesis and length control

Centrioles are core structures of the centrosome. They are formed by nine microtubules cylinders organised as triplets and doublets. In addition, complexes required for centriole formation, integrity and cilia biogenesis are attached to centriolar microtubules, forming the mother and daughter centrioles (Figure 2). Our lab is particularly interested in understanding how centriolar distal appendages (DAs) are formed at the mother but not at the daughter centriole, as these macromolecular protein complexes are essential for the initial steps of cilia formation (Streubel and Pereira, 2023). In collaboration with the group of Elmar Schiebel (ZMBH, Heidelberg), we showed that DA formation requires in part the centrosomal protein CEP350. Interestingly, CEP350 is present at both, mother and daughter centrioles, and its absence leads to unstable and overelongated centrioles that lack DAs (Figure 2). The current model proposes that CEP350 is recruited to newly formed (daughter) centrioles to provide their stability. As the daughter centriole matures into the old, mother centriole, CEP350 functions as a scaffold to allow DA assembly by a mechanism that is currently unknown (Karasu et al., 2023).

Ciliogenesis is a tightly regulated process that relies on the coordinated interplay of cell cycle regulators, vesicular trafficking and the transport of cilia building blocks to the mother centrosome. An open question in the field is how this coordination enables both the formation and maintenance of ciliary structure and length, which are optimised for the signalling function of cilia. We could show that mammalian primary cilia axonemes exhibit distinct proximal (PS) and distal segments (DS), characterised by tubulin polyglutamylation-rich and -poor regions, respectively. Analysis of their segmentation highlights how disruptions causing cilia overelongation affect PS or DS lengths differently, influencing cilia stability and behaviour. We have identified septins, GTPases with functions in cell division, as novel inhibitors of DS growth, regulating the localisation of transition zone proteins, and the cilia tip accumulation of the microtubule-capping kinesin KIF7, an inhibitor of cilia growth. Live-cell imaging and sonic-hedgehog (SHH) signalling activation assays further demonstrate that DS overextension increases cilia ectocytosis events while reducing SHH activation. These findings emphasise the importance of understanding cilia segmentation and the role of septins in length regulation and cilia-mediated signalling pathways (Kanamaru et al., 2022).

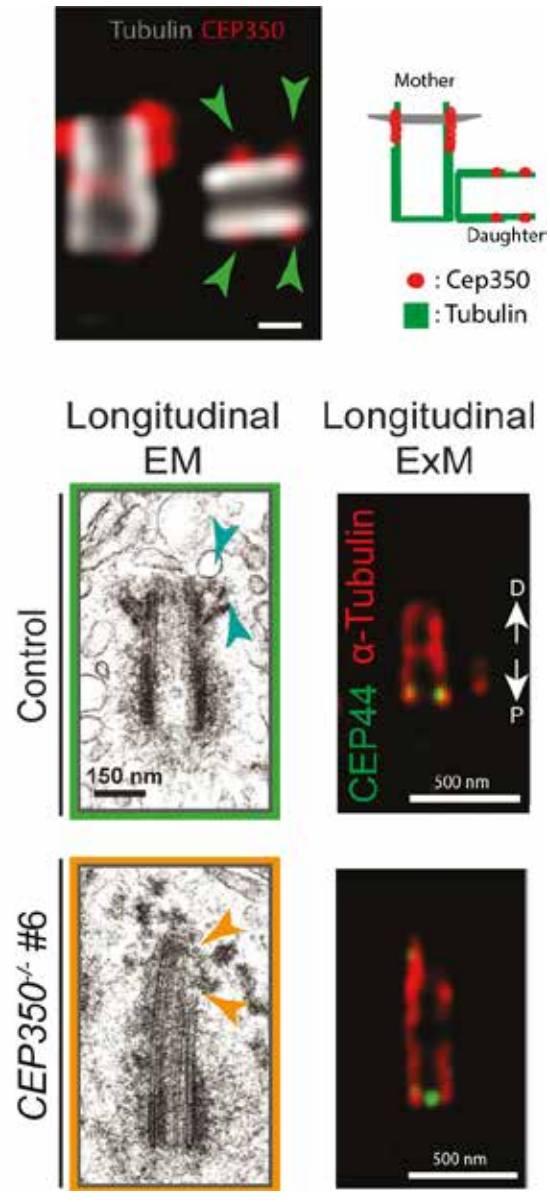

Figure 2:

CEP350 controls centriole stability and DA formation. Expansion microcopy (ExM) show Cep350 at mother and daugther (green arrows) centrioles, as depicted in the cartoon. Electron microscopy (EM) micrographs depict DAs at the centriolar tip in control (blue arrows) but not in CEP350-knockout cells (green arrows). Distal appendages are present in control (blue arrows), but not in CEP350-/- cells (orange arrows). ExM display centriolar walls (red), with centrioles extending from the distal end (D) in CEP350-/-. CEP44 marks the proximal end (P).

Future directions
Our future research will continue to explore the pivotal role of centrosomes in cellular processes, with a particular focus on centriole maturation, cilia biogenesis and cell cycle regulation.

Although we have made significant progress in identifying novel SPOC components, the cellular mechanisms that sense spindle orientation remain elusive. Additionally, the process by which the SPOC is silenced following spindle re-orientation is not well understood. We plan to further investigate the molecular function of novel SPOC regulators and use alternative genetic screens to identify SPOC inhibitors involved in checkpoint silencing. These studies aim to elucidate the pathways by which cells coordinate spindle orientation with cell cycle progression, ensuring accurate chromosome segregation and preventing aneuploidy.

We will also focus on the mechanisms underlying centriole maturation and the formation of centriolar DA complexes. Using a combination of biochemical and cell biology approaches, we aim to dissect the molecular pathways involving CEP350 in DA assembly and function. By unraveling these mechanisms, we hope to provide a comprehensive view of how centrioles and their associated structures are regulated and how mutations in these components contribute to cilia-related diseases.

Moreover, cilia biogenesis and length control are central to our future research plans. Our study demonstrating that septins are key inhibitors of distal segment growth opens new questions about how cilia segmentation is regulated and how it influences signalling pathways such as SHH. Cilia length varies among different cell types. Analysis of cilia from ciliopathy patients demonstrates that inactivating mutations in cilia-related genes can either prevent cilia extension or lead to abnormally elongated cilia. In both cases, cilia signalling is impaired, likely explaining the disease phenotypes. Our long-term aims are to understand how different cell types regulate cilia behaviour (length and function) and the mechanistic role of septins in this process. Building upon our previous work on the role of DAs in the initial steps of cilia formation, our future work will also focus on the role of novel regulators of axoneme extension, the impact of extracellular matrix perturbations on ciliogenesis, and how signalling pathways, including WNT and SHH, influence cilia biogenesis in 2D and 3D-organoid models.

Selected publications since 2021
Streubel, J. and **Pereira, G.** (2023). Control of centrosome distal appendages assembly and disassembly. Cells&Dev 174, 203839.

Caydasi, A.K., Khmelinskii, A., Darieva, Z., Kurtulmus, B., Knop, M. and **Pereira, G.** (2023). SWR1 chromatin remodeling complex prevents mitotic slippage during spindle position checkpoint arrest. Mol. Biol. Cell 34, ar11.

Karasu, O.R., Neuner, N., Atorino, E.S., **Pereira, G.**, Schiebel, E. (2022). The central scaffold protein CEP350 coordinates centriole length, stability, and maturation. J. Cell Biol. 221, e202203081.

Rathi, S., Polat, I., **Pereira, G.** (2022). The budding yeast GSK-3 homologue Mck1 is an essential component of the spindle position checkpoint. Open Biol. 12, 220203.

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Publication statistics
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G Greifen und Klettern
Grabbing and climbing
V Wühlen und Graben
Digging and burrowing

2.17 JUNIOR PROF. DR. LAUREN SAUNDERS
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Fields of Interest
Developmental biology, genetics, evolution, neural crest, single-cell genomics, lineage tracing, zebrafish, medaka

Summary and outlook
The Saunders lab opened in January 2024! We are interested in understanding how a series of developmental events produces a complex, multicellular organism. The diversity of form and function in animals ultimately traces back to evolutionary modifications at the cellular level, mediated by genetic and environmental constraints on cell lineages during development. Thus, we must study these processes at multiple scales (molecular, cellular, organismal). Single cell genomics has rapidly expanded our understanding of the continuum of molecular cell states present during development; yet, we still do not understand how these states are constrained by lineage, the developmental history of each cell tracing back to fertilization. For example, are histologically similar tissues with separate lineage origins driven by the same developmental programs? How do lineage-specific fates contribute to robust development in the face of diverse perturbations? We hypothesize that lineage-specific contributions to the same cell types and tissues impart robustness and provide a flexible substrate for adaptation. My lab studies lineage-specific constraints on and plasticity of two diverse cell lineages, neural crest (NC) and mesoderm, which convergently populate tissues throughout the animal. To investigate these questions, we apply an innovative, multi-disciplinary approach, using single cell genomics, developmental genetics, genome engineering, computational biology, and the zebrafish model system (Fig. 1).

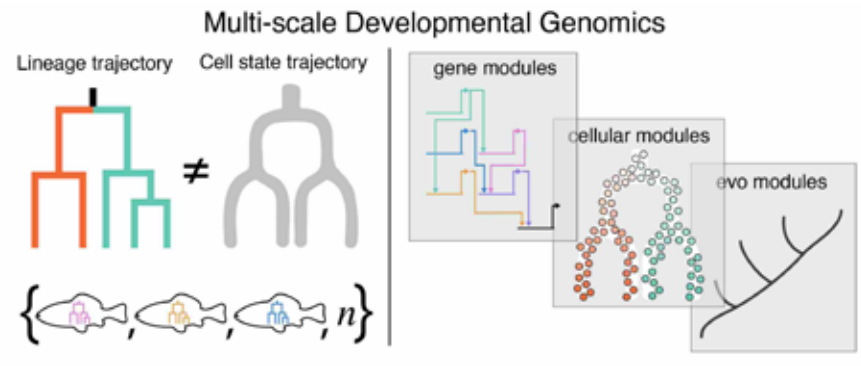

Figure 1:
Towards and organismal view of cell lineage histories, gene expression programs, and chromatin dynamics that contribute to evolutionary innovations in vertebrates.

Research highlights since 2021
As a postdoctoral fellow from 2021-2024, I studied the relationship between genetics, development and morphological features in zebrafish. In a collaborative effort, I worked to uncover the developmental mechanisms of a characteristic post-embryonic trait: fish scales. This study revealed how ancient, conserved signaling pathways can be elaborated upon in specific cell populations to produce diverse dermal appendages, a defining morphological feature of vertebrates (Aman, Saunders et al. 2023).

In order to apply single cell genomics to diverse perturbations across many individual organisms, we needed a scalable method that coupled single cell measurements to specific samples. I pioneered a high-throughput scRNA-seq approach to measure transcriptomes from thousands of individually-barcoded whole embryos in a single experiment, enabling multi-scale (molecules + cells + organism) phenotyping of development. In particular, individual animal measurements enable us to quantify changes in cellular abundance in response to diverse perturbations and ultimately relate these to gene expression changes, providing a deep molecular and cellular view of how a whole organism responds to genetic or environmental changes. I deployed this technique to study the effects of 23 perturbations at multiple time points during zebrafish development, also generating the most comprehensive atlas of wildtype zebrafish development to date (>3M cells; ~2000 individuals) (Saunders, Srivatsan et al. 2023). I also developed new computational tools to measure differential cell type abundances with newfound statistical power, enabling a quantitative readout of cell type-specific responses across individuals. Comprehensive analyses of multiple genetic perturbations on related cell types revealed key insights into vertebrate skull evolution via the mesoderm. The collaborative, cross-disciplinary projects I led as a postdoctoral fellow in Seattle are a template for the integrative research program I am building in my own lab at COS, employing both experimental and computational methods.

Future directions
As a new Junior Professor and Emmy Noether fellow at COS, the lab has big plans for the coming years. Each multicellular animal starts as a single cell, meaning that every cell has reached that position through a series of cell divisions that constitute its lineage history. Classic embryological approaches to uncover lineage histories with dye injections provided some of the first fate maps of vertebrate embryos. Modern methods to capture genetically encoded lineage recording systems have been developed for zebrafish, but still have major technical limitations. Still, this technology holds great potential; single-cell transcriptome measurements capture just a snapshot of a cell's state at a given time, and incorporating lineage information alongside transcriptomes would massively expand our ability to learn about the relationships between cell histories and cell potential *in vivo*. Building on my preliminary data, the lab will now use cranial cartilage as a tractable experimental paradigm to characterize the relationship between cell lineage history and cell state/type. Importantly, there are other instances in which different cell lineages converge on a single cell type. This convergence happens multiple times for neural crest and mesodermally-derived cells; examples are fibroblasts, chondrocytes, and cardiomyocytes. Yet, it is not known how the cellular history impacts cell state and whether this convergence functions to bolster embryonic robustness, as has been suggested in post-embryonic heart regeneration. One objective in the lab is to globally map the convergent cellular identities of NC and mesodermal lineages organism-wide. We will do this using a combination of genetic engineering, imaging and single-cell phenotyping approaches. This work will provide the basis to investigate mechanisms and roles of these convergent differentiation processes.

Another objective of the lab is to leverage the power of the zebrafish system in combination with medaka, another well-established vertebrate model, to investigate the evolution of lineage-specific mechanisms during cartilage development. Medaka is a teleost model system that share key developmental similarities as well as differences with zebrafish. Medaka offers outstanding opportunities to study mechanisms of genotype-to-phenotype relationships because of a large number of inbred strains that possess different adaptive, morphological characteristics, including differences in cranial morphologies. In collabora-

tion with Medaka experts at COS, my lab will apply single-cell phenotyping to characterize the development of multiple medaka inbred strains to dissect lineage-specific changes that have occurred over ~120 million years of evolution. These studies will pave the way for long-term future directions of my laboratory.

Selected publications since 2021

Saunders, L.M.*, Srivatsan, S.R.*, Duran, M., Dorrity, M.W., Ewing, B., Linbo, T.H., Shendure, J., Raible, D.W., Moens, C.B., Kimelman, D., et al. (2023). Embryo-scale reverse genetics at single-cell resolution. Nature 623, 782–791.

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Publication statistics

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2.18 PROF. DR. KARIN SCHUMACHER
CELL BIOLOGY

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Fields of Interest
pH-homeostasis, proton-pumps, membrane transport, vesicular trafficking, vacuole biogenesis, cell growth, V-ATPase evolution

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 their 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 we have made substantial progress in understanding structure, function and regulation of this highly conserved eukaryotic proton-pump in the model plant Arabidopsis. In seed plants, 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 Ca2+ and are making use of these tools in dissecting how the V-ATPase is integrated in diverse cellular and metabolic networks. Moreover, we have used multiple approaches to purify V-ATPase complexes along with interacting proteins and pursue our interest in tracing the evolution of V-ATPase isoforms in the plant kingdom.

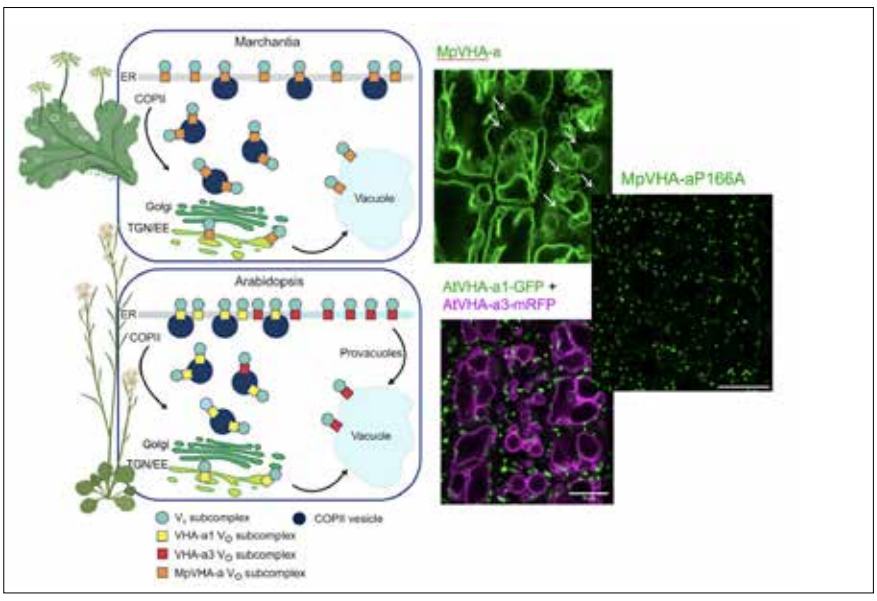

Figure 1:
Targeting and evolution of V-ATPase isoforms

Research highlights since 2021

Targeting and evolution of V-ATPase isoforms

Differential targeting of the V-ATPase is mediated by isoforms of subunit a, the largest of the V-ATPase subunits that consists of a C-terminal hydrophobic domain with eight transmembrane domains and a large N-terminal domain that is accessible for cytosolic interaction partners. We have shown previously that VHA-a1 targets the V-ATPase to the TGN/EE via a targeting domain that servers as both an ER-exit and as a TGN/EE-retention motif. Analysis of CRISPR/Cas9 generated null alleles revealed that VHA-a1 has an essential function for male gametophyte development but acts redundantly with the tonoplast isoforms VHA-a2 and VHA-a3 during vegetative growth. In contrast, the genome of the liverwort *Marchantia* encodes a single VHA-a isoform (MpVHA-a) that localizes predominantly to the tonoplast. Recently, we have identified a PLL motif that is required for tonoplast sorting of VHA-a3 as well as MpVHA-a. Based on this finding, we propose that loss of the PLL motif was the first step towards the development of a dedicated TGN/EE VHA-a isoform that is found in all seed plants. In collaboration with the lab of Takashi Ueda (NIBB, Okazaki) we will test the hypothesis that the PLL-motif is involved in Retromer-mediated cycling of the V-ATPase between TGN/EE and tonoplast. Analysis of CRISPR/Cas9 generated null alleles revealed that VHA-a1 has an essential function during male gametophyte development but acts redundantly with the tonoplast isoforms during vegetative growth providing a striking example that differential localization does not preclude functional redundancy. Our further analysis revealed that *vha-a1* mutant microspores fail to develop the protective pollen coat and thus collapse soon after the tetrad is dissolved. The situation in the vha-a1 sporophyte would therefore be reminiscent of the ancestral state in which a single isoform was sufficient for acidification of both, the TGN/EE and the vacuole. Given that the duplication that gave rise to the divergent clades of VHA-a1 and VHA-a3 can be traced back to the last common ancestor of gymnosperms and angiosperms, our finding that VHA-a1 is essential during male gametophyte development argues that the invention of a dedicated TGN/EE-isoform of the V-ATPase might have been a key innovation in the evolution of seed plants (Lupanga et al., unpublished).

Vacuole biogenesis

Plant vacuoles play key roles in cellular homeostasis, performing catabolic and storage functions, regulating pH and ion balance. Despite their essential role, there is still no consensus on how vacuoles are established. Our model proposing that the endoplasmic reticulum is the main contributor of membrane for growing vacuoles in meristematic cells has been challenged by a study proposing that plant vacuoles are formed de novo by homotypic fusion of multivesicular bodies (MVBs). We combined *in vivo* high-and super-resolution (STED) microscopy and customized FRAP assays to demonstrate the presence of tubular and connected vacuolar structures in all meristematic cells. Moreover, we showed that in Arabidopsis seedlings expressing the RUBY casette, betalains accumulating inside vacuoles are ideally suited for life-imaging of vacuole morphology (Figure 2). Taken together our results support strongly that vacuoles cannot originate from MVBs alone but must receive membrane material from other sources (Scheuring et al., reference to come).

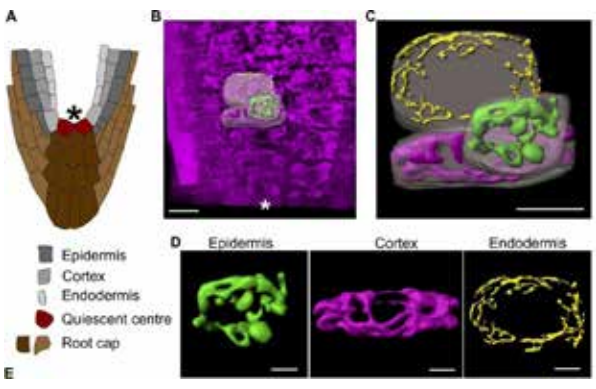

Figure 2:

Vacuole morphology in different cell types of the root visualized by betalain fluorescence. (A) Schematic overview of the root tip close to the QC. (B) Position of rendered vacuoles in respect to the QC. (C) 3D vacuole models from epidermal (green), cortex (magenta) and endodermis cells (yellow). Respective cellular volumes are highlighted (transparent white). (D) Different vacuole morphologies from neighboring cells with different identities.

Cryo-EM

Cryo-EM maps of the intact multiprotein-complex from mammals and yeast have revealed the arrangement and subunit stoichiometry, along with the dynamics related to rotational catalysis. While the structure and biochemical activity of all eukaryotic V-ATPases are generally similar, their biological reach has been greatly diversified by cell-type specific expression and differential subcellular localisation. The high-affinity interaction between the Legionella pneumophila effector SidK and V-ATPase subunit A forms the basis for efficient affinity purification of intact V-ATPase complexes and enabled Cryo-EM of the lemon enzyme as the first plant V-ATPase (Tan et al., 2022). Although several unique features were identified, the obtained resolution was limited and analysis was complicated by the lack of a well-annotated reference genome. In the meantime, we have established SidK-based purification in Arabidopsis and based on negative stain electron microscopy and first cryo-EM images obtained in collaboration with John Rubinstein (Toronto), we should be able to obtain a high-resolution structure in the near future.

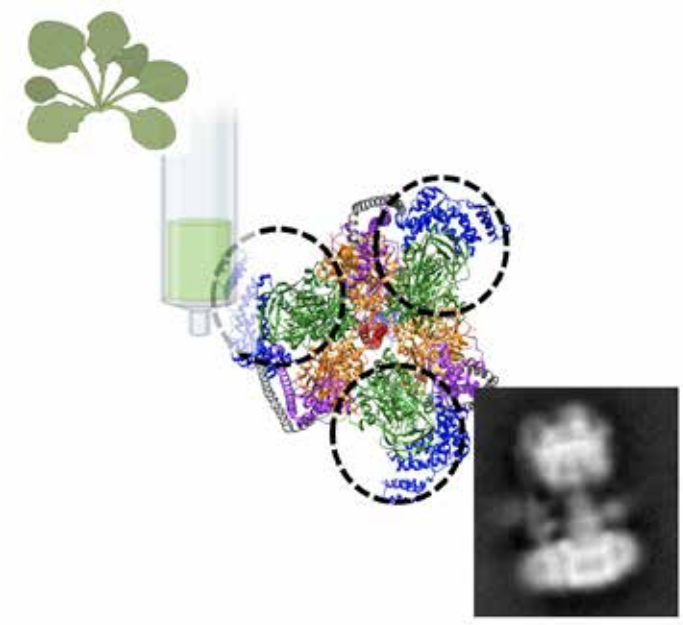

Figure 3:
SidK-based purification of the Arabidopsis V-ATPase - First snapshots

Future directions

Cellular energy homeostasis

Both, the catabolic and the storage function of plant vacuoles are essential for cellular energy homeostasis and energy invested in vacuolar acidification need to be tightly controlled. SidK-based purification of V-ATPase complexes has not only allowed us to pursue structural analysis via cryo-EM (Fig 3) but has also allowed us to identify V-ATPase interacting proteins by mass-spectrometry. Among these proteins, TLDc-domain proteins are of particular interest as they have been shown to link the V-ATPase to Target of Rapamycin (TOR) kinase. Future projects will address the relationship between V-ATPase and TOR activity as well as the obvious link to autophagy. To address whether there is a direct link between cellular energy signaling and V-ATPase assembly, we will use a FRET-FLIM approach to image dynamics of V-ATPase assembly state. Although reversible assembly appears to be conserved among higher eukaryotes, no direct evidence for its existence in plants has been reported. We have created a transgenic line co-expressing VHA-A-mRFP (V1) and the tonoplast localised VHA-a3-GFP (VO) which allows us to monitor the assembly state of the holoenzyme by FRET-FLIM analyses and preliminary results provide proof of principle that the method is suitable.

pH-dependent fluorescence lifetime imaging microscopy (pHLIM)
Although we have successfully used the ratiometric sensor pHusion to measure pH in acidic environments such as the TGN/EE, Golgi and apoplast, both sensitivity and resolution are limited due to the low signal-to-noise ratio and the pKa of 5.8. To increase the sensitivity and resolution of our pH imaging in plant cells we want to establish E2GFP and mRuby as fluorescence lifetime-based pH indicators in plant cells. Both fluorophores have been reported to exhibit pH-dependent lifetime changes ideally suited for the neutral to mildly alkaline and the acidic pH range respectively. As only one fluorophore is measured, the measurement procedure is simpler than with ratiometric pH indicators and can be easily combined with other fluorophores for multiplex imaging. With our recently aquired CLSM that is set up for fast fluorescence lifetime microscopy we plan to overcome these limitations. We have used established targeting strategies to direct both fluorescent proteins to different cellular compartments along the plant secretory and endocytic pathway and in the cytosol to perform pHLIM-based measurements in the respective transgenic lines (>Melanie Krebs).

Selected publications since 2021

Scheuring, D, Minina, E.A., Krüger, F., Lupanga, U., Krebs, M., and **Schumacher, K.** (2024) Light at the End of the Tunnel: FRAP Assays Combined with Super Resolution Microscopy Confirm the Presence of a Tubular Vacuole Network in Meristematic Plant Cells. The Plant Cell https://doi.org/10.1093/plcell/koae243.

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Fields of Interest
Membrane transport, membrane trafficking, proton pumps, pH homeostasis, calcium signaling, fluorescent indicators, live imaging

Summary and outlook
To study plant adaptation mechanisms at the molecular level, we combine fluorescence indicator-based techniques, classical biochemistry, genetics, and more recently, computational modelling. Using an integrative approach, combining mathematical modelling and experimental work, we developed models that describe pH control in the vacuole, assessing the extent to which vacuolar H^+-ATPases (V-ATPase) located at the vacuolar membrane and the trans-Golgi network/early endosome (TGN/EE) can contribute to vacuolar acidification. In addition, we could demonstrate that two endomembrane anion transporters, CLCd and CLCf, fulfil essential functions and are required to maintain pH homeostasis in Golgi stacks. Furthermore, our research hast contributed to identify a mechanism by which plants adapt to varying NaCl stress levels via a Ca^{2+} sensor switch.

Research highlights since 2021
Fluctuating environmental conditions trigger diverse physiological and developmental adaptations in plants and our efforts aim to understand how plants sense and process such environmental information. To study physiological adaptations and signalling events at the 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 investigate the mechanism of vacuolar acidification in *Arabidopsis* more efficiently than previously established methods, we developed a plate-reader-based platform to measure vacuolar pH using the pH-sensitive fluorescent dye 5-CFDA (Figure 1). The increased sample throughput allowed us to generate response kinetics of vacuolar pH at varying concentrations of the V-ATPase specific inhibitor, Concanamycin A (ConcA), which were then used to generate different models to assess the extent to which V-ATPases located at the vacuolar membrane and the TGN/EE might contribute to vacuolar acidification. This approach provided an integrative perspective on vacuolar pH regulation in *Arabidopsis* and will guide further experimental work (Holzheu *et al.* 2021). In addition to studying vacuolar pH control, our research has focused on the function of CLCd and CLCf, two distantly related members of the Arabidopsis Cl- channel (ClC) family that localize to the TGN/EE. Our findings revealed that CLCd and CLCf have redundant functions that are essential for male gametophyte development. Using an inducible knockdown approach combined with *in vivo* pH measurements, we demonstrated that reduced ClC activity does not affect pH in the TGN/EE but is necessary to maintain pH homeostasis in Golgi stacks. These results underline the importance of functional ion homeostasis within endomembrane compartments (Scholl *et al.* 2021). Physiological adaptations in plant cells are associated not only with changes in cellular pH but also with transient concentration changes of the second messenger calcium. Based on our previous research, in which we developed protocols for calibrating genetically encoded Ca^{2+} indicators in intact plant tissues, we contributed to identify a molecular mechanism by which plants use a Ca^{2+}-based molecular switch to adapt their responses to different concentrations of NaCl (Steinhorst *et al.*

2022). Additionally, our previously developed Ca^{2+} imaging tools were instrumental in investigating the relationship between analogues of the phytohormone auxin on Ca^{2+} signalling and their influence on the endocytosis of auxin efflux transporters of the PIN family (Wang *et al.* 2022).

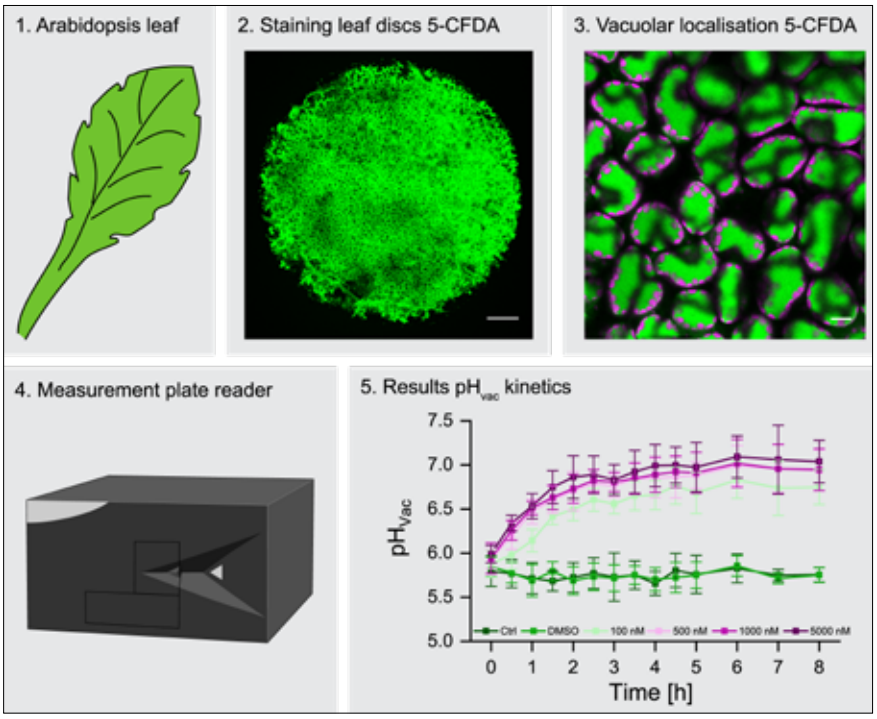

Figure 1:

Medium throughput platform for fluorescence based measurements of vacuolar pH kinetics in Arabidopsis leaf discs. (1) Leaf discs are transferred to 96-well plates and (2) stained with the ratiometric pH indicator 5-CFDA. The dye exclusively labels the large central vacuole in Arabidopsis leaf cells (3). Plate reader analysis of fluorescence intensities (4) allows medium sample throughput of vacuolar pH kinetics under different conditions, e.g. analysis of response kinetics after treatment with different concentrations of the V-ATPase-specific inhibitor Concanamycin A (5).

Future directions

Our previous work suggested a novel mechanism for vacuolar acidification, where vacuolar stored Ca^{2+} acts as a battery to fuel H^+ uptake via reverse transport activity of vacuolar Ca^{2+}/H^+ exchangers. We aim to prove this hypothesis and determine, if plants possess this alternative mechanism alongside the established proton pump-mediated vacuolar acidification machinery. We will also continue to refine and expand our existing toolkits for Ca^{2+} and pH imaging in plants. To address Ca^{2+} responses at the cellular level with greater precision in a tissue context, we will express Ca^{2+} indicators in a cell type-specific manner (Figure 2). To investigate the relationship between Ca^{2+} signalling and regulation of V-ATPase activity, we will develop genetic tools for precise manipulation of cytosolic Ca^{2+}. Additionally, we will establish low-affinity Ca^{2+} indicators that can withstand acidic environments to study the roles of vacuole and apoplastic space as major Ca^{2+} stores in plant cells. Furthermore, we will generate fluorescence lifetime-based pH indicators to overcome the limitations of existing intensity-based imaging, to further investigate the role of cellular pH as a determinant of endocytic and secretory trafficking in plant cells.

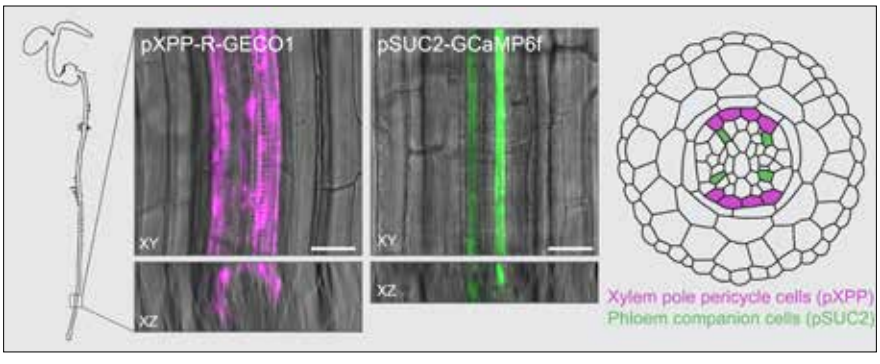

Figure 2:

Cell-type specific expression of spectrally distinct Ca2+ indicators for multi-spatial analyses of Ca2+ responses in intact tissue context. Shown are confocal laser scanning microscopy images of red and green fluorescent genetically encoded Ca2+ indicators that are expressed under control of the cell-type specific promoters XPP (xylem pole pericycle cells) and SUC2 (phloem companion cells) respectively. Shown are overlays of the brightfield images (grey LUT) and the maximum projections of the fluorescent Ca2+ indicators (R-GECO1, magenta LUT, z = 30 µm; GCaMP6f, green LUT, z = 20 µm). Scale bars indicate 20 µm.

Selected publications since 2021

Scheuring, D., Minina, E.A., Krueger, F., Lupanga, U., **Krebs, M.**, and Schumacher, K. (2024). Light at the end of the tunnel: FRAP assays combined with super resolution microscopy confirm the presence of a tubular vacuole network in meristematic plant cells. Plant Cell 00: 1–9.

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Publication statistics

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Fields of Interest
Glycobiology; glycosyltransferases and functions of glycans in yeast and mammals; interconnection between different glycosylation pathways; congenital disorders of glycosylation

Summary and Outlook
Over the past three years, our laboratory has made significant strides in understanding glycosylation, one of the most prevalent and complex protein modifications. Our research has particularly focused on O-mannosylation and N-glycosylation, essential processes in eukaryotes, which play critical roles in severe congenital disorders.

Our group has made considerable progress in elucidating the intricate roles of protein O-mannosyltransferases (PMTs/POMTs). Specifically, we have investigated the Pmt4 protein in Chaetomium thermophilum (ctPmt4), uncovering its structural and functional properties. Key discoveries include a unique β-hairpin insertion crucial for its O-mannosyltransferase activity and the mannose donor substrate binding within ctPmt4's cytosolic pocket. These insights extend to the human PMT4 homolog, POMT1, linking mutations to congenital muscular dystrophy.

Additionally, we have delved into the interplay between N-glycosylation and O-mannosylation, essential for protein folding and stability. We provided strong evidence that defects in the N-glycosylation pathway (ALG3 deficiency) lead to secondary O-mannosylation defects, offering new insights into congenital disorders of glycosylation (CDGs).

Our third research focus has explored the impact of POMT deficiency on cell-extracellular matrix (ECM) adhesion and migration, revealing significant changes in gene expression and integrin β1 maturation. This research has provided a deeper understanding of protein O-mannosylation in cellular motility and its implications for conditions like Walker-Warburg syndrome. Looking ahead, our group aims to further elucidate the molecular mechanisms underlying these pathways and their broader biological implications. We plan to continue our collaborative efforts, leveraging advanced structural biology techniques to deepen our understanding and potentially identify new therapeutic targets.

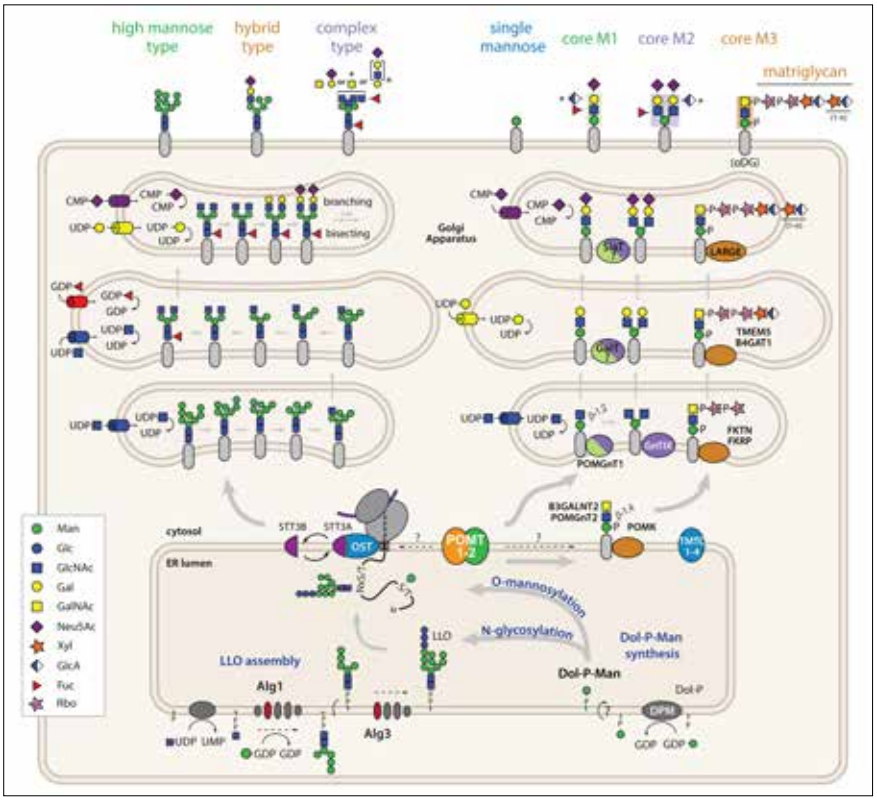

Figure 1:

N-glycosylation and O-mannosylation in mammals. ER: assembly of the lipid-linked glycan donors (LLO). 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.

Research Highlights

Protein O-Mannosyltransferases (PMTs)

Our work on protein O-mannosyltransferases (PMTs), particularly the Pmt4 protein in *Chaetomium thermophilum* (ctPmt4), has yielded key insights into its structural and functional properties. In collaboration with the group of I. Sinning (BZH, Heidelberg University), we performed structure-function analyses revealing the unique arrangement of the luminal MIR domains in ctPmt4 and a distinctive β-hairpin insertion not present in Pmt2's MIR domains. This β-hairpin insertion was shown to be crucial for Pmt4's O-mannosyltransferase activity, highlighting its vital role in the O-mannosylation of substrate proteins. Furthermore, our research identified that the mannose donor substrate binds within a cytosolic pocket of ctPmt4, a critical interaction for the enzyme's function. Notably, mutations in this region in the human PMT4 homolog, POMT1, are associated with congenital muscular dystrophy, highlighting an evolutionary conservation of these mechanisms from yeast to humans. (manuscript submitted)

Interplay Between N-Glycosylation and O-Mannosylation

A second research focus has been understanding the complex interplay between N-glycosylation and O-mannosylation. These vital processes are evolutionarily conserved, underscoring their importance in cellular and organismal biology. Defects in these pathways lead to severe diseases like CDGs, manifesting with symptoms affecting multiple organ systems. In collaboration with C. Thiel (Heidelberg University Hospital), we conducted studies on patient-derived fibroblasts suffering from congenital disorder of glycosylation (ALG3-CDG) and observed significant effects on the abundance of POMT1 and POMT2. To further investigate the underlying mechanisms, we generated an ALG3 knockout (ΔALG3) in HEK293 cells, benefiting from an isogenic background and clearer defect characterization. We found that ΔALG3 cells exhibit reduced POMT protein levels and aberrant N-glycosylation of POMTs.

Additionally, we gained strong evidence that defects in the N-glycosylation pathway that affect POMT stability lead to secondary O-mannosylation defects. This discovery offers a novel explanation for complex CDG manifestations and highlights the intricate network of glycosylation pathways. These findings on aberrant N-glycosylation and its impact on O-mannosylation represent a significant milestone in glycobiology. (manuscript in preparation)

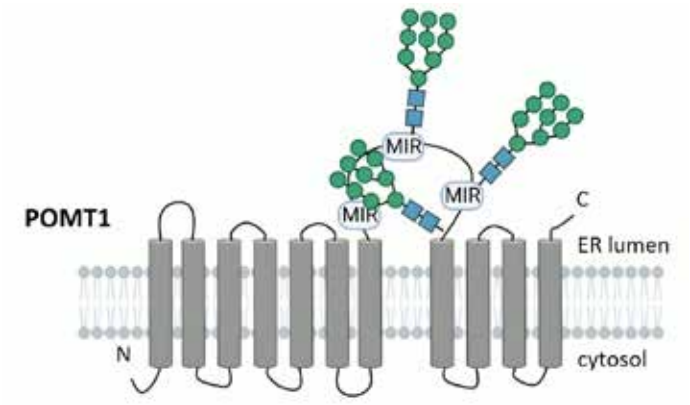

Figure 2:
Human POMTs are N-glycosylated membrane proteins with 11 transmembrane domains. Created by BioRender.

Impact of POMT Deficiency on Cell-ECM Adhesion and Migration
Another major focus has been on the impact of POMT deficiency on cell-ECM adhesion and migration, specifically using HEK293 cell models deficient in POMT2. We observed significant changes in genes involved in cell adhesion, confirmed by functional assays that showed a marked decrease in integrin-mediated cell adhesion in POMT-deficient cells. At the molecular level, we explored the maturation of integrin β1, a key transmembrane receptor for ECM adhesion, and found altered ratios of precursor to mature integrin β1 isoforms in POMT-deficient cells, with significantly reduced levels of mature integrin β1 on the cell surface. This impaired maturation was also observed in skin fibroblasts from patients with Walker-Warburg syndrome (WWS), a condition linked to protein O-mannosylation deficiency. We further unraveled that the impaired maturation of integrin β1 in POMT-deficient cells resulted from defective N-linked glycosylation. We noted an increase in high mannose N-glycan structures and a decrease in complex N-glycan structures in POMT-deficient cells. Finally, the downregulation of ER mannosidase MAN1B1 in POMT-deficient cells was identified as the cause of the altered N-glycan profile and impaired integrin β1 maturation. In summary, our research has uncovered a previously unknown crucial role of protein O-mannosylation in integrin-mediated cell-ECM interactions. These findings provide a deeper understanding of the molecular mechanisms underlying dystroglycanopathies. (manuscript in preparation)
Overall, our studies highlight potential therapeutic targets and open new avenues for potential therapeutic interventions for CDG and related disorders.

Future Directions
Building on our recent discoveries, our future research will focus on advancing our understanding of glycosylation processes and their implications in health and disease. In collaboration with the Sinning group, we aim to conduct comprehensive studies on the functional importance of the conserved cytosolic cavity in PMT4. This will involve mutational analyses and advanced structural biology techniques to explore the interactions between PMTs and their substrates in greater detail.

Over the next three years, it is essential that ongoing projects are completed effectively and unpublished work is compiled and published. This will ensure that our research legacy remains sustainable. In the final phase of my tenure, I will also place a strong emphasis on teacher training and development within the Faculty of Biosciences and the Heidelberg School of Education. This includes developing new courses and improving existing curricula to provide future educators with a comprehensive understanding of biology and prepare them for the diverse challenges of today's school life. By doing so, I aim to help create a solid foundation for the next generation of scientists and educators.

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Publication statistics

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2.20 JUNIOR PROF. DR. BRITTA VELTEN
BIOLOGICAL DATA SCIENCE

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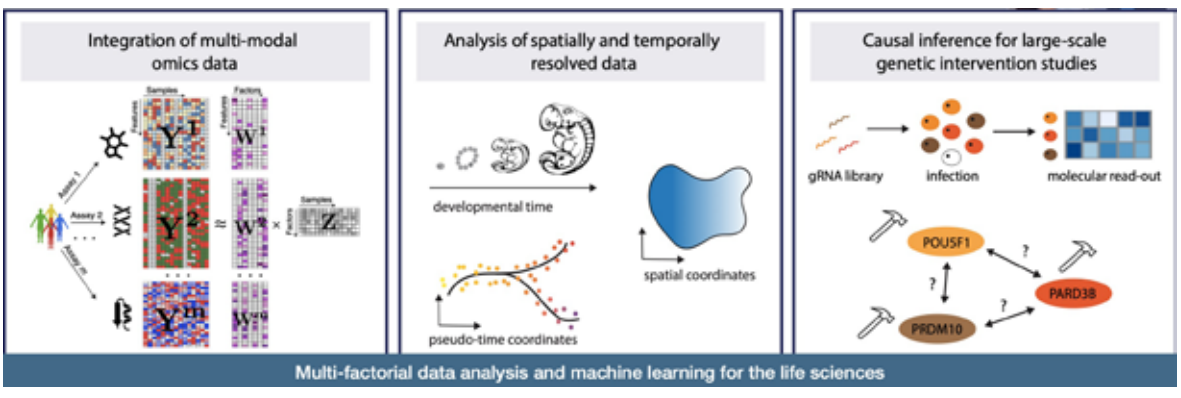
Fields of Interest
Probabilistic machine learning, multi-factorial data analysis, data integration and visualization, multi-omics, single cell biology, causal inference, gene regulatory networks, spatio-temporal omics

Summary and outlook
The Velten group develops statistical methods and machine learning approaches to move from large-scale molecular data ('omics data') to biological insight on gene regulation and decipher the heterogeneity of biological processes across molecular layers, biological contexts and temporal and spatial scales. Modern technologies enable researchers to study biological organisms and processes simultaneously on different molecular layers and in diverse biological contexts. To enable a joint analysis of the resulting data we develop methods for the supervised and unsupervised analysis of multi-modal omics data using probabilistic machine learning. Our methods facilitate the joint analysis of data arising from multiple omics technologies and different biological contexts in a data-driven manner. At the same time, we also accommodate technological advances that increasingly enable time- and space-resolved molecular measurements at scale. These data offer new opportunities to study the dynamic and contextual properties of a biological system and can uncover novel traits that would not be visible without the temporal or spatial context. To extract such insights from the data we develop methods for the identification of temporal and spatial patterns and their comparison across contexts. Finally, we aim to leverage the combination of molecular read-outs with targeted or non-targeted interventions to gain new insights into regulatory mechanisms of key biological processes. For this purpose, we build upon causal modelling and use of statistical invariance principles in order to pinpoint causal mechanisms on the molecular level and reveal common principles across biological contexts.

Research highlights since 2021
Omics technologies with temporal and spatial resolution have recently emerged as a new powerful tool to study dynamics of biological processes, tissue architecture, cell-cell communication and spatial co-localization of molecules in diverse model systems and at cellular or even subcellular scale. From a computational perspective, such data offers new opportunities and challenges to account for and leverage the temporal and spatial information.

Figure 1:
Overview of the research lines



To accommodate such data and identify major temporal and spatial patterns from the noisy molecular data we have developed MEFISTO (Velten et al., 2022), a computational framework for the analysis and integration of temporal and spatial omics data. This framework extends prior work on latent variable models for the integrative analysis of multi-omics data by explicitly leveraging and accounting for the temporal and spatial information and thereby can accurately identify relevant spatial patterns and temporal dynamics as well as underlying molecular drivers. For example, this enabled us to identify conserved and non-conserved gene expression programs along mammalian development across different species and organs as well as major anatomical regions in the mouse brain based on spatial co-expression patterns of genes. To further accommodate spatial data with subcellular and single molecule resolution (e.g., as produced by multiplexed FISH-based technologies) we extended the model, developing a new method (FISHFactor, Walter et al., 2023) that can identify major subcellular gene expression patterns based on the localization of single molecule in a cell and use them to identify heterogeneity across cells based on their subcellular gene expression patterns.
To accommodate complex multi-sample single cell atlas data and combine this information with spatial read-outs we have furthermore extended our previous strategies for integration of multi-omics data to facilitate the identification of multi-cellular programs, capturing coordinated responses across different cell types in response to perturbation or disease (MOFAcell, Ramirez-Flores et al., 2023). In collaborations, we have used our expertise on data integration as well as spatial and temporal modelling for example in the analysis of a multi-omics and spatial data collection to describe the maternal–fetal interface during early pregnancy in humans and analyze the epigenetic and transcriptional changes along differentiation and invasion of trophoblast (Arutyunyan et al., 2023). Furthermore, similar modelling strategies applied to temporal profiles have enabled us in collaboration to analyze and describe the mechanisms that underly the effects of osmolytes on the thermal stability of proteins in situ. (Pepelnjak et al., 2024)

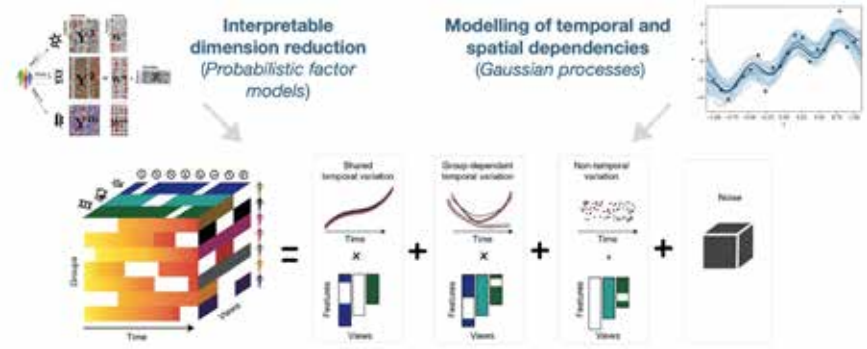


Figure 2:
Illustration of MEFISTO for the integration of temporal and spatial multi-modal data and identification of spatial and temporal patterns (here illustrated for time)

A second major technological breakthrough is the combination of omics read-outs with targeted interventions based on CRIPSR in high-throughput using pooled single cell sequencing (scCRISPR). Such assays promise to yield novel insight into gene regulation by facilitating to study the molecular consequences of genetic interventions and are by now widely applied across model systems. For example, in collaboration with the group of Leopold Part (Sanger Institute, UK) we are currently using a single cell CRIPSR interference screen to map the gene expression changes induced by downregulation of target genes at scale in induced pluripotent stem cells. For a reliable and powerful analysis of such data several challenges need to be addressed, including the assignment of cells to individual guides that link a cell to a given genetic intervention based on sparse sequencing data as well as the identification of causal effects and gene regulatory networks underlying the observed gene expression changes and their comparison across different systems. As a first step in this direction, we have developed a Python package crispat that provides an easy-to-use interface to different methods for the guide assignment step in single cell

CRIPSR screens. We have used it to compare different guide assignment strategies on public data from single cell CRISPR interference screens and could highlight the implications of this choice for the power of the analysis (Braunger et al., 2024), with crispat providing a flexible framework to apply and compare different strategies and ensure optimal power.

Future directions

As part of a recently awarded DFG Emmy Noether research grant we aim to continue our work on the development of tailored statistical methods for single cell CRISPR assays to facilitate the reliable estimation of the molecular effects of a perturbation from the data and their comparison across different biological systems. For this we aim to develop a flexible, powerful and statistically sound analysis framework for the discovery of regulatory mechanisms from high-content CRISPR screens. Thereby, we aim to (i) improve the estimation of total causal effects of a perturbation on the molecular features, addressing shortcomings in calibration, power and flexibility of existing methods, (ii) benchmark and develop methods to learn directed causal networks from the data that can reveal the molecular mechanism underlying the observed changes, highlight novel roles of genes and guide the design of future experiments as well as (iii) dissect the interplay of cellular state and biological context with the effect of a perturbation. We will apply such method to data from our CRISPR interference assay in induced pluripotent stem cells as well as data from other cell lines and model organisms to dissect gene regulatory mechanisms underlying organismal function, plasticity and development.

In addition, we will continue our work in the area of analysis and method development for multi-omics and spatial omics data. In particular, we aim to extend our previously developed approaches to study subcellular localization patterns in heterogeneous cell populations and test for changes across different biological conditions. Furthermore, we will address questions related to the spatial alignment and comparison of patterns across multiple tissue samples. Finally, in collaboration with the group of Ingrid Lohmann at COS we aim to develop computational methods for a robust and sensitive inference of gene regulatory networks from multi-omic data in heterogeneous cell populations and apply them dissect the heterogeneity in muscle nuclei underlying correct muscle development.

Selected publications since 2021

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Arutyunyan, A., Roberts, K., Troulé, K., Wong, F., Sheridan, M.A., Kats, I., Garcia-Alonso, L., **Velten, B.**, et al. (2023). Spatial multiomics map of trophoblast development in early pregnancy. Nature 616 (7955), 143-151.

Ramirez Flores, R.O., Lanzer, J.D., Dimitrov, D., **Velten, B.**, and Saez-Rodriguez, J. (2023). Multicellular factor analysis of single-cell data for a tissue-centric understanding of disease. eLife 12, e93161.

Walter, F., Stegle, O., and **Velten, B.** (2023). FISHFactor: A Probabilistic Factor Model for Spatial Transcriptomics Data with Subcellular Resolution. Bioinformatics 39 (5), btad183.

Velten, B., Braunger, J.M., Argelaguet, R., Arnol, D., Wirbel, J., et al. (2022). Identifying temporal and spatial patterns of variation from multi-modal data using MEFISTO. Nature Methods 20 (10), 1462-1474.

Publication statistics

https://scholar.google.com/citations?hl=de&user=sJ2IkaAAAAAJ

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

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Fields of Interest
Molecular genetics, stem cells in development, growth, regeneration (eye, heart, brain, intestine; medaka: *Oryzias latipes*, zebrafish: *Danio rerio*), genome environment interactions in physiology morphogenesis, regeneration and behaviour. Vertebrate organoids, new constraints and developmental potential. Technology development: Crispr/Cas, imaging, organoids

Summary and outlook
The lab has been focussing on three main axes, 1) the coordination of stem cell activity in niche establishment and contribution to organ growth. We find evidence that the massive pruning by the immune system eliminates potentially deleterious cells, while simultaneously reducing cell density to allow the stem cell niche to initiate the secondary growth phase, continuously expanding the retina.

2) The systematic analysis of complex genetic traits in the interaction of genome and environment alongside with the continuous development of tailor made Crispr/Cas9 tools to functionally test the mapping derived hypotheses. We are exploiting our (COS-)MIKK panel of medaka inbred lines and have mapped and validated loci responsible for genome/environment (GxE) interactions in collaboration with our partners at KIT (Loosli, Baumbach) in the UK (Birney) and in Japan (Naruse). The systematic exploitation of our medaka inbred panel with special interest in heart and retina function as well as regeneration will remain central focus.

3) We use organoids to address alternative developmental routes with analogous outcomes, representing a third pillar of activities in the lab. Fish retinal organoid development under specific conditions results in the formation of retinal structures resembling simple ommatidia in invertebrates rendering the organoid into an “evolutionary time machine”.

Research Highlights since 2021
In recent years, my laboratory has made significant progress in deciphering „the genetics of individuality,“ supported by an ERC Synergy grant and NIH funding. Our close collaborations within the Cluster of Excellence 3DMM2O have catalyzed novel approaches to identifying and repurposing evolutionary building blocks in the context of retinal development in organoids.

Continuing our efforts to elucidate the action of the retinal stem cell niche, we have focused on two key areas, the origin of retinal stem cells and the role of the immune system in initiating activity during the transition from embryonic to post-embryonic retina. Our research revealed that progenitor cells from the peripheral zone of the optic vesicle are reprogrammed into stem cells. Notably, these cells give rise to progenitors that can be reprogrammed again, if stem cells are specifically depleted.

A substantial portion of the lab has focussed on the systematic analysis of complex polygenic traits contributing to heart function, visual acuity, and nervous system regeneration. Central to this analysis are quantitative high-throughput approaches, which we have been developing. To uncover the genetic basis for various traits, we have quantified phenotypes across all lines of our Medaka inbred panel (MIKK panel). Our analysis of cardiac function uncovered striking genome-environment (temperature) interactions.

For each phenotype, we established F2 offspring from extreme phenotypes across the inbred panel and performed whole-genome sequencing on over 2,000 quantitatively phenotyped F2 individuals. This allowed us, in collaboration with E. Birney's group, to correlate genotype and phenotype. We validated identified loci using CRISPR-based targeted mutagenesis, to assess the relative contribution, additive effects, and synergistic effects of the linked loci.

We have applied this approach to a range of quantitative assays: e. g. heart function, visual acuity, impact of environmental toxins (ethanol, disulfiram, caffeine, ziram), brain and body morphology. All data linking genome to phenome will constitute a continuously growing, cross-referencing database. This resource aims to establish connections between species, from fish to humans and back, ultimately creating tractable models for development and disease.

The development of medaka retinal organoids (Zilova, Weinhardt et al., 2021) marked a milestone in our research. It allowed to expose pluripotent stem cells to instructive biochemical or physical cues that mimic embryonic conditions, recapitulating organogenesis or, alternatively, subject cells to cues outside their evolutionary context, uncovering evolutionarily constrained building blocks.

A prime example of such a building block is the stereotypic assembly of retinal cells into rosettes of 8 cells and various retinal cell types (Figure 1). While fish retinal organoids

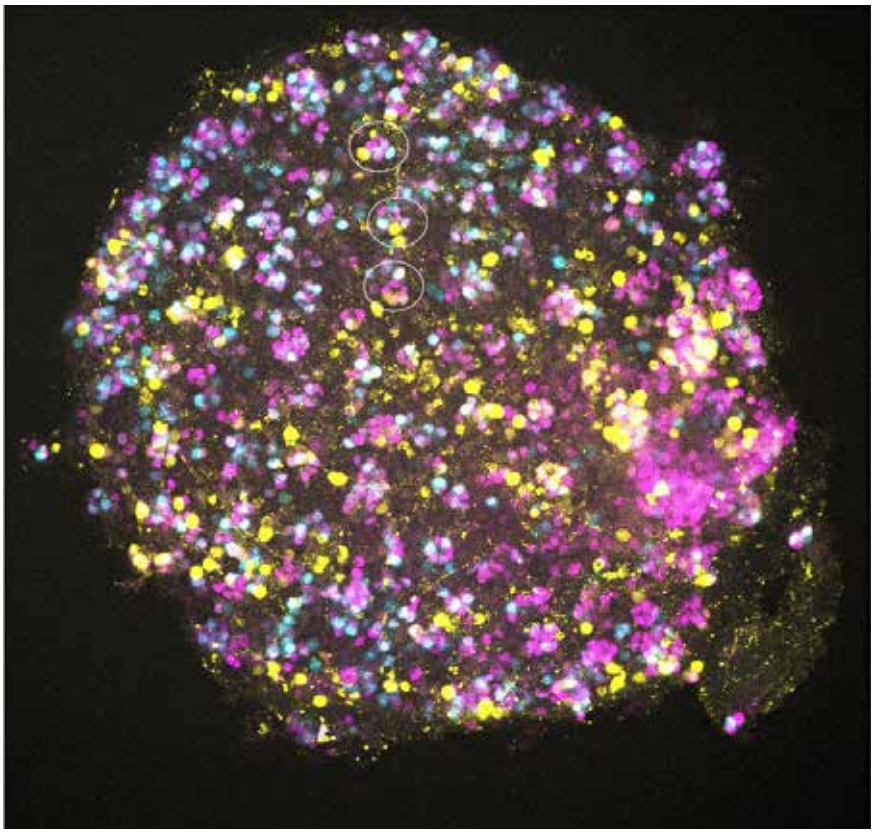

Figure 1:

Rosette structures (white circles) resembling simple invertebrate ommatidia established by vertebrate retinal cell types after seven days of organoid culture. Cyan: cone photoreceptors. Magenta: photoreceptors and bipolar cells. Yellow: retinal ganglion cells.

typically establish a layered structure similar to their *in vivo* counterparts, we revealed that interfering with polarity cues led to a re-organization into minimal units, strikingly reminiscent of basic invertebrate ommatidia. This discovery opens up the possibility of using organoids under relaxed constraints as an „evolutionary time machine," an angle we are actively pursuing.

To address the lack of precise manipulation tools for organoids, we've been collaborating intensively within the 3DMM2O cluster.

In collaboration with K. Goepfrich we established and employed DNA micro-beads to deliver a morphogen cargo in a temporo-spatially controlled manner. We demonstrated UV-triggered morphogen release, forming a gradient that induces retinal pigmented epithelium (RPE) while maintaining neuroretinal cell types. This technique allows precise engineering of organoid differentiation and introduction of specific functionalities.
In collaboration with E. Blasco we changed the physical constraints within the developing embryo. We achieved that by 3D printing within the developing fly or fish embryo. This required an injectable, biocompatible ink that does not trigger any immunogenic responses. Finally the non-polymerised material should be resorbed by the embryo to actually expose the surrounding cells to the physical constraints caused by the *in organismo* printed structures. In a proof of concept study we achieved all that and are now en route to apply *in organismo* 3D printing to challenge the embryo with physical cues it has not had time to adapt to in evolution.

All of those activities crucially depended on continuous method development across scales. One big competitive advantage allowing to develop quantitative high throughput assays for the analysis of complex traits was the extraordinary collaboration with G. Hofmann ((micro) mechanical workshop). Long term collaborations with E. Birney (EBI) and K. Naruse (NIBB), the cluster 3DMM2O (C. Selhuber-Unkel, E. Balsco, K. Göpfrich) and T. Baumbach and F. Loosli (KIT) provide the complementary expertise as basis for shifting the frontiers into the uncharted territories.

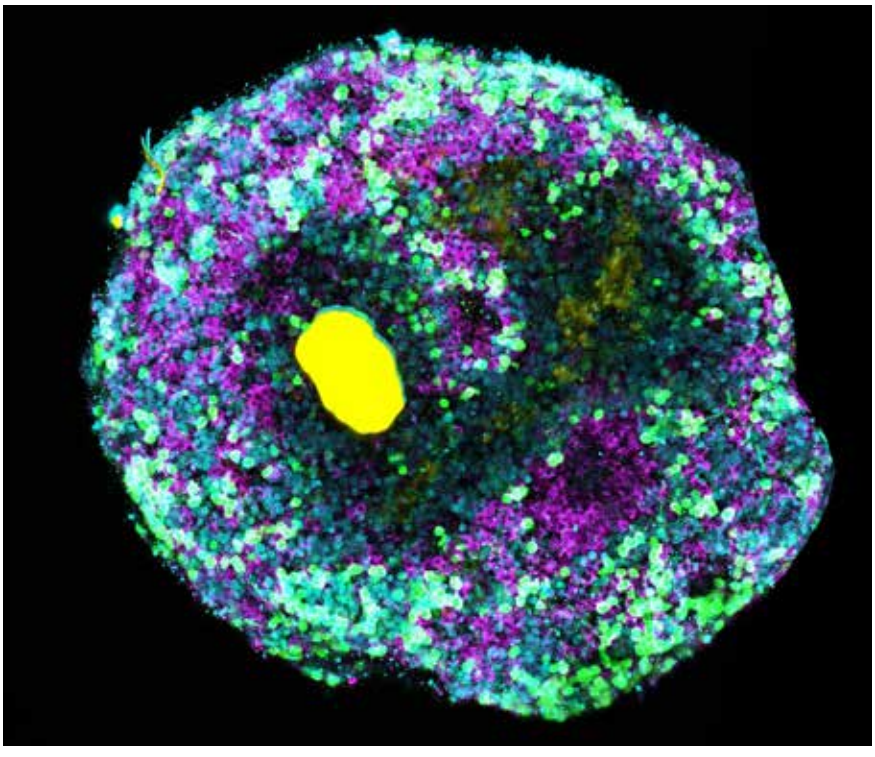

Figure 2:

Cargo loaded, fluorescently labelled DNA bead (yellow) inside a medaka retinal organoid. Light induced release exposes cargo to various retinal cell types (green, cyan, magenta)

Future directions

In the coming years the focus of the lab will be on cluster topics as well as on the exploitation of the COS-MIKK panel, the inbred lines kept at COS we have established from a wild population in Japan. The interface to engineering, physics and chemistry in 3DMM2O has established a vibrant environment for new discoveries. Based on our projects on DNA-bead-mediated morphogen release and on *in organismo* printing together with the discovery of evolutionary building blocks, we will reach out to design and program functions. Rather than recapitulating development we aim to use them as a substrate for the engineering of new functionalities building on the collaborative spirit and synergistic expertise within the cluster.

The analysis of polygenic traits will be continued and extended. We are just concluding the analysis of the impact of (environmental) drugs on heart rate and whole body morphology. We will pursue mapping and validation by targeted genome editing (base editing, snp-flip). The responses to the four compounds tested (ethanol, disulfiram, caffeine, ziram) is highly strain specific, not centrally mediated by the general detoxication machinery. The connection between highly specific responses and well established detoxication pathways will be of particular interest.

The analysis of the genetic basis of visual features exploits the opto-motor-response of fish larvae (Figure 3). The high throughput setup we have established allows to tune all relevant parameters and automatically retrieve the quantitative features of the individual fish tested in individual infinity pools.

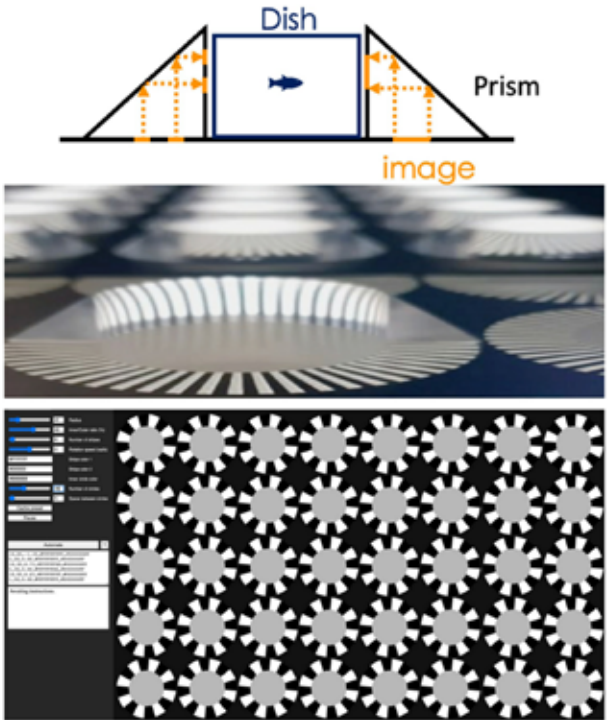

Figure 3:
High throughput setup quantifies visual parameters of individual larvae. Prism projects tuneable patterns from 4K monitor to walls of small petri dishes (infinity pools) for recording larval opto-motor-response.

Addressing retinal regeneration, we had found that the standard lab strain (Cab) does not regenerate but rather repairs retinal injuries. In contrast to zebrafish, upon injury Cab Mueller Glia cells form rod photoreceptors without being restored (repair). Performing the manual injury assay across selected lines of the COS-MIKK panel showed that individual lines display distinct features ranging from repair to full retinal regeneration. The current manual assays don't have the throughput for the phenotype/genotype correlation required.

In close collaboration with the micro-mechanical workshop (Gero Hofmann) we will establish new high throughput quantitative phenotyping pipelines allowing to map the contributing loci to tackle long standing questions in the field.To ensure progress in all fields we will collaboratively continue developing the cutting edge technology across scales enabling our disruptive approaches.

Selected publications since 2021

*equal contribution
co-correspondence

Afting, C.*, Walther, T.*, Drozdowski, O.M., Schlagheck, C., Schwarz, U.S., **Wittbrodt, J.**#, and Göpfrich, K.# (2024) DNA Microbeads for Spatio-Temporally Controlled Morphogen Release within Organoids. Nature Nanotechnol. 10.1038/s41565-024-01779-y.

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Publication statistics

https://scholar.google.de/citations?user=8QOk6-QAAAAJ&hl=de

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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 put in place, 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 concentrations of anthropogenic contaminants. Given the trend towards alternative test methods, fish cell culture and embryo toxicity testing systems are being developed by the COS 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 2021
Since the 2021 COS report, major activities of the Aquatic Ecology and Toxicology Group at the COS have focused on the following topics:

General and neurotoxicity testing: A whole set of behavior-based endpoints in (zebra)fish embryos has now been established to complement our list of endpoints for the identification of (developmental) neurotoxicity. Meanwhile, we have entered the stage of international validation preceding the submission of appropriate draft test guidelines to the OECD Test Guideline committee. Major progress has been made to identify potential reasons for the failure of zebrafish embryos to detect neurotoxic substances during early stages of development: Given that until the age of approx. 72 h, zebrafish embryos breathe *via* the skin, but not *via* the gills, the “respiratory failure syndrome” might be a major trigger; a novel method for the simultaneous detection of breathing frequency and amplitude in zebrafish (*Danio rerio*) embryos and larvae has been developed (Figure 1). Together with colleagues from Australia, a major review into mechanistic foundations in aquatic eco-neurotoxicology has been published (e.g., Figure 2).

Teratogenicity testing: Within the Horizon 2020 project EU-ToxRisk, further arguments have been added to establish the zebrafish embryo as an alternative to mammalian teratogenicity testing. Atlases have been published to systemize and categorize morphopathological alterations in zebrafish development, and first attempts to discriminate between chemicals of similar molecular structure *via* the response syndrome during chemical testing have been successful.

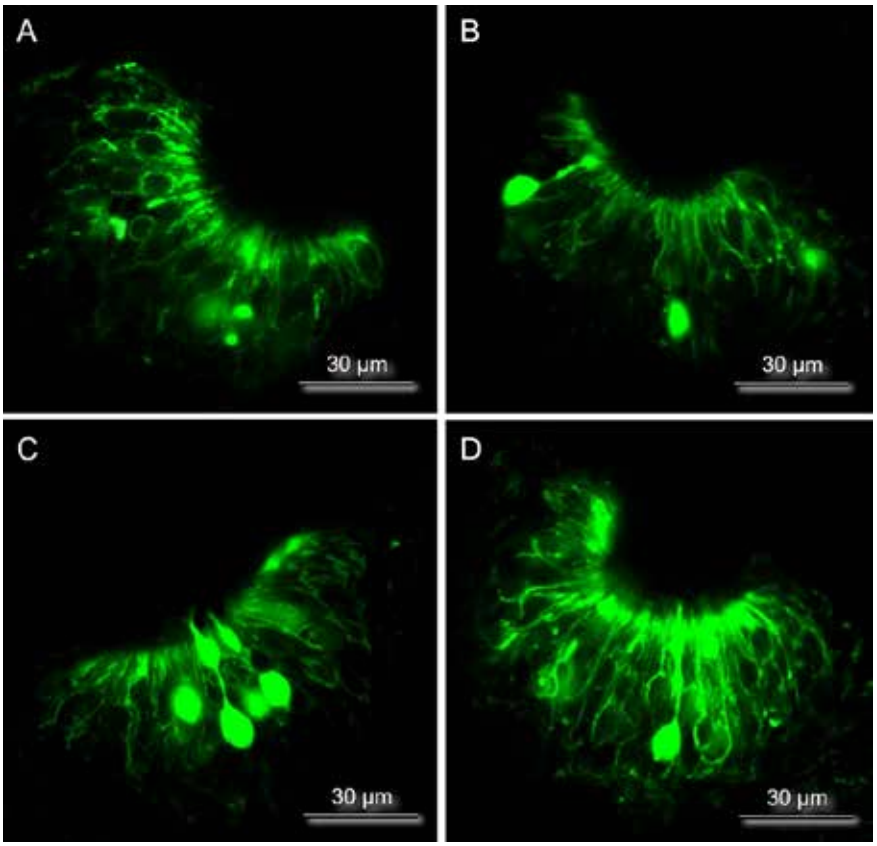

Figure 1:

Z-stack images (A–D) of the olfactory epithelium of 72 h old embryos of zebrafish (*Danio rerio*) under the confocal laser scanning microscope after staining with rhodamine 123 (2-(6-amino-3-imino-3H-xanthene-9-yl)-benzoic acid methyl-ester in 1.5% DMSO). (E) Concentration-dependent decline of fluorescence intensity after exposure to cobalt chloride. From: Wlodkowic et al. 2022.

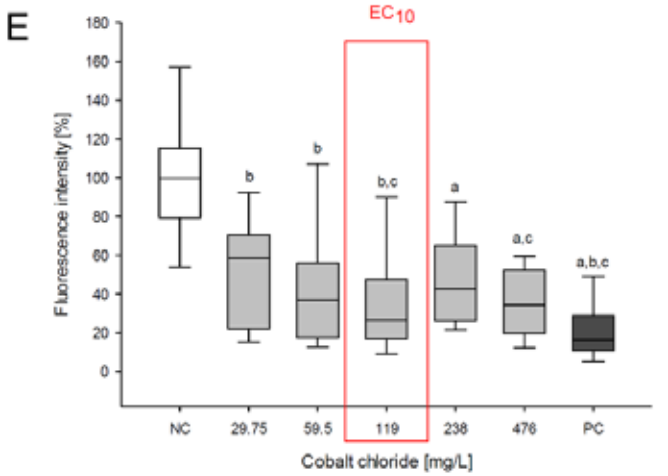

Figure 2:

Graphical representation of the adverse outcome pathway (AOP) leading from thyroperoxidase inhibition to altered visual function *via* altered retinal layer structure (AOP 363). Numbers preceded by # refer to AOP-Wiki IDs. Overall confidence levels resulting from the weight-of-evidence evaluation considering both the biological plausibility and the empirical evidence of the key event relationships have been included (see also Table 1). KER = key event relationship; MIE = molecular initiating event; TH = thyroid hormone; TPO = thyroperoxidase. From: Gölz, L., Baumann, L., Pannetier, P., Braunbeck, T., Knapen, D. and Vergauwen, L. (2022) AOP report: Thyroperoxidase inhibition leading to altered visual function in fish *via* altered retinal layer structure. Environ. Toxicol. Chem. 41: 2632-2648.

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. Together with the MiWa consortium, a battery pf biological tests has been successfully applied to identify long-term effects of microplastic exposure and to initiate a debate on rethinking the relevance of microplastics as vectors for anthropogenic contaminants.

Endocrine disruption: Within the scope of EU-funded programs, a novel test combining adult and early developmental exposure to thyroid-manipulating endocrine disruptors has been established. For developmental malformations of the eyes of zebrafish embryos, an Adverse Outcome Pathway has been developed (Figure 3).

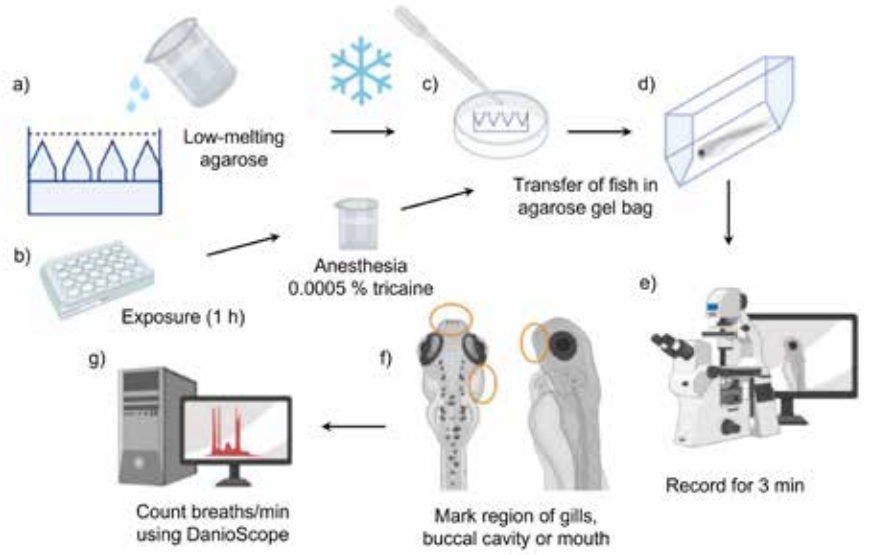

Figure 3:
Overview of experimental set-up of breathing frequency (fv) measurement and analyses with DanioScope™. (a) Agarose gel bags are prepared using low melting agarose and a polymethylmethacrylate casting mold with wedge-shaped "teeth" (Sabaliauskas et al., 2006). (b) After zebrafish (*Danio rerio*) were exposed to 5 ml of the lindane solution for 1 h in a 12-well-plate, they were anesthetized with 50 mg/L tricaine (≙ 0.005%) and transferred to one of the agarose gel bag (c). Fish were oriented in a way that either the opercula or the mouth were visible from top (d)). After 3 min of video recording (e) and conversion with the software Handbrake, the region of interest is marked (f) with the DanioScope™ software, using the "Chorion" button at activity settings. Finally, breaths per min are counted automatically (g). From: Kämmer, N., Reimann, T., Ovcharova, V. and Braunbeck, T. (2023) A novel automated method for the simultaneous detection of breathing frequency and amplitude in zebrafish (*Danio rerio*) embryos and larvae. Aquat. Toxicol. 258: 106493.

Selected publications since 2021

Gölz, L., Baumann, L., Pannetier, P., **Braunbeck, T.**, Knapen, D. and Vergauwen, L. (2022) AOP report: Thyroperoxidase inhibition leading to altered visual function in fish *via* altered retinal layer structure. Environ. Toxicol. Chem. 41: 2632-2648.

Gölz, L., Blanc-Legendre, M., Rinderknecht, M., Behnstedt, L., Coordes, S., Reger, L., Sire, S., Cousin, X., **Braunbeck, T.** and Baumann, L. (2024) Development of a zebrafish embryo-based test system for thyroid hormone system disruption: 3Rs in ecotoxicological research. Environ. Toxicol. Chem.; doi: 10.1002/etc.5878.

Hanslik, L., Huppertsberg, S., Kämmer, N., Knepper, T.P. and **Braunbeck, T.** (2022) Rethinking the relevance of microplastics as vector for anthropogenic contaminants: Adsorption of toxicants to microplastics during exposure in a highly polluted stream - Analytical quantification and assessment of toxic effects in zebrafish (*Danio rerio*). Sci. Total Environ. 816: 151640.

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Publication statistics

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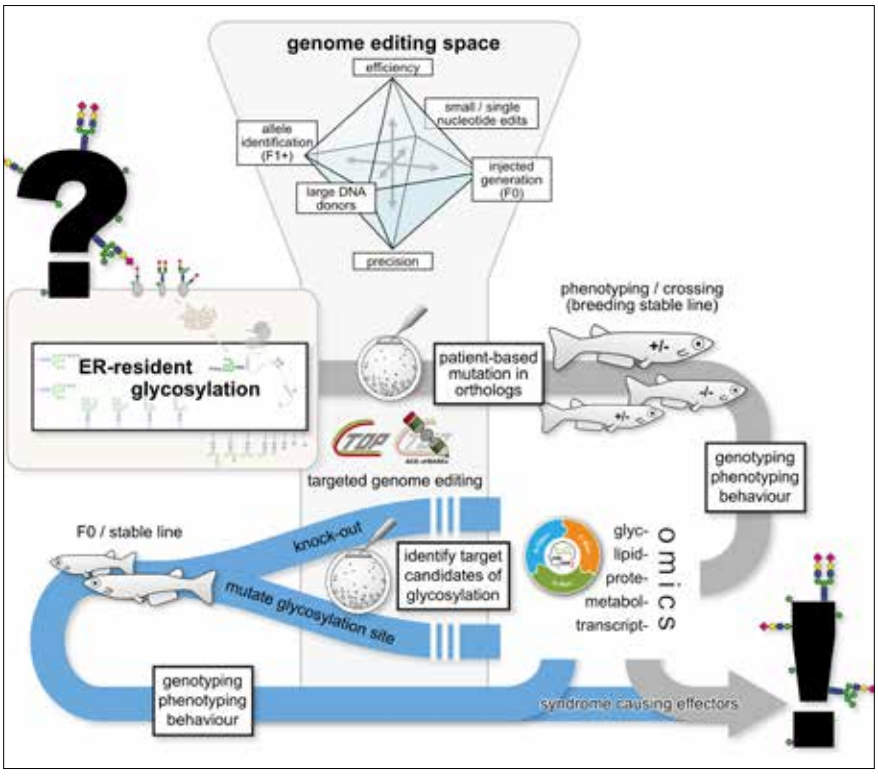
Fields of Interest
Developmental biology, targeted genome editing, CRISPR/Cas9, base editing, congenital disorders of glycosylation, patient allele-based modeling, glycobiology, population genetics, GWAS

Summary and outlook
Glycosylation, the addition of sugar moieties to proteins, is an essential post-translational modification. Changes within its endoplasmic reticulum(ER)-resident machinery severely impact organismal development and physiology. Patients with rare congenital disorders of glycosylation (CDG) exhibit multisystemic phenotypes primarily in skeletomuscular and neuronal structures. Using our advanced targeted genome editing toolbox, we generate fish models based on CDG-patient genetics to investigate embryonic development and homeostasis under hypo-glycosylation. These models recapitulate the multisystemic patient phenotypes. By integration of phenotyping, genetics, and omics (collaborative within FOR2509), we unravel how aberrant glycosylation differentially affects cell types in an organismal context and throughout development.



Modifying key enzymes of the *N*-glycosylation, *O*-Mannosylation, and *C*-Mannosylation pathways to recapitulate patients' hypo-glycosylation states, we identify target glycoprotein candidates that drive the phenotypic development when misglycosylated.

Research highlights since 2021

Modeling patient-specific alleles in medaka (*Oryzias latipes*) is challenging due to the generation of allelic variety in canonical CRISPR/Cas9 approaches. In the Wittbrodt lab, we enhanced the Cas9 fidelity using the "hei-tag" that improves genome editing efficiency and reduces allelic variance (Thumberger et al., 2022). In most CDG-patients however, single nucleotide polymorphisms are causative for the hypo-glycosylation state. To model these, base editors are available that introduce predictable nucleotide transitions. We upgraded our online CRISPR design tool CCTop *feat.* ACEofBASEs to evaluate target sites for the use with base editors (Cornean et al., 2022). These rely on a protospacer adjacent motif (PAM) at a specific distance, rendering modeling of many patient mutations inaccessible. We overcame this by sequential, combinatorial base editing termed "inception": Using an A-to-G base editor to create a novel PAM (step1) and simultaneously applying a second base editor that binds to the new PAM for the desired editing (step2; Pakari et al., 2023). "Inception" broadens the base editing scope by ~60%, enabling previously impossible specific edits.

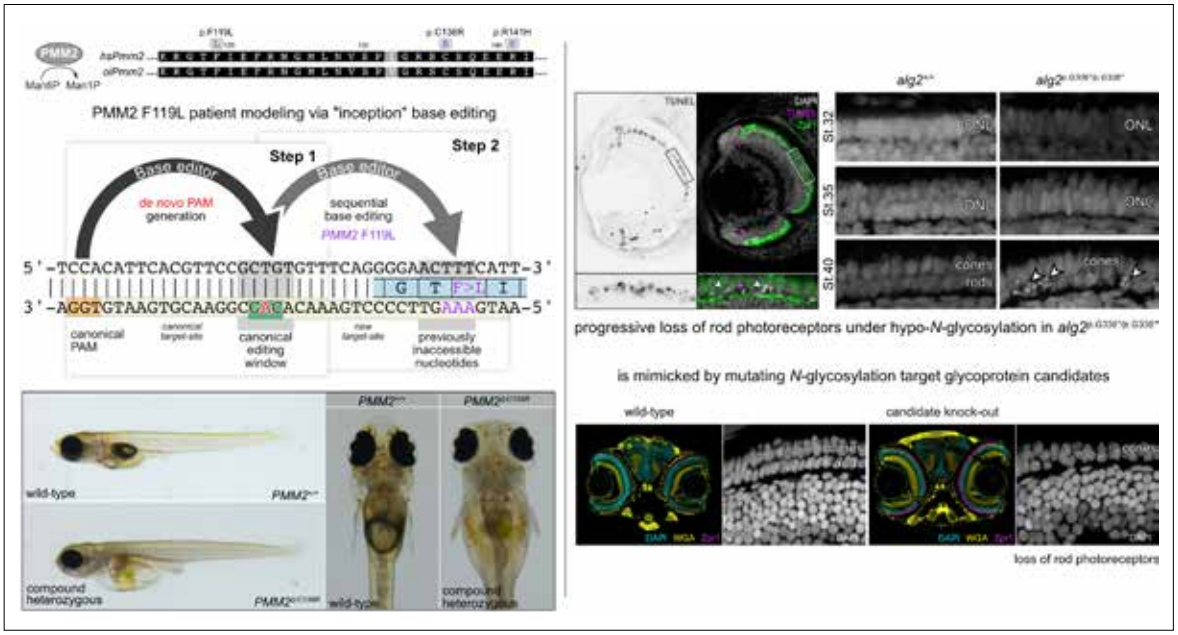


Figure 2:

Modeling of common PMM2-CDG mutation (F119L) in medaka via sequential, combinatorial base editing ("inception") and further alleles phenocopy the patient syndrome. Progressive loss of rod photoreceptors under hypo-N-glycosylation recapitulated by loss of candidate glycoprotein.

With these methods, we create hypo-glycosylation fish models at different steps in the ER-resident glycosylation machinery. Availability of Mannose-1-Phosphate is the basis for this machinery provided by the Phosphomannomutase-2 (PMM2). Man1P serves as precursor during *N*-glycan-synthesis, as well as for *C*- and *O*-Mannosylation. PMM2-CDG are most abundant with differing degrees of severity. Modeling patient, point-mutation and deletion alleles in medaka PMM2 underscore a structure-function relationship reflected by PMM2 activity and severity of phenotypes. This allows to tackle key effectors of the syndrome and to understand the biology of supplementation rescues in an organismal context.

In a specific hypo-*N*-glycosylation model recapitulating the multisystemic phenotypes of ALG2-CDG-patients, the systemic reduction of glycans differentially impacts on different cell types (Gücüm et al., 2021). Initially formed rod photoreceptors fail to persist while cone photoreceptors remain. Collaboratively within the FOR2509 research unit, we used

omics to identify potential target glycoproteins for this progressive loss. Comparing knock-out and specific modification of the glycan binding sites in these candidates provides structure-glycosylation-function relationship, revealing novel effectors ensuring continuous photoreceptor viability.

Future directions

CDG patients with identical mutations often show varying phenotypic severity, while our fish models exhibit remarkably consistent phenotypes. This contrast indicates the importance of the genetic background in phenotypic expression. Building on our discoveries of stringent sequence-glycosylation-phenotype relationships, I want to investigate the genomic regulation of glycosylation. Analyzing allelic series revealed that different changes of the glycosylation machinery enzymes (structure, abundance, stability, kinetic activity) impact embryonic development and individual phenotypes at varying degrees. With the unique medaka inbred panel (MIKK-panel; Fitzgerald et al., 2022) at hand, that is based on inbreeding lines derived from a wild population of medaka, I want to identify the underlying genetic regulation of glycosylation beyond pathological conditions. In a healthy state, each individual has a unique glycome fingerprint, i.e. the composition of all glycan structures. We uncovered a genetic foundation since the glycomes among individuals of a particular MIKK-line are more similar compared to individuals of different lines. Based on my involvement in quantitative trait/genome association projects across the MIKK-panel in the Wittbrodt lab, along with my glycosylation studies, patient model results, and genome editing expertise, I want to investigate the fundamentals of the glycosylation machinery to explore broader implications of glycosylation in developmental biology.

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Publication statistics

https://scholar.google.com/citations?user=_1q0rHkAAAAJ

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Fields of Interest
Development of 3D/4D imaging techniques, X-ray tomography, correlative imaging, digital anatomy

Summary and outlook
The field of structural cell biology has been transformed in recent years. On one side, we can now examine genes and the molecular composition of individual cells within the human body. On the other hand, high-resolution imaging techniques have enabled us to locate and understand the anatomy of specialized cells, tissues and organs.

Among various visualisation methods, X-ray imaging has emerged as a powerful technology. The high penetration depth of X-rays and their wide range of energies make them adaptable to a variety of scientific challenges. Recent developments in advanced imaging modalities, such as phase contrast, soft X-rays, and X-ray microscopy, have opened up unexplored directions for life science applications, particularly in nanoscale imaging of whole human cells and creating digital anatomical atlases for education and modelling. When combined with other imaging techniques like fluorescence and electron microscopy, X-ray imaging holds great promise for basic life science research, understanding diseases, and developing novel therapeutic approaches in medicine.

At present, my group is focused on the development of correlative imaging approaches which enable simultaneous localisation of proteins of interest within tissue and organs of whole model organisms, including X-ray tomography of single cells with tens of nanometres spatial resolution.

Research highlights since 2021
Understanding the spatial distribution of gene activity is crucial in developmental and cell biology, offering insights into an organism's developmental processes and disease mechanisms. For example, to address the progression of retinal tissue development in fish primary embryonic pluripotent cells, we employed live imaging with light-sheet microscopy. In collaboration with Lucie Zilova and members of the Wittbordt lab, we have analysed the mode of cell migration at the early stages of retinal organoid development (Zilova, Weinhardt, et al. 2021). While 4D live imaging enables analysis of molecular mechanisms within tissue, the information on the natural and often crowded environment where cells migrate was missing. To address this limitation, we developed a novel approach that combines light sheet microscopy with established previously contrast-enhanced X-ray microtomography (Weinhardt et al. 2018). The combination of these techniques enables the simultaneous detection of fluorescent markers and visualization of tissue structure at micrometer-scale resolution in organisms and organoids spanning hundreds of micrometers (Figure 1). Our multimodal imaging approach demonstrates versatility in visualizing gene expression through both protein-specific antibody labeling and fluorescence RNA in situ hybridization (Kairišs, Sokolova et al., 2024). This capability allows to reveal cellular and molecular composition in whole organisms and less structurally defined *in vitro* models, such as organoids.

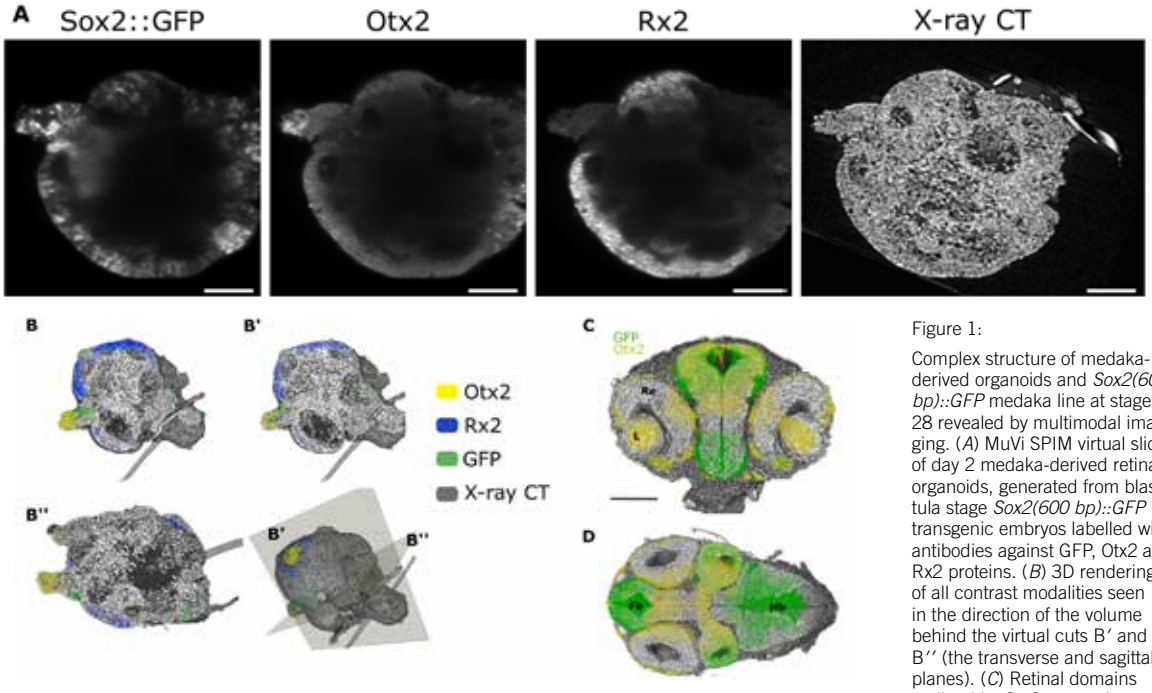

Figure 1:

Complex structure of medaka-derived organoids and *Sox2(600 bp)::GFP* medaka line at stage 28 revealed by multimodal imaging. (*A*) MuVi SPIM virtual slices of day 2 medaka-derived retinal organoids, generated from blastula stage *Sox2(600 bp)::GFP* transgenic embryos labelled with antibodies against GFP, Otx2 and Rx2 proteins. (*B*) 3D renderings of all contrast modalities seen in the direction of the volume behind the virtual cuts B′ and B′′ (the transverse and sagittal planes). (*C*) Retinal domains outlined by Otx2 expression only and non-retinal neuronal domains expressing sox2 and Otx2 in axial cuts along the eye and eye with optical nerve. (*D*) Specificity of sox2 expression to the neuronal non-retinal domain in coronal sections of the brain region. Organs are labelled as follows: lenses (L), retina (Re), forebrain (Fb), and hindbrain (Hb). The scale bar is 100 µm.

To understand the composition and phenotypic differences at the cellular level, my group is developing a unique imaging method – soft X-ray tomography – to visualise whole eukaryotic cells with a few tens of nanometers spatial resolution, minimal sample preparation, and quantitative contrast of protein-rich intracellular structures (Weinhardt et al, 2020). The technique is particularly suitable to study cells under various pathological conditions, for example, cell remodelling by SARS-CoV-2 (Loconte et al., 2021) and Herpes viruses (Chen et al., 2022). Recently, we have introduced a new imaging geometry called half-acquisition tomography to enable imaging of large human cells, commonly seen in cancer research (Ekman et al, 2023). This approach offers imaging of twice larger cells without compromising spatial resolution or image contrast. X-ray projection, virtual slice through the 3D volume and 3D renderings obtained by our method of anatomical details on single cells are shown in Figure 2. At the moment, this technology is available in five laboratories around the world. To broaden its use further, in collaboration with CoCID consortium (www.cocid.eu), we are working on the development of a laboratory soft X-ray microscope (Fahy et al., 2021). Currently, laboratory soft X-ray microscopy enables 3D ultrastructural imaging of dried and cryogenically preserved cells (Chatzimpinou et al., 2023).

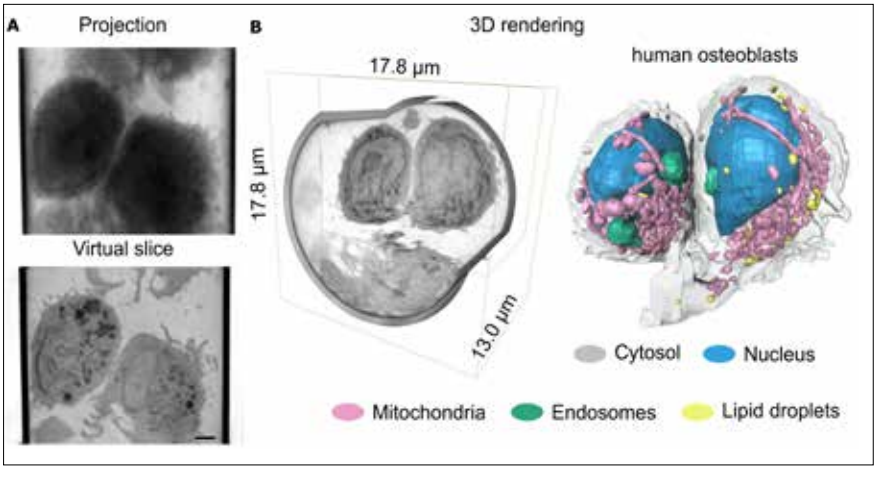

Figure 2:

Extension of the SXT imaging volume using the HA scheme with a 50 nm FZP. (A) Merged mirroring projections (top) and virtual slice through the reconstructed SXT volume (bottom) of two U2OS cells inside a glass capillary. (B) 3D rendering of the SXT volume (left) and segmentation of cells (right) shown in SXT volume of 17.8 µm × 17.8 µm × 13.0 µm contains two organelle-rich U2OS cells, ice, and surrounding dense glass capillary.

Future directions
In the coming years, the focus of the group will be on the further development of correlative modalities at the level of whole organisms and single cells. We will continue exploring a combination of light-sheet and X-ray tomography for imaging of cleared and expanded specimens. This will enable understanding of whole organisms, in particular mouse and human organoids, in 3D at the molecular and structural level. These activities should enable the generation of digital atlases with both functional and anatomical information present.
At the microscopic level, we will continue to work on the development of high-throughput laboratory soft X-ray microscopy of single cells. In particular, we will focus on further development of automatic segmentation of cell anatomy. Using convolution neuronal networks, we have developed automatic segmentation of cell cytoplasm (Erozan et al., 2024). This work will be extended to automatic analysis of the nucleus structure, including hetero- and eu-chromatin distribution, analysis of mitochondria states and analysis of lipid droplet composition.

To link this structural information to the function of genes and proteins, we will follow our approach at the organismal scale and combine soft X-ray microscopy with super-resolution fluorescence microscopy. In particular, we are interested in the development of axial super-resolution fluorescence tomography for small organisms, tissues and single cells.

Selected publications since 2021
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Loconte, V., Chen, J.-H., Cortese, M., Ekman, A., Le Gros, M., Larabell, C., Bartenschlager, R., **Weinhardt, V.** (2021). Using soft X-ray tomography for rapid whole-cell quantitative imaging of SARS-CoV-2-infected cells, Cell reports methods, https://doi.org/10.1016/j.crmeth.2021.100117

Publication statistics
https://scholar.google.com/citations?user=Jg0F2wgAAAAJ&hl=en

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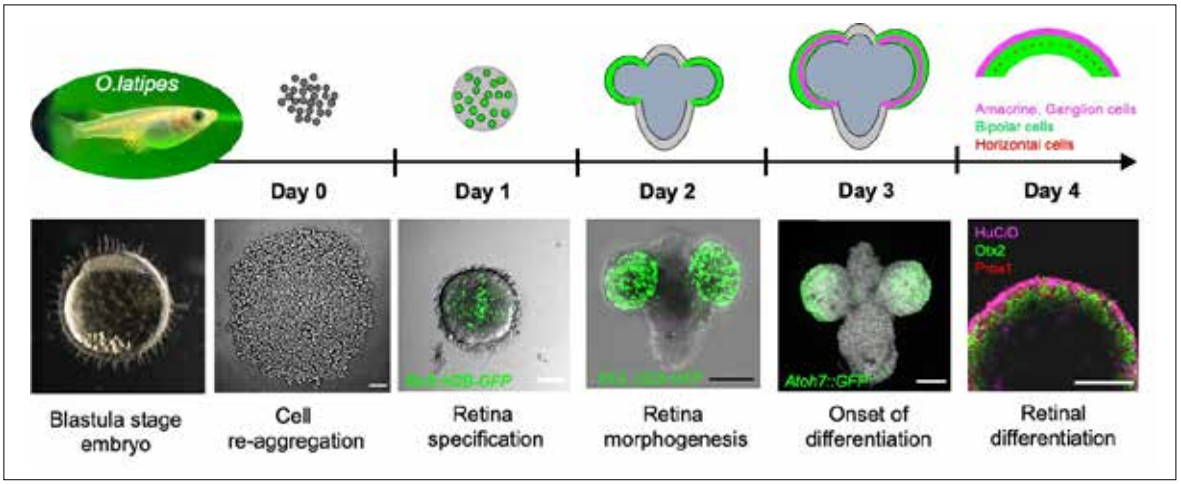
Fields of Interest
Eye development, pluripotent embryonic stem cells of medaka fish (*Oryzias latipes*), organoids, self-organization of pluripotent embryonic stem cells into organ-like structures, cell fate decisions, retinal stem cells

Summary and outlook
During embryonic development, cells are exposed to changing environment that together with intrinsic genetic programs shape cell fate decisions, and tissue morphogenesis. Pluripotent embryonic stem cells display remarkable ability to self-assemble into organ-like structures when cultured *in vitro*, providing a model to study mechanisms regulating organ formation. Using fish (*Oryzias latipes*) pluripotent cells, we are establishing non-mammalian organoid models to elucidate fundamental biological processes that enable pluripotent cells to aggregate, take a specific fate and eventually form complex organ-like structures such as eye. By exploring cell-cell, cell-ECM interaction, hard-wired genetic programs and cellular metabolism, we aim to understand how these processes cooperatively control cell fate choices and eventually organ formation *in vitro* and *in vivo*.

Figure 1:
Generation of organoids from medaka pluripotent cells



Research highlights since 2021
Fish pluripotent cells assemble in retinal tissue mirroring early steps of eye development
Although organoids derived from mammalian embryonic stem cells are well established, their use shows some limitations such as long generation time, complicated genetic manipulation and low reproducibility. To overcome those limitations, we established non-mammalian retinal organoid models using pluripotent embryonic cells derived from fast developing medaka fish. We showed that blastula stage embryo-derived cells efficiently assemble into retina tissue mirroring key steps of early eye development *in vivo* including

eye field specification (expression of *Rx3* gene), optic vesicle morphogenesis and differentiation of individual retinal cell types such as photoreceptors, retinal interneurons and retinal ganglion cells. Importantly all of those processes follow natural developmental timing of medaka fish resulting in retina formation in only 4 days. Taking the advantage of a toolkit to perform genetic manipulation in medaka (developed in the group of Joachim Wittbrodt and Thomas Thumberger) to generate gene loss-of-function and gene reporter lines allows us to study gene function on one site and monitoring specific cell fates on the other site.

Fish pluripotent cells assemble into complex organoids composed of retina and lens
Fast generation time of fish organoids allowed us to probe multiple parameters of the culture in short period of time and discover conditions that promote formation of complex organoids composed of lens wrapped by the retina, a feature that has not yet been described in other organoid models.

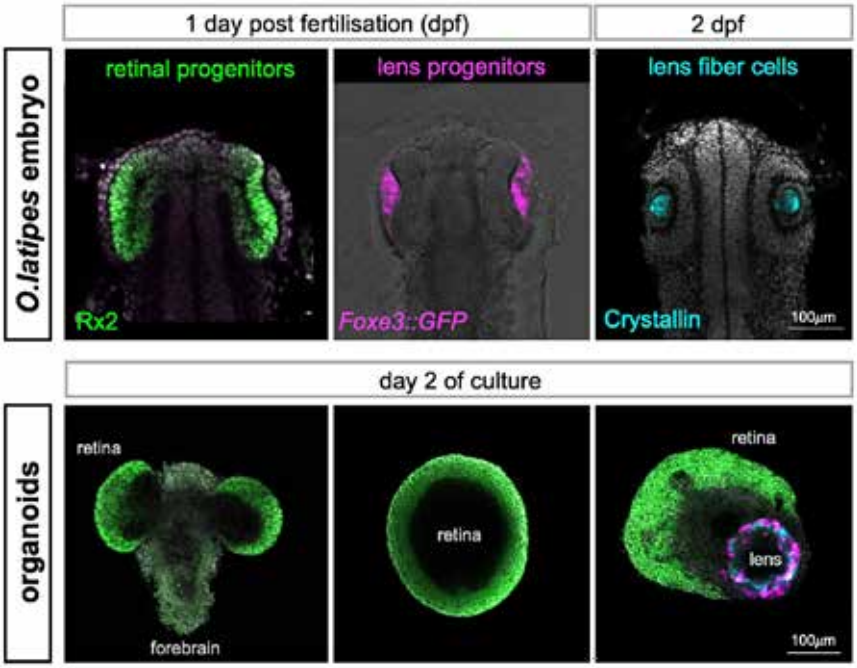


Figure 2:
Distribution of retinal, lens and brain tissues in the embryo; and organoids derived under different conditions.

Genetic background of pluripotent cells impacts on cell fate choices in vitro
Pluripotent embryonic cells have the ability to differentiate in any cell type of the body. Interestingly, we observe that cells originating from the fish with different genetic backgrounds (established in the group of Joachim Wittbrodt) display variable ability to differentiate into retinal tissue and show the bias toward specific cell fates, if cultured *in vitro* pointing to the importance of intrinsic genetic programs as an underlying factor determining responsiveness of cells to the environment. By analyzing transcriptomics and metabolic profile of pluripotent cells (in collaboration with Claudia Muhle-Goll, KIT) of respective fish lines we aim to understand by what molecular mechanisms genetic background impacts on cell fate decisions.

Future directions

The new insights obtained by studying mechanisms of organ formation in fast developing fish organoids will be subjected to the comparative studies to address the level of conservation between different species and the possibility to transfer the newly obtained knowledge into the mammalian organoid system. For example, the generation of lens-carrying retinal organoids from mouse embryonic stem cells will be assessed (with the group of Martin Bastmayer, KIT).

Ease of genetic manipulations together with fast generation time are offering unique possibility of using fish organoids as a screening system to address how environmental and genetic factors impact on cell fate decisions during retinal development. Generation or synthetic retina composed of cells incorporated in 3D printed scaffold is one of the objectives of the excellence cluster 3DMM2O. In collaboration with the group of Pavel Levkin (KIT) we aim to establish a high-throughput assay to probe a large array of biomolecule hydrogel combinations for their ability to support differentiation of individual retinal cell types. Similar screening approach will be employed to explore molecular composition of the retinal stem cell niche in medaka fish and its ability to establish, maintain and regenerate retinal stem cell domain (in collaboration with the group of Joachim Wittbrodt).

Selected publications since 2021

Kairišs, K., Sokolova, N., **Zilova, L.**, Schlagheck, C., Reinhardt, R., Baumbach, T., Faragó, T., van de Kamp, T., Wittbrodt, J., Weinhardt, V. (2024). Visualisation of gene expression within the context of tissues using an X-ray computed tomography-based multimodal ap proach, Scientific Reports, https://doi.org/10.1038/s41598-024-58766-5

Sokolova, N., **Zilova, L.**, and Wittbrodt, J. (2023) Unravelling the Link between Embryogenesis and Adult Stem Cell Potential in the Ciliary Marginal Zone: A Comparative Study between Mammals and Teleost Fish; Cells Dev. 174:203848. doi: 10.1016/j.cdev.2023.203848.

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Publication statistics

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RESEARCH CONSORTIA

3

3.1 DFG CRC873
MAINTENANCE AND DIFFERENTIATION OF STEM CELLS IN DEVELOPMENT AND DISEASE

2010-2022

Spokesperson:
Prof. Dr. Jan Lohmann,
COS, Department of Stem Cell Biology

www.sfb873.de

The overarching goal of the CRC873, which ran from 2010-2022 was to define the regulatory principles underlying the balance between maintenance, expansion and differentiation of stem cells in diverse systems on a mechanistic level.

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. Therefore, the CRC873 consortium took full advantage of a diverse set of model systems from plants to human to illuminate the cellular and molecular mechanisms governing stem cell function. Using organismal diversity to derive important insights into the evolution of stem cells and the underlying regulatory modules, made the CRC873 into a truly pioneering consortium.

The CRC873 studied 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 focused on diseases such as cancer or infection, since they represented important models for stem cell dysregulation and served as steppingstones for translational research. During the three funding periods, the members of the CRC873 developed into a tightly connected community. Consequently, our subprojects were productive and 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 third 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, has been a focus in the final years of the consortium. Similarly, the question of the evolutionary origin of stem cells and their regulatory mechanisms attracted 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 made evolutionary studies more meaningful, since they allowed direct comparisons of highly defined cell types and thus filter out the noise caused by divergent cell compositions.

In summary, the CRC873 focused 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 approached these features across species and kingdom boundaries *in vitro* and *in vivo*. Our mission was to mechanistically elucidate the components identified so far and to rigorously compare the defined modules between the diverse systems. A follow up CRC with a focus on growth control and coordination is currently planned with broad support from the COS community.

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Prof. Dr. Thomas Greb
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Prof. Dr. Anna Marciniak-Czochra

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

Members of the CRC873 giving outreach lectures in the Karlstor Bahnhof Bar, Heidelberg.

Key publications

Wallner, E.S., Tonn, N., Shi, D., Luzzietti, L., Wanke, F., Hunziker, P., Xu, Y., Jung, I., Lopéz-Salmerón, V., Gebert, M., Wenzl, C., Lohmann, J.U., Harter, K., Greb, T. (2023). OBERON3 and SUPPRESSOR OF MAX2 1-LIKE proteins form a regulatory module driving phloem development. Nat Commun. 14(1): 2128. doi: 10.1038/s41467-023-37790-5.

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3.2 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

http://sfb1324.de/

The Wnt signaling pathways are of crucial importance for many biological processes and diseases in animals and humans. They play a crucial role in early development, cell differentiation and regeneration. Misregulation of Wnt signaling can lead to severe developmental disorders and diseases, including tumorigenesis. The overall aim of the Collaborative Research Centre (CRC) 1324 is to gain a mechanistic understanding of Wnt signaling pathways and to investigate their physiological consequences during development and disease in a representative range of model systems. Wnt ligands are secreted, lipidated proteins that occur exclusively in animal systems. Different 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. Despite considerable progress in this field, fundamental questions remain about the specificity and context-dependence of signaling mechanisms and the control of signaling during development and disease.

As part of the CRC 1324, COS PIs have established a close and productive collaboration between research groups working on the Wnt signaling pathway with other Heidelberg research institutions such as the Medical Faculties in Heidelberg and Mannheim, the Biochemistry Centre (BZH), the Institute for Applied Mathematics, the European Molecular Biology Laboratory (EMBL) and the DKFZ, but also with the Karlsruhe Institute of Technology (KIT) and the University of Göttingen. Through complementary approaches and the integration of state-of-the-art quantitative technologies, CRC 1324 is in a unique position to answer fundamental questions in Wnt signaling. A unique aspect of CRC 1324 is also the broad spectrum of developmental model systems such as *Hydra*, *Xenopus*, *Drosophila*, mouse and human cell models.

In the second funding period, we have successfully continued our interdisciplinary approach with a large number of projects and are now concentrating on fundamental aspects of Wnt signaling at the organism level. There are two main areas of research here: (A) Wnt secretion and receptor-ligand interactions. Here we analyze 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 trigger different signaling cascades. (B) Wnt coupling to downstream and context-dependent signaling. Here we address the important question of how different Wnt signaling pathways trigger different biological responses or how Wnt signaling is coupled to different downstream factors. Furthermore, we analyze the spatio-temporal dynamics of Wnt signaling to understand how oscillations and wave patterns are generated and regulated.

In summary, the CRC 1324 pursues an interdisciplinary approach that integrates different modelling systems and state-of-the-art technologies to investigate fundamental questions of Wnt signaling secretion and transmission as well as their spatio-temporal dynamics in a context-dependent manner. In this way, we contribute to the understanding of the complexity of Wnt signaling pathways and their role in development, regeneration and tumorigenesis. The CRC 1324 has gained a great international reputation, which is also manifested in the great interest in the international Wnt conference in September 2024, which will take place in Heidelberg again after 2018.

Members of the 1st and 2nd funding period

Heidelberg University
Prof. 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 (Institute of Applied Mathematics)
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. Christof Niehrs
Prof. Dr. Ana Martin-Villalba

EMBL
Dr. Alexander Aulehla

University of Göttingen (UMC)
Prof. Dr. Holger Bastians
Dr. Julia Gross

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

SFB 1324 Retreat, Young Scientists and PI Meeting at Grenzhof in Heidelberg, 10.-11. May 2023
(Photo: Christoph Bastert Photographie)

Key publications

Holzem, M., Boutros, M., Holstein, T.W. (2024). The origin and evolution of Wnt signalling. Nat Rev Genet. 25, doi: 10.1038/s41576-024-00699-w.

Veschgini, M., Suzuki, R., Kling, S., Petersen, H.O., Bergheim, B.G., Abuillan, W., Linke, P., Kaufmann, S., Burghammer, M., Engel, U., Stein, F., Özbek, S., Holstein, T.W., Tanaka, M. (2023) Wnt/ß-catenin signaling induces axial elasticity patterns of Hydra extracellular matrix. iScience, 26(4):106416. doi: 10.1016/j.isci.2023.106416.

Tursch, A., Bartsch, N., Mercker, M., Schlüter, J., Lommel, M., Marciniak-Czochra, A., Özbek, S., Holstein, T.W. (2022). Injury-induced MAPK activation triggers body axis formation in *Hydra* by default Wnt signaling. Proc Natl Acad Sci U S A, doi: 10.1073/pnas.2204122119.

Ziegler, B., Yiallouros, I., Trageser, B., Kumar, S., Mercker, M., Kling, S., Fath, M., Warnken, U., Schnölzer, M., Holstein, T.W., 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, J.J.M., Tietje, M., Villacorta, L., Benes, V., Fernández-Sáiz, V., Acebrón, S.P. (2021) USP42 protects ZNRF3/RNF43 from R-spondin-dependent clearance and inhibits Wnt signalling. EMBO J 22(5):e51415.

3.3 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 tremendous advancements in large-scale genome sequencing may suggest that understanding life is solely tied to our genome. Yet, there are additional layers of complexity, one of the most diverse being the modification of proteins with various sugar moieties, a process observed across all domains of life. Among the most conserved and widespread glycosylation pathways are N-glycosylation, O-mannosylation, and C-mannosylation. The precise coordination of these processes is critical for proper protein function, influencing everything from accurate folding and localization to the formation of specific gradients of secreted glycosylated morphogens. These glycosylation processes initiate in the endoplasmic reticulum, and it is common for a single protein to be modified by multiple types of glycans. The essential nature of this process is underscored by the wide array of hereditary disorders (congenital disorders of glycosylation, or CDGs) caused by disruptions in these pathways, leading to severe, multisystem syndromes.

The intricate complexity of glyco-modification has posed significant challenges to systematic studies that aim to decipher its function across various biological scales. To tackle this, the Research Unit FOR2509 has assembled experts who explore glycosylation from structural, molecular, cellular, organ, and organismal levels. Their work integrates genetics, structural biology, lipid biochemistry, glycomics, proteomics, molecular and cellular biology, and developmental biology, with an emphasis on patient outcomes. The interdisciplinary approach of FOR2509 has led to notable success, fostering collaborations from structural analysis to animal model research, resulting in numerous joint projects and publications. In December 2020, the project was extended with an annual funding of €1,339,511, and it is set to conclude in February 2025.

The close collaboration during both funding periods yielded new mechanistic insights and innovative approaches to understanding the molecular underpinnings of complex glycosylation disorders. Newly developed animal models for CDGs provide a platform for integrating the group's expertise to explore how altered glycosylation impacts target protein decoration. Evidence has revealed a strong interconnection among the three types of glycosylation, underscoring the importance of a synergistic, team-based approach to unravel structure-function relationships and their broader biological implications. This foundation is crucial for the development of novel diagnostic tools and therapeutic strategies. One example is the discovery of an effective treatment for GFUS-CDG, a newly identified glycosylation disorder: the administration of L-fucose, as detailed in the study by Feichtinger et al. (EMBO Mol Med, 2021).

Members

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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
Prof. Dr. Falk Büttner

MPI for Dynamics of Complex Technical Systems, Magdeburg
Dr. Erdmann Rapp

FOR2509 Teaching Workshop "Protein Glycosylation in Health and Disease" 2023 in Heidelberg

Key publications

Sakson, R., Beedgen, L., Bernhard, P., Alp, K.M., Lübbehusen, N., Röth, R., Niesler, B., Luzarowski, M., Shevchuk, O., Mayer, M.P., Thiel, C., Ruppert, T. (2024) Targeted Proteomics Reveals Quantitative Differences in Low-Abundance Glycosyltransferases of Patients with Congenital Disorders of Glycosylation. Int J Mol Sci. 25:1191. doi: 10.3390/ijms25021191.

Kale, D., Kikul, F., Phapale, P., Beedgen, L., Thiel, C., Brügger, B. (2023) Quantification of Dolichyl Phosphates Using Phosphate Methylation and Reverse-Phase Liquid Chromatography-High Resolution Mass Spectrometry. Anal Chem. 95:3210-3217. doi: 10.1021/acs.analchem.2c03623.

Himmelreich, N., Kikul, F., Zdrazilova, L., Honzik, T., Hecker, A., Poschet, G., Lüchtenborg, C., Brügger, B., Strahl, S., Bürger, F., Okun, J.G., Hansikova, H., Thiel, C. (2023) Complex metabolic disharmony in PMM2-CDG paves the way to new therapeutic approaches. Mol Genet Metab. 139(3):107610. doi: 10.1016/j.ymgme.2023.107610. PMID: 37245379

Hütte, H.J., Tiemann, B., Shcherbakova, A., Grote, V., Hoffmann, M., Povolo, L., Lommel, M., Strahl, S., Vakhrushev, S.Y., Rapp, E., Buettner, F.F.R., Halim, A., Imberty, A., Bakker, H. (2022) A Bacterial Mannose Binding Lectin as a Tool for the Enrichment of C- and O-Mannosylated Peptides.Anal Chem. 94:7329-7338. doi: 10.1021/acs.analchem.2c00742. PMID: 35549177

Feichtinger, R.G., Hüllen, A., Koller, A., Kotzot, D., Grote, V., Rapp, E., Hofbauer, P., Brugger, K., Thiel, C., Mayr, J.A., Wortmann, S.B. (2021). A spoonful of L-fucose-an efficient therapy for GFUS-CDG, a new glycosylation disorder. EMBO Mol Med. 13(9):e14332. doi: 10.15252/emmm.202114332.


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3.4 DFG RESEARCH UNIT FOR2581
QUANTITATIVE MORPHO-DYNAMICS OF PLANTS

2017-2023

Spokesperson:
Prof. Dr. Alexis Maizel
COS, Department Cell and Developmental Biology

http://www.for2581.de/

Morphogenesis of organisms 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 the cell scale can translate into complex structural changes at the tissue or organ scale. Since they continuously form new tissues and organs, plants are ideal models to study how shape is generated, with the added feature that plant cells are strongly mechanically coupled, forcing them to constantly adjust their behaviour relative to each other. Despite significant progress in recent years, an integrated view on how plant organ shape is controlled has been missing.

The multidisciplinary research unit FOR2581, "Quantitative Morphodynamics of Plants", brought together nine biologists, physicists and computer scientists to understand plant morphogenesis quantitatively. Within a short time frame, this small group has catalysed remarkable advances in understanding how plants shape their organs. Through developing and deploying quantitative methods, they produced several high-profile publications that contributed significantly to the state of the art. Important new tools developed by the RU that allow volumetric segmentation of plant cells and advanced quantification of cells in complex tissues are now established standards and benchmarks. Reflecting the relevance of morphodynamics and the attractiveness of the expertise of the RU members, four have moved to (permanent) PI positions since 2019. Finally, the RU has significantly impacted the community by training two dozen young scientists, organising technical workshops on segmentation and modelling tools, and holding the international symposia "Plant Growth & Form" (2019, 2022).

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 2022 at COS.

Key publications

Vijayan, A., Mody, T.A., Yu, Q., Wolny, A., Cerrone, L., Strauss, S., Tsiantis, M., Smith, R.S., Hamprecht, F.A., Kreshuk, A., et al. (2024) A deep learning-based toolkit for 3D nuclei segmentation and quantitative analysis in cellular and tissue context. Development 151 doi: 10.1242/dev.202800

Mody, T.A., Rolle, A., Stucki, N., Roll, F., Bauer, U. & Schneitz, K. (2024) Topological analysis of 3D digital ovules identifies cellular patterns associated with ovule shape diversity. Development 151 doi: 10.1242/dev.202590

Ramos, J.R.D., Reyes-Hernández, B.J., Alim, K. & Maizel, A. (2024) Auxin-mediated stress relaxation in pericycle and endoderm remodeling drives lateral root initiation. Biophys J doi: 10.1016/j.bpj.2024.06.017

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Stöckle, D., Reyes-Hernández, B.J., Barro, A.V., Nenadic, M., Winter, Z., Marc-Martin, S., Bald, L., Ursache, R., Fujita, S., Maizel, A., et al. (2022) Microtubule-based perception of mechanical conflicts controls plant organ morphogenesis. Sci Adv 8: eabm4974 doi: 10.1126/sciadv.abm4974

CORE FACILITIES

4.1 BOTANIC GARDEN & HERBARIUM

BOTANIC GARDEN & HERBARIUM
Director: Prof. Dr. Marcus Koch
Curator Living Collection: Dr. Andreas Franzke
Technical Curator Herbarium HEID: Dr. Peter Sack
Technical Manager: René Bruse
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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 45,000 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 programs.

The Botanic Garden has been at its present location—in the heart of the campus—for over 100 years. The general renovation program that began in 2022 is more than the urgently needed technical measures, but also the beginning of a new epoch in the Garden's history, on its way to a strong and successful future.

General refurbishment project: structural frames of the interim houses awaiting completion.

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 material 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 norms and regulations covering specific topics such as phytosanitary certification, for example. The scientific and horticultural 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.

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.

Selected Activities & Achievements 2021–2024
Living Collection Development
≈ 340 new plant accessionings
≈ 700 new seed accessionings
≈ 350 accession identifications *(det./rev./conf.)*
≈ 460 nomenclatural changes
≈ 4,000 curator-revised data sets

Herbarium Collection Development
≈ 5.000 mountings/databasing of new specimens
≈ 5.000 mountings/databasing of historic specimens
≈ 1.600 vouchers digitised
≈ 30 newly identified/rediscovered type vouchers

Wild Plant ex-situ Conservation Projects
Our collections also include *ex-situ* cultivations derived from populations of endangered native plant species: the last Odenwald population of the royal fern (*Osmunda regalis*) is found in Heidelberg and has been included into the Heidelberg municipal biodiversity

Wild plant *ex-situ* conservation project: the royal fern (*Osmunda regalis*)

strategy, under which we propagate endangered populations and undertake first reintroduction attempts where possible. Also in cooperation with counterpart administrative institutions, we continued our *ex-situ* maintenance and resettlement activities for endangered cheddar pink (*Dianthus gratianopolitanus)* populations from the Swabian Alb Biosphere Reserve. These efforts were again financially supported by the WIPs-De program, a national network for the conservation of endangered plant species, itself funded in turn by the German Federal Agency for Nature Conservation (BfN) in the context of Germany's National Biodiversity Strategy. In addition, we hold an *ex-situ* Baden-Württemberg population of the dwarf white water lily (*Nymphaea candida*). This species is extremely rare in the state and threatened by intensified water management and displacement through the introduction of cultivars bred from North American *Nymphaea* species. It is also feared that the genetic integrity of *Nymphaea candida* may become blurred through hybridisation with these cultivars, as well as with the more abundant native European white water lily (*N. alba*). Cytogenetic and genomic markers to identify hybrids and introgressed populations were developed and applied in collaboration with institutions of the federal states of Bavaria and Baden-Württemberg. We recently expanded these concepts to species protection strategies focusing on the least water lily (*Nuphar pumila*) in Germany and Switzerland in collaboration with an expanded network of experts and institutions.

AgroBioDiv Project
The »Organic Farming Research Program« established by the State of Baden-Württemberg funded the project »Organic Varieties for Biodiversity and Climate Protection« (AgroBioDiv, 2020 to 2024), that brought together plant biodiversity research and political sciences. The Herbarium (HEID) played an important role in this initiative by documenting the conducted comparative floristic analyses, archiving and indexing nearly 1,000 herbarium vouchers, and through the establishment of an extensive reference collection for seeds from segetal species. The »Gartenfest« held in 2022 was also a successful science outreach event for the interdisciplinary AgroBioDiv project.

Atacama Project
The bromeliad *Tillandsia landbeckii* occurs in the hyper-arid Chilean Atacama Desert, is dependent on fog as its predominant source of water and forms characteristic regular linear stands—so called lomas. Since 2020 these lomas are part of ongoing studies by the Koch Group at COS within a DFG (German Research Foundation) funded Collaborative Research Centre initiative: »CRC 1211 Earth – Evolution at the Dry Limit«. The aim is to elucidate the genetic structure and the spatio-temporal gene-flow within these unique population structures and their correlation with various environmental parameters. Growth measurements with a large number of population-wide collected individuals were carried out in the Botanic Garden, which provided expertise for cultivating and databasing these *ex-situ* experimental plant collections. Up to today ca. 250 individuals collected in the wild to enrich our living and research collections.

General Refurbishment Project
During the reporting period our work was more than heavily impacted by the general renovation project for the entire Garden—glass houses and outdoor area—that is expected to be fully completed in 2033. Besides our own extensive and detailed internal »plant-logistic« planning for the upcoming demolition and reconstruction phases during operation, we also engaged extensively with the state executive building authority, which acts as the governing body for this construction undertaking, with the University department of construction, architects and other specialist planners, such as building services engineers. These efforts were especially intensive for the Garden‘s technical manager, René Bruse. In addition, the entire Garden management also attended all regular staff planning meetings of the aforementioned state building authority. The first major activity of this long-term project, started in early 2022, was the dismantling of the Systematic Beds that played an important role in both academic and public teaching.These serve now as our temporary work yard, since our regular work yard space is unavailable for the duration of the project. The commissioning of two interim greenhouses (480 m^2) that set the starting point in mid 2022 were finished with some delay in October 2023. This exemplies the challenges for the following years while demolition and construction work, allocation of plants and functions are rotated through the Garden for several growing seasons and during ongoing daily horticultural and curational work.
During the period under review, organisational, planning, and hands-on horticultural work in direct context with the general refurbishment project amounted to ca. 2.400 staff-hours.

Material Transfer for Scientific Purposes & Support of Local Research Programs
As a member of the International Plant Exchange Network (IPEN), the Garden provides material for international research programs conforming to the Convention on Biological Diversity (CBD). In the period under review, material from over 350 accessions was transferred to international scientific research projects. A list of external publications that made use of such transfers can be found on the Garden´s homepage. In addition, we shared material from over 600 accessions with other Botanic Gardens within the IPEN circle. The Botanic Garden also supported Heidelberg-based research programs with the provision of plant material and horticultural expertise. The Garden’s Scientific Plant Cultivation Service (SPCS) cultivated around 3,000 individual plants annually from a great variety of wild species. This also led to a substantial number of SPCS-based publications, listed in the Garden´s annual reports.

General refurbishment project: clearing out the Madagascar house.

Academic Teaching & Theses
The Botanic Garden plays an important role in academic teaching programs: From 2021 to 2023 the Garden’s collections hosted academic courses with ≈ 1.000 students on ≈ 300 days and provides plant material for ≈ 300 course days with ≈ 1.250 students. The Herbarium is also integrated into education programs. The Student’s Herbarium of plants,

collected during field excursions during the last 15 years, incorporates more than 12,000 (fully digitised) specimens and in the winter semester 2023/24 a new course on herbarium curation and collection-based research was developed. The Garden provided plant material or horticultural support for a variety of academic works. Garden-related theses submitted from 2021 to 2023 include two PhD theses, six master theses, and 11 bachelor theses, listed in the Garden´s annual reports.

Vocational and Public Education Programs, Visitors & Public Events
Excellent horticulturists are a prerequisite for the maintenance of scientific living plant collections. The training of gardeners, therefore, is another prominent function for botanical gardens. In the period under review, 12 gardener apprentices successfully completed their training in our Garden. In addition, we provided gardening work experience placements for ≈ 75 persons, mainly pupils. The »Green School« of the Botanic Garden represents a comprehensive outreach program that reached about 4,000 children and adults in the period under review; In 2021 nearly all of these programs had to be cancelled due to COVID-19. Our outreach program addressing children and teenagers is 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, with ca. 50,000 visitors annually enjoying all that our Garden has to offer. The Garden was closed for visitors for nearly the whole of the year 2021 due to Corona regulations and also our annual »Gartenfest«—attracting each time ca. 1,000 visitors—had to be cancelled.

CURATORIAL RESEARCH ACTIVITIES IN LIVING COLLECTIONS

BOTANIC GARDEN
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Fields of Interest
Living collenction management, evolution of Brassicaceae, evolutionary biology, philosophy of biology

Brief Summary of Work & Major Contributions Since 2021
Botanic gardens maintain documented living plant collections for the purposes of scientific research, education, conservation and display. Documenting collections is a facet of botanical gardens that fundamentally distinguishes them from other plant collections like parks, which have very limited reference value. 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. A constant task of my curational work is to continuously improve the documentation of our 12.000 living accessions based on critical (re)evaluation of data utilising our own archives (old entry books, fieldbooks, index cards, lists, literature) as well as to cross-reference (retrospectively) our accession numbers with corresponding numbers and data of other Botanical Gardens that shared material with us in the past. The aim is to boost the impact of our collections on research and society through improved online access to this data. In the period of review I updated ≈ 4,000 data sets. Since March 2022 the Botanic Garden is obliged to be a registered »Business« (DE-BW2000252) at the Administrative District Governmental Department in Karlsruhe (RPK) in the context of EU regulation 2016/203 on protective measures against pests of plants. This registration means annual reporting for and audits by the RPK. It was a laborious and in particular nerve-fraying endeavour to fully understand all the relevant regulations and documentary duties,

and implementing these into our garden-wide curational and databasing workflows (e.g. handling phytosanitary certification, EU plant passports etc.). Major projects in my work concerning display included the completion of the eco-geobotanical sections project with a new concept for the beech forest section and a pilot project on using Quick Response (QR) codes to increase knowledge gain by visitors. The flexibility of such a QR code system will play an important role in the context of the recently started general refurbishment project (cf. Botanic Garden chapter) and the corresponding changes for visitors resulting from the construction works. Planning efforts related to our general refurbishment project had also a significant influence on my workload during the last years. Other permanent tasks arise with regard to the organisation and visibility of our public events as well as corporate communications. A major project here was the complete revision of our homepage and the implementation of a new content management system used by the university. My courses and lectures dealt with basic plant anatomy, anatomy and evolution of reproductive organs in land plants, plant-identification, basic biology, and general evolutionary biology.

Publication

Hendriks, K.P., Kiefer, C., Al-Shehbaz, I.A., Bailey, C.D., Hooft van Huysduynen, A., Nikolov, L.A., Nauheimer, L., Zuntini, A.R., German, D.A., Franzke, A. et al. (2023). Global Brassicaceae phylogeny based on filtering of 1,000-gene dataset. Curr Biol 33, 4052–4068

https://scholar.google.de/citations?user=c6Ua0e8AAAAJ&hl=de

4.2 DEEP SEQUENCING CORE FACILITY

DEEP SEQUENCING CORE FACILITY
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CellNetworks Deep Sequencing Core Facility
The CellNetworks Deep Sequencing Core Facility was founded in September 2010 to provide access to Next Generation Sequencing (NGS) technologies for the Heidelberg University research community. The Excellence Cluster CellNetworks, the Centre for Organismal Studies (COS Heidelberg) and the Heidelberg Molecular Life Sciences (HMLS) research council, had previously supported the core facility. Since October 2019 the facility has been supported by Field of Focus 1, and more recently by the newly ratified CellNetworks Core Technologies Platform, (CCTP) as permanent successor to CellNetworks.

Core Facility Concept and Services Provided
The Deep Sequencing Core Facility was established to satisfy the NGS needs of the Heidelberg Life Science Campus, and is open for access for members of Heidelberg University, the Medical Faculties Heidelberg and Mannheim, DKFZ, EMBL as well as the Max Plank Institute for Medical Research.

In recent years, the facility established a workflow that new and old users are required to follow in order to facilitate project management and sample handling. Users are encouraged to discuss their needs with the facility members, who in turn can provide a plan best suited for the project and the user's budget. Next, the samples should be registered in the Agilent iLab system to help assist with sample tracking, and physically handed over for preparation. The majority of samples submitted are extracted RNA or single-cell preparation, where-upon library preparation and sequencing are carried out as a full service, including all required quality controls. However, as we maintain an open facility, user prepared libraries can be sequenced and facility-prepared libraries can be taken elsewhere for sequencing. Furthermore, we provide training for those who wish to learn NGS protocols or need advice in developing custom protocols for their own research projects.

It should be noted, that our facility does not offer bioinformatic support due to lack of qualified staff.

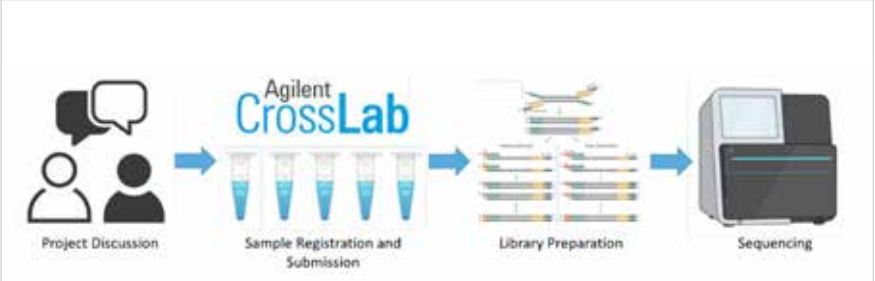

Figure 1:
Showing the sample handling workflow of the Deep Sequencing Core Facility

Standard Library Preparations on Offer:
– Stranded bulk RNA-Seq
– Small RNAseq
– Chip-Seq
– gDNA-Seq (de novo, resequencing)
– ATACseq– Target Enrichment
– Target Enrichment
– Methyl-Seq / Bisulfite Sequencing
– Single-Cell Sequencing (10X Genomics and Parse Biosciences)

Sequencing Services and Instrumentation
The facility has currently three sequencing instruments, the MiniSeq purchased as part of the MULTI-SPACE Project (Health and Life Science Alliance Heidelberg Mannheim), and the NextSeq 550. Given the increasing demand for single cell 'omics, and the increasing costs of running the NextSeq 550, the installation of the NextSeq 2000 has begun, and should be operational within the summer 2024. This enables to the facility to continue to support its users with middle – moderate high sequencing capacity for their projects in a timely and cost-effective manner.
Further to this, the facility has an ongoing collaboration with the EMBL Genomics Core Facility, which enables access to long read technologies of PacBio and Oxford Nanopore. This collaboration has been long term and is particularly fruitful in that there is a bidirectional transfer of knowledge and techniques which helps both facilities. Since mid-2023, we have access to the NovaSeq 6000 of the NGS Facility of the University Clinic of Mannheim, which can be used for especially large projects, needing multiple billion reads. However, due to the different financial circuits between University Clinic of Mannheim and University of Heidelberg, billing issues are hindering the willing collaboration of the two facilities.

Instrument	Year of Purchase	Short Description
Illumina MiniSeq	2023	NGS instrument for small scale projects and project optimisation (up to 25 million reads)
Illumina NextSeq 550	2019	NGS instrument – mid-output sequencer - For legacy projects (up to 400 million reads)
Illumina NextSeq 2000	2024	NGS instrument offering a scalable range of sequencing output including large projects. Especially suitable for medium range single cell projects (up to 1.8 billion reads)
Covaris S2	2010	Sonicator – shearing of DNA – User accessible
Agilent BioAnalyzer		On chip capillary electrophoresis, used for quality control of incoming samples and during sample preparation – User accessible
Qubit	2024	Quantification of DNA/RNA of incoming samples and sample library preparations
Oxford Nanopore MinIon	2018	Long read sequencer has uses for scaffold genome building, native RNA sequencing
10 x Chromium Controller	2019	Microfluidic instrument to partition cells into an emulsion – Legacy instrument situated in DKFZ Single Cell Open Lab
10 x CytAssist	2024	Instrumentation enabling the processing of tissue sections (FFPE) for high definition spatial transcriptomics and protein expression (panel based)
10 x Chromium iX	2024	Microfluidic instrument to partition cells into an emulsion – offers ability to process fixed cells (human/mouse)

Implementation of Single-cell Sequencing Services
In 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, and can be used to generate libraries from single cell suspensions. In more recent years, the facility has not only implemented the standard 3' Gene Expression protocols, but has implemented further protocols including 5' protocols to enable the capture of V(d)J regions of the TCR, ATAC-seq, and Multiome. Furthermore, the facility has been actively working with users to establish cell hashing methods, which allow the loading of multiple samples on a single lane, hence decreasing the price per sample. The tested cell hashing methods included the MULTI-seq protocol using oligo-lipid conjugates and antibody-based CITE-seq. A particular highlight is with close work with AG Jäger of the National Tumour Center (NTC) Heidelberg, the implementation of MediMer (Meyer et al, 2024).

Due to the rapidly developing field of single-cell sequencing, multiple new commercialized solutions appeared on the market. In order to keep providing the best solutions to our customers, we are constantly benchmarking new methods. In 2023, we begun a collaborative project with AG Leuschner of the Cardiology Department and AG Schapiro of the Medical Faculty to implement the commercialized SPLiT-Seq, Parse Biosciences Evercode. This method works on fixed cells, which makes sample collection possible over a long period of time, incredibly valuable in a clinical setting. Furthermore, Evercode is highly scalable as it is able to profile up to one million cells in one experiment.

Currently the facility is also conducting feasibility tests on the Rhapsody system from Becton Dickinson and Company, (BD). Given the strong connection the University Clinic has to BD, and that there are tailored panels of antibodies which are of interest to several groups within the clinic, the Rhapsody is a promising new method to be implemented into our core facility's portfolio.

As before, the facility also offers the possibility to perform single cell analysis on individual cells with the SMART-seq technology (both adapted from published protocols, and the commercial solution from Takara). In contrast to the highly-parallel solutions mentioned above, SMART-seq is low-throughput, however, it is significantly more sensitive in regards to rare-transcript detection and gene number detection.

User Base
As mentioned above the facility interacts with users across the Heidelberg campus and the Medical Faculty of Mannheim. Our facility is currently not allowed to take external users except for DKFZ, EMBL and Max Plank Institute for Human Medicine. In 2024 we have currently over 20 active groups, and over 26 active users from those groups as determined by our registration system. In the time between 1st January 2022 and July 2024, the facility has handled the samples from over 43 different groups on campus, comprising of over 70 users.

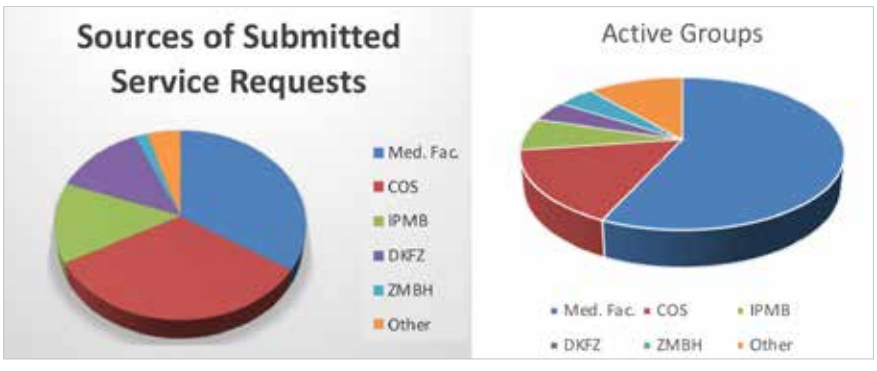

Figure 2:
Charts showing the origin of requests being received by the Sequencing Facility as a percentage of actual orders submitted (left), and location of active groups between 2022 and 2024 (right)

User Fees
The facility has to recuperate its' running costs as best as it can, and as previously, the facility uses the guidelines of the DFG for its' price calculations and fees. Today given that the facility is part of the CCTP, one of the requirements is that the facility is to be registered in the DFG RIsources, and can be found under RIsource number (RI_00573). The costs are regularly checked, and undergo a revision at least once a year. However, the regulations required for the facility pricing mean the facility cannot recuperate its costs, ie those costs incurred for maintenance and service contracts, infrastructure, and personnel.

Further Highlights
Since 2022, the facility participates in the teaching of single cell techniques to Masters Students, which is organized by Dr. Josephine Bageritz. This course is a yearly two-week course of up to 16 students, wherein the facility is active in the first week aiding the students to learn the 10x Genomics 3' Gene Expression technique along with sequencing, and isolation of nuclei. Further to this in 2022 the Facility helped co-ordinate the Spatial Omics Summer School, aimed at PhD students and Post-Docs who had an interest in different Spatial Omic Technologies.
In 2022, the facility was requested to join the MultiSpace Project of the Health and Life Science Alliance Heidelberg Mannheim. The idea of the project was to attempt to establish spatial omic technologies in the Mannheim Heidelberg area as part of State supported project. Given its' sequencing expertise the facility was to focus on spatial transcriptomics to which manner, it now hosts a new Staff Scientist, Dr. Bianka Berki, who joined in September 2022. Dr. Berki has thus far established the 10x Visium method for the facility and is developing the non-biased higher resolution method SeqScope using the MiniSeq as basis. In combination of the cross-institutional collaboration of MultiSpace, the facility has closer networking with the nCounter Core Facility of the Human Genetics Department, given their acquisition of the GeoMX.
In the same year the Alliance introduced the ExploreTech Grants, which are available to core facilities and researchers wishing to use core facilities to either use a facility with existing techniques or to help explore and develop new techniques. The facility has had the opportunity to be part of four such grants (two are ongoing this year), and has indirectly led to increased interest in the facility.

Future Perspectives
The facility is a well utilised core, and with the introduction of ExploreTech Grants its' visibility on campus has been increasing, and we have seen the influx of new groups requesting meetings for future projects with us, something which prior to 2022 rarely was the case. With the aquisition of the NextSeq 2000 sequencing instrument from Illumina, the facility feels strengthened for the foreseeable future with its' sequencing capacities. This linked to the collaborations we have with two NGS facilities within the region only strengthens the capabilities of the facility. Furthermore, the facility is about to improve its' position in what it can provide in terms of single cell transcriptomics following the successful internal grant application for a 10x Genomics iX, and the CytAssist. This latter instrument enables the high definition analysis of fixed tissue sections, something that has been requested independently by at least three groups from the Medical Faculty.
With a revived infrastructure, we are optimistically looking into the future, and look forward to increasing our capabilities and offerings for the campus at wide.

Selected Publications
Binmöller, L. *et al.* (2024). Differential expression and evolutionary diversification of RNA helicases in Boechera sexual and apomictic reproduction. Journal of experimental botany. 75(8):2451-2469.

Liu, K., *et al.* (2024) Dynamic YAP expression in the non-parenchymal liver cell compartment controls heterologous cell communication. Cell. Mol. Life Sci. 81 (115).https://doi.org/10.1007/s00018-024-05126-1

Meyer, M. *et al.* (2024) MediMer: a versatile do-it-yourself peptide-receptive MHC class I multimer platform for tumor neoantigen-specific T cell detection. Frontiers in Immunology 14.

Huth, T., *et al.* (2023) Chromosome 8p engineering reveals increased metastatic potential targetable by patient-specific synthetic lethality in liver. Cancer.Sci.Adv. 9:1442

Yousuf S, *et al.* (2023) Spatially Resolved Multi-Omics Single-Cell Analyses Inform Mechanisms of Immune Dysfunction in Pancreatic Cancer. Gastroenterology. 165(4):891-908.

Poster
Huth, T., *et al.* (2023) Chromosome engineering and CRISPR-Cas9 viability screening reveals increased metastatic capacity targetable by patient-specific synthetic lethality. Journal of Hepatology 78: p.S547

Submitted –available on BioRxviv:
Zhang, Y. *et al.* (2023) Single-step discovery of high-affinity RNA ligands by UltraSelex. BioRxviv, 2023.

Binmöller, L., *et al.* (2022) Differential expression analysis of sexual and apomictic Boechera uncovers *FAS4* as crucial for gametogenesis BioRxviv, 2022.


RMIT

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 requirements at the subcellular level. The facility was established to provide electron microscopy support to all researchers, including undergraduates in the Heidelberg life science community. The EMCF is therefore open to all researchers on the Heidelberg University campus and is a DFG registered facility (RI 00565) associated with the CellNetworks Core Technology Platform (CCTP), which acts as an umbrella organization for core facilities at Heidelberg University. The EMCF is committed to education and offers regular training courses and user training on many aspects of electron microscopy. It also offers courses for students from various disciplines, such as the Heidelberg Biosciences International Graduate School (HBIGS).

The EMCF currently operates a number of electron microscopes that cover a wide range of applications. With the scanning electron microscope (SEM) we can image surface structures of cells and tissues, which is useful for fine structures on single cells as well as for viewing details on the surface of tissues or whole (mm-sized) organisms. In contrast, transmission electron microscopy (TEM) allows subcellular structures to be visualized with the highest resolution through ultra-thin slices. In addition to a standard TEM, the EMCF also has an instrument with a high acceleration voltage TEM (200 kV) that enables 3D tomography. This involves taking a series of images of the section at different angles of inclination. This technology can be used to analyze the spatial arrangement of small structures within cells. In cases where the 3D structure of a larger volume is required, the facility 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 staining and transmission electron microscopy to gain insights into their molecular structure. In essence, the EMCF offers EM techniques covering scales from mm-surfaces to ultrastructure of protein complexes.

In addition to the electron microscope, the EMCF recently acquired a micro-CT in collaboration with Professor Wittbrodt, which can be used to scan centimeter-sized samples and provide information about the internal structure of a sample without having to cut it open. The resolution corresponds to histological information and can provide volume data for tissues. This device thus represents a bridge between histology and electron microscopy.

Many biological questions require the combination of structural information with information on protein expression. To support projects in this area, the EMCF has extensive experience in the application of immunogold labelling with antibodies to identify areas of protein localization on sectioned material. Importantly, we also offer correlative light and electron microscopy (CLEM) methods in which the expression of a fluorescently labelled protein is used to identify a small structure or rare event.

With all the different options available for imaging and sample preparation, it is important to find the most appropriate technique for each project and sample. The EMCF has extensive experience in the application of a wide range of technologies and can therefore assist in the development of protocols suitable for different samples and scientific questions. The workflow at the EMCF is therefore based on close and continuous collaboration between scientists and facility staff. This starts with an initial meeting with EMCF facility heads Charlotta Funaya and Réza Shahidi, and continues until the successful publication of the data. From the publications with papers citing EMCF staff as supporters or authors [37 in the current COS reporting period], it is clear that many groups on campus have benefited from the EMCF. The number of co-authorships by EMCF scientists [12 in this period] also underscores the importance of scientific consultation, which is an important aspect of our mission and can be a valuable asset for groups that rarely use electron microscopy.

The broad range of applications and technologies offered and supported by the EMCF is critical to the scientific success of our community. Pooling resources in an open campus facility is the most efficient form of providing technologies that require expensive equipment and specialized expertise, as it increases instrument use and improves user support. Importantly, user training requires a high level of hands-on experience and ongoing support on the various instruments, which is clearly possible within a centralized facility. The decision to consolidate the EM facilities within the EMCF and create a centralized EM laboratory for the community was therefore a very important step. For the near future, we are looking forward to the upcoming inauguration of our microscope rooms at INF 345, so that we can finally offer a better electron microscopy service at our site. Together with the investment to replace outdated equipment, this will provide a solid foundation for our users to actively learn and utilize electron microscopy.

Selected Publications

Bezares Calderón, L.A., Shahidi, R., Jékely, G. (2024) Mechanism of barotaxis in marine zooplankton. eLife, 13:RP94306.

Prasad, V., Cerikan, B., Stahl, Y., Kopp, K., Magg, V., Acosta-Rivero, N., Kim, H., Klein, K., Funaya, C., Haselmann, U., et al. (2023). Enhanced SARS-CoV-2 entry via UPR-dependent AMPK-related kinase NUAK2. Molecular cell, 83(14), 2559–2577.

Kehrer, J., Formaglio, P., Muthinja, J. M., Weber, S., Baltissen, D., Lance, C., Ripp, J., Grech, J., Meissner, M., Funaya, C., Amino, R., & Frischknecht, F. (2022). Plasmodium sporozoite disintegra-tion during skin passage limits malaria parasite transmission. EMBO reports, 23(7), e54719.

Klaus, S., Binder, P., Kim, J., Machado, M., Funaya, C., Schaaf, V., Klaschka, D., Kudulyte, A., Cyrklaff, M., Laketa, V., Höfer, T., Guizetti, J., Becker, N. B., Frischknecht, F., Schwarz, U. S., & Ganter, M. (2022). Asynchronous nuclear cycles in multinucleated *Plasmodium falciparum* facilitate rapid proliferation. Science advances, 8(13), eabj5362.

Simon, C. S., Funaya, C., Bauer, J., Voß, Y., Machado, M., Penning, A., Klaschka, D., Cyrklaff, M., Kim, J., Ganter, M., & Guizetti, J. (2021). An extended DNA-free intranuclear compartment organizes centrosome microtubules in malaria parasites. Life science alliance, 4(11), e202101199.


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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 and is primarily funded through third-party grants. It is located at COS within the Department of Molecular Biology of Plants, directed by Rüdiger Hell and managed by Gernot Poschet.

The mission of MCTP is to develop and provide analytical services across the strategic Field of Focus I of Heidelberg University, including the two Medical Faculties and the non-university institutions DKFZ, MPImF and EMBL. MCTP has established scientific metabolomics approaches and developed novel analytical tools enabling custom-tailored services for research projects. MCTP accompanies projects in close cooperation from early development to publication and funding applications. The substantial impact of MCTP is evident from its contributions to over 110 publications, including top-tier journals (Cell, Nature, Science), and the acquisition of approximately €5 million in funding.

Since 2021, metabolite analyses have been conducted for more than 80 research groups across Heidelberg and Mannheim Life Sciences. This effort has been supported by ongoing third-party funding, alongside investments in personnel and equipment. Key achievements include standardized targeted measurements of over 1,000 compounds, non-targeted metabolomics, and 13C tracer/flux analyses. Future developments will focus on consolidating high-end instrumentation and novel analytical tools, developing a mass spectrometry-based imaging platform (“spatial metabolomics”), training postdoctoral researchers in metabolomics, and expanding bioinformatics capabilities.

Research Highlights since 2021
During this reporting period, we significantly enhanced and expanded our analytical instrumentation, primarily through third-party funding. In 2021, we acquired two MicroLC systems, which allow for analyses with higher sensitivity. While chromatography using micro and nano flow rates has been widely used in proteomics for years, this underutilized technique has not yet been fully explored for metabolomics applications. In addition to our ongoing successful collaboration with DKFZ, in 2022, a ZenoTOF instrument, purchased and owned by a DKFZ research group, was installed at MCTP. The main capacity of this highly sensitive high-resolution QTof system is utilized by the DKFZ group, while surplus capacity is available to MCTP for additional projects on campus, providing mutual benefits for DKFZ, MCTP, and the entire life sciences community on campus.

In 2023, MCTP acquired a new state-of-the-art GC-MS/MS system, generously funded through the C-Tech call of FoF1. This enables us to offer highly sought-after untargeted metabolomics screening with more than a tenfold increase in sensitivity, allowing for enhanced coverage of the metabolome. As part of the “MULTI-SPACE” project of the Heidelberg & Mannheim Life Science Alliance, MCTP successfully collaborated with Prof. C. Opitz (DKFZ) and Prof. C. Hopf (Hochschule Mannheim) to establish a “Mass Imaging Platform” for spatial metabolomics in Heidelberg. This platform, comprising a timsTOF flex

MALDI-2 mass spectrometer, a laser microdissection system, and a cryotome, is currently being set up in newly renovated rooms at BioQuant. Altogether, the new analytical instrumentation represents an investment of €2.5 million.

Currently, MCTP is managed by Gernot Poschet and supported by three full-time researchers and three part-time engineers/technicians. On average, MCTP performs over 5,000 analyses per year, with a particularly high demand for metabolomics from the Medical Faculties and DKFZ besides university institutes. Special applications have been developed and continuously improved to address specific scientific questions for diverse research projects from Heidelberg life science institutes, such as the quantification of major neurotransmitters, analysis of the "NAD-ome", and the "Dipeptid-ome" within the framework of joint Explore!Tech grant applications, among others.

In addition to the already established 13C or 15N tracing analysis of central carbon and nitrogen metabolism via heavily labeled isotope tracers of *in vitro* samples, we aim to offer this technique for *in vivo* samples and are currently evaluating it with several research groups. Several projects based on tracing studies have already been successfully completed, and future developments will target additional metabolic pathways and enhanced sensitivity.

The MCTP's scientific services have enabled a significant number of publications in high-quality journals by institutions across the campus and several international cooperation partners. MCTP scientists have also published their own research projects as first and last authors. In various projects, we have optimized and established methods for large-scale quantitative metabolomics using standardized MxP Quant 500 (Biocrates) kits for major model organisms like mouse, zebrafish, and Drosophila (Gegner et al. 2022), as well as studies using cell culture, primary cells, or human tissue (Andresen et al. 2022). In collaboration with teams from the medical faculty, DKFZ, and EMBL, we examined the effects of pre-analytical factors on metabolomic and proteomic analyses of human plasma and serum samples (Gegner et al. 2022). As part of the BMBF-funded project SMART-CARE, we developed and evaluated "MTBE-SP3," a unified workflow for combined proteome and metabolome analysis from a single sample, integrating established protocols for metabolomic (EtOH/MTBE) and proteomic (autoSP3) profiling. This workflow demonstrates equivalence in proteome profiles regardless of prior metabolite extraction, can be applied to various biological matrices (FFPE tissue, fresh-frozen tissue, plasma, serum, and cells), and offers advantages such as reduced sample variation, decreased input amounts, and enhanced standardization for large-scale studies (Gegner et al. 2024). We also anticipate significant scientific impact from the recent implementation of the newly developed UP-LC-MS/MS MxP Quant 500 XL (Biocrates) kits, which provide even broader metabolome coverage (>1,000 metabolites). This assay is already being applied to large sample cohorts and studies in collaboration with the departments of virology, cardiology, and other local partners.

Future directions
The future development of MCTP will focus on 1) renewing existing and identifying new funding sources, 2) training young scientists and fostering scientific development, 3) establishing "spatial metabolomics," and 4) addressing bottlenecks in bioinformatics and laboratory space.

1. We are currently renegotiating the contract with the German Cancer Research Center (DKFZ) Heidelberg as an associated external core facility, due at the end of 2024. Furthermore, we are continuously discussing potential funding opportunities (e.g., Explore!Tech calls) with our collaboration partners to ensure state-of-the-art methodology and instrumentation.
2. The scientific excellence of MCTP is a core aspect of our mission and heavily relies on young researchers. However, standard analyses based on user fees are typically not attractive for young researchers at the postdoctoral stage, who, due to MCTP's funding

structure, all have limited contracts. Since user fees cannot legally be converted into positions (and are required for consumables, instrument maintenance, and replacement), the request for services and scientific development often do not benefit each other, severely impacting MCTP's long-term development.

3. As outlined briefly, MCTP will establish a high-end platform for "spatial metabolomics" and implement the respective techniques in Q3/Q4 2024. The first collaborative projects with partners from COS and other local research institutions are expected to commence in Q1 2025.
4. The analysis of primary data in mass spectrometry must be curated by scientists. The individual and often tailor-made data processing is highly time-consuming for MCTP and usually cannot be transferred to users. Therefore, MCTP's services would greatly benefit from at least a 50% position for a bioinformatician, who would manage software and workflows for data analysis and implement algorithms for further data processing, enhancing data mining capabilities and reducing turnaround times for results. A long-term challenge is the limited laboratory and office space, which prevents any further growth. MCTP currently occupies nearly half of the space of the Department of Molecular Biology of Plants. Additionally, the technical capabilities of INF 360 have reached their limits (media supplies, safety, aeration, air conditioning). Consequently, discussions have been initiated with the rectorate and BioQuant directorate regarding a potential move of MCTP to BioQuant in 2025.

Selected publications since 2021

Gegner, H. M., Naake, T., Aljakouch, K., Dugourd, A., Kliewer, G., Müller, T., Schilling, D., Schneider, M. A., Kunze-Rohrbach, N., Grünewald, T. G. P., Hell, R., Saez-Rodriguez, J., Huber, W., Poschet, G., & Krijgsveld, J. (2024). A single-sample workflow for joint metabolomic and proteomic analysis of clinical specimens. Clinical proteomics, 21(1), 49.

Andresen, C., Boch, T., Gegner, H. M., Mechtel, N., Narr, A., Birgin, E., Rasbach, E., Rahbari, N., Trumpp, A., Poschet, G., & Hübschmann, D. (2022). Comparison of extraction methods for intracellular metabolomics of human tissues. Frontiers in molecular biosciences, 9, 932261.

Gegner, H. M., Mechtel, N., Heidenreich, E., Wirth, A., Cortizo, F. G., Bennewitz, K., Fleming, T., Andresen, C., Freichel, M., Teleman, A. A., Kroll, J., Hell, R., & Poschet, G. (2022). Deep Metabolic Profiling Assessment of Tissue Extraction Protocols for Three Model Organisms. Frontiers in chemistry, 10, 869732.

Gegner, H. M., Naake, T., Dugourd, A., Müller, T., Czernilofsky, F., Kliewer, G., Jäger, E., Helm, B., Kunze-Rohrbach, N., Klingmüller, U., Hopf, C., Müller-Tidow, C., Dietrich, S., Saez-Rodriguez, J., Huber, W., Hell, R., Poschet, G., & Krijgsveld, J. (2022). Pre-analytical processing of plasma and serum samples for combined proteome and metabolome analysis. Frontiers in molecular biosciences, 9, 961448.

Heidenreich, E., Pfeffer, T., Kracke, T., Mechtel, N., Nawroth, P., Hoffmann, G. F., Schmitt, C. P., Hell, R., Poschet, G., & Peters, V. (2021). A Novel UPLC-MS/MS Method Identifies Organ-Specific Dipeptide Profiles. International journal of molecular sciences, 22(18), 9979.

https://scholar.google.com/citations?user=n-bgJBIAAAAJ&hl=de


NIKON

4.5 NIKON IMAGING CENTER

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Fields of interest
The Nikon Imaging Center at the Heidelberg University (NIC) offers advanced light microscopy 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.

Summary and outlook
The NIC was founded in 2005 as a collaboration between the Heidelberg University and Nikon and has grown to include 15 microscopes. The techniques offered have diversified as new technologies emerged. Meanwhile the NIC has managed to update its Confocal Laser Scanning and Spinning Disk Microscopes to the 3rd generation. More recent applications comprise Light Sheet Microscopes and since this year Single Molecule Localization Microscopy (SMLM). We also offer several advanced wide field imaging setups (Table 1 shows major instrumentation), where automated multi-point imaging or optogenetic activation is the focus.
Our user base is spread across 86 groups in institutes from the Biosciences, Medical and Engineering Faculty. We have so far trained more than 2000 researchers and supported them in their projects, around 400 in the last 4 years. 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.

We are engaged in CRC 1324 on Wnt signaling (speaker Michael Boutros). Here we are involved in the individual projects, especially the group of Acebrón in studying mitotic fidelity (Bufe et al. 2021 doi: 10.1073/pnas.2108145118, De Jaime-Soguero et al. in press), the Holstein/ Özbek group (Garg et al. 2023 doi: 10.1016/j.isci.2023.106291, Veschingi et al. 2023 doi: 10.1016/j.isci.2023.106416) and the Boutros group (Schubert et al. 2022 doi: 10.1073/pnas.2122476119).

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 Disco, 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 (2x 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 micro-scope	2	Laser scanning confocal systems (Nikon AX and Nikon A1R) on an automated inverted microscope (Ti2) with perfect focus and multipoint acquisition. On AX a structured detector for super-resolution is available. Laser lines: 405, 440, 488, 514, 561, 640 nm.
SMLM	1	N-STORM with astigmatic lens for 3D STORM with NA 1.40 objective and Hamamatsu B95. STORM, PALM and PAINT with 20 nm resolution. Imaging Lasers: 488, 561, 640, activation with 405 nm.
Spinning disc con-focal Yokogawa W1	1	Spinning disk confocal systems with sensitive EM-CCD detection or sCMOS 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 con-focal CREST V3	1	Spinning disk confocal systems with large field of view (FOV 25 mm) combinded 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
Laser lines: 405, 440, 488, 514, 561, 640, 750 nm	1	2-photon system (LaVision Biotec) on upright fix-stage microscope (Nikon FN-1) for physiological deep-in-tissue observation. Detection on non-descanned all-GaAsP ultra-sensitive-PMT port (4 channels). Exciation of UV-dyes (fura) up to red fluorescent proteins (e.g. mCherry). Dipping lenses.
Simulation lines: 395, 440, 488, , 550, 640, 750 nm	1	Nikon structured illumination microcope (N-SIM) for multichannel imaging with a lateral resolution of 110nm. 2D-SIM, 3D-SIM and TIRF –SIM illumination modes are available. Laser lines: 405, 488, 561, 640

Table 1: Major Instrumentation at Nikon Imaging Center

Research Highlights since 2021

The Nikon Imaging Center at the Heidelberg University provides access to advanced light microscopy for a broad range of applications. These range from organ imaging to subcellular dynamics, FRAP and FRET. A highly used application is volumetric tissue imaging on the Ultramicroscope II 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). This system bridges the gap between low resolution whole animal imaging and laser scanning confocal microscopy.

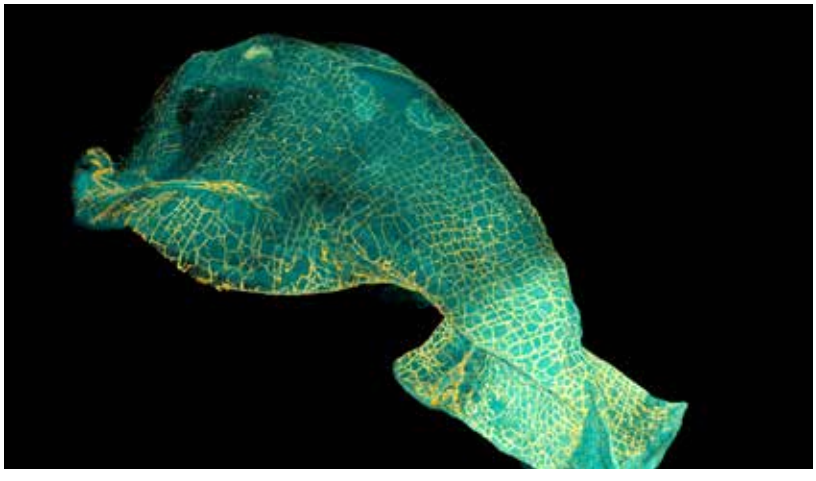

Figure 1:

Piece of murine colon imaged by light sheet microscopy (Ultramicroscope II) after optical clearing. Vasculature (orange) and autofluorescence (teal). Specimen courtesy Vera Thiel, Andreas Trumpp DKFZ)

Two new instruments have been acquired with a large instrument grant (DFG GG91b) led by Ulrike Engel: The spinning disk CSU-W1 for live imaging and SMLM instrument N-STORM. The CSU-W1 improves our live cell imaging, which so far relayed on smaller field of view systems (CSU-X) and its integration on a Ti-2 allows us to perform successful multipoint and multi-well imaging.
Laser scanning microscopy remains an important pillar of the NIC and here we were able to bring in a new large field version, the Nikon AX, which we also equipped with a super-resolution detector N-SPARC. We make use of novel silicone immersion objectives, which allow for imaging deeper into the tissue due to refractive index matching (Figure 2).

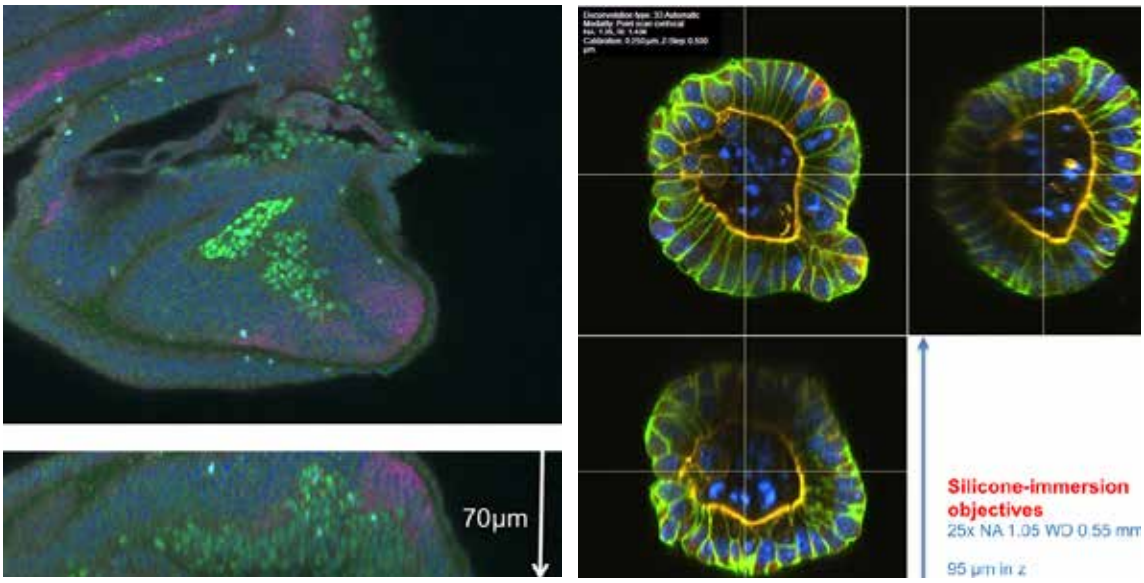

Figure 2:

Examples 3D imaging of organs/ organoids with silicone immersion objectives using CLSM. Drosophila imaginal disks with markers of wnt expression with a 40x Silicone immersion objective NA 1.25. Due to the good match of refractive index of tissue, glycerol embedding and Silicone-immersion it is possible to obtain good images well over 50µm in the tissue. Sample: Josephine Bageritz, COS. B) Imaging of organoids with 25x NA silicone immersion objective. Sample: Kim Boonekamp/ Prof. Boutros, DKFZ)

The N-STORM is our first SMLM microscope, which we run based on our expertise on TIRF microscopy where we also explored blinking fluorophores (in collaboration with the Grossmann lab, Fuchs et al. bioRxiv 2021) We also collaborate with Charlotte Kaplan, who runs the CellNetworks MIN-FLUX system. She set up exchange-PAINT on this microscope, and we hope to also employ this technique on the STORM for multicolor imaging. The MIN-FLUX system is operated in association with the two other imaging facilities of the university (IDIP in CIID, and the ZMBH imaging facility). This has led to more interaction between the facilities and a synergy in the field of super resolution, e.g. in teaching a dedicated course that would encompass all relevant techniques (STED, MIN-FLUX, N-STORM).

We also received seed funds to establish dynamic imaging at the single molecule level to monitor interaction between microtubule-associated proteins and the cytoskeleton from the Life Health Science Alliance in collaboration with a group at the DKFZ. These inter-institutional funds are welcome as they allow to exploit cooperation when collaborating with e.g. the Medical Faculty or the DKFZ.

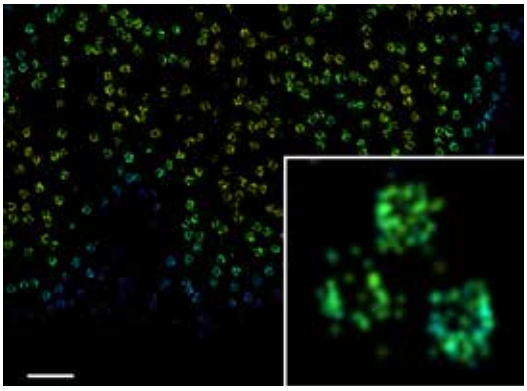

Figure 3:
3D-STORM rendering taken with the N-STORM installed in April 2024. The color encodes depth (0-800nm). Scale bar is 800nm and 100nm in insert.

While we have been successful in obtaining funding to bring our instruments up to date (3 high-end instruments in last 4 years!) we lack sufficient staff to support all applications. The usage has been slowly but steadily rising over the years and has almost doubled within the last 10 years, while the number of people supporting users and applications has been the same in this period.

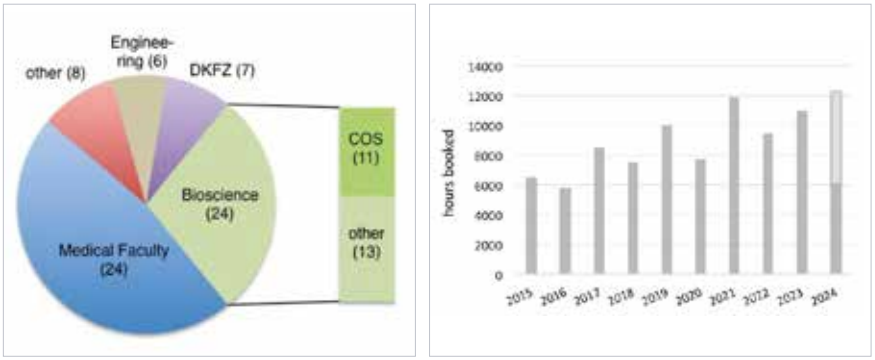

Figure 4:
A) distribution of groups which are supported by the NIC. B) Hours booked by users (2017-2024). For 2024 numbers were extrapolated from data collected in the first 6 months.

Planned research and new directions
The new setups which were installed this year open up new possibilities. An important aim is to bridge live microscopy (by spinning disk microscopy) with superresolution imaging by SMLM or expansion microscopy using confocal microscopy which is necessarily done on fixed specimen.

STORM and Expansion Microscopy
The NIC is setting up imaging workflows for SMLM on the N-STORM setup, which can image molecular blinking (e.g. dSTORM or PALM) or DNA-PAINT, where binding and unbinding of small complementary DNA-strands are used. Both require some optimization for each application.
An alternative approach to obtain superresolution information is expansion microscopy which several groups on Campus have now established for their specimen. Here confocal imaging or enhanced resolution by N-SPARC will be used. The main resolution gain comes from the expansion of the structures. Here objectives with high working distance and high numerical aperture are required, to reach both the intended resolution and physically reach the focus plane of the structure inside cells (e.g. after 4x expansion). Here our water immersion objectives on spinning disk and confocal microscopes are well-suited as the gels are expanded in a watery environment. First trials are currently conducted with 4x expanded marine animals by the Jekely lab (COS) using the new spinning disk CSU-W1.

Correlative workflows.
To bridge information between live imaging at diffraction-limited resolution and SMLM we aim to process specimen for SMLM after live imaging and re-find regions of interests. We are able to import point-lists from one setup to the other and also identify regions of interest based on image analysis (feedback microscopy). However these workflows have to be established and optimized for each application and require stringent testing. This will be done in collaboration with interested groups.

Assay for molecular machines: Funded by a Explore Tech grant from the Life Health Science Alliance this interinstitutional project together with Dr. Maiwen Caudron-Herger at DKFZ we exploit our expertise with TIRF microscopy to establish an assay where binding and function of GFP-tagged proteins can be explored on the single molecule level.

Selected publications
Publications supported by the NIC (as acknowledged in article) or with co-authorship. The total number of publications since 2021 has reached 73.

Seidl, C., Da Silva, F., Zhang, K., Wohlgemuth, K., Omran, H., and Niehrs, C. (2023) Mucociliary Wnt signaling promotes cilia biogenesis and beating. Nat Commun. 14:1259. doi: 10.1038/s41467-023-36743-2

Schubert, A., Voloshanenko, O., Ragaller, F., Gmach, P., Kranz, D., Scheeder, C., Miersch, T., Schulz, M., Trumper, L., Binder, C., Lampe, M., Engel, U., and Boutros, M. (2022) Superresolution microscopy localizes endogenous Dvl2 to Wnt signaling-responsive biomolecular condensates. Proc Natl Acad Sci U S A. 119:e2122476119. doi: 10.1073/pnas.2122476119

Linster, E., Forero Ruiz, F.L., Miklankova, P,. Ruppert, T., Mueller, J., Armbruster, L., Gong, X., Serino, G., Mann, M., Hell, R., and Wirtz, M. (2022) Cotranslational N-degron masking by acetylation promotes proteome stability in plants. Nat Commun. 13:810. doi: 10.1038/s41467-022-28414-5

Kanamaru, T., Neuner, A., Kurtulmus, B., and Pereira, G. (2022) Balancing the length of the distal tip by septins is key for stability and signalling function of primary cilia. EMBO J. 41:e108843. doi: 10.15252/embj.2021108843
Hodapp, A., Kaiser, M.E., Thome, C., Ding, L., Rozov, A., Klumpp, M., Stevens, N., Stingl, M., Sackmann, T., Lehmann, N., Draguhn, A., Burgalossi, A., Engelhardt, M., and Both, M. (2022) Dendritic axon origin enables information gating by perisomatic inhibition in pyramidal neurons. Science. 377:1448-1452. doi: 10.1126/science.abj1861

Google Scholar to view Ulrike Engel's publications:
https://scholar.google.com/citations?user=VU71vo0AAAAJ&hl=de


Säugetiere
Mammals

4.6 ZOOLOGICAL COLLECTION TIMELINE EVOLUTION

ZOOLOGICAL COLLECTION TIMELINE EVOLUTION
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From the > 200-year-old collection of the Zoological Institute to a state-of-the-art exhibition on the evolution of animals and mankind

Due to the necessary extensive renovation of the 50-year-old building INF 230, the "Zoological Collection of Heidelberg University" was not accessible to the public from 2012 - 2023. In spring 2023, the collection became available again with a timely concept focussing on the latest progress in molecular evolution of animals. Embedded in the modernised teaching and research facilities, the newly designed, interactive permanent exhibition 'Timeline Evolution' was established, which builds on the over 250 years old famous zoological collection of Heidelberg. Many specimens were part of the "Zoologisches Cabinett" founded by Friedrich Tiedemann (1819), a committed fighter against slavery, and it contained specimens collected by Carl Gegenbaur (1826-1903), Otto Bütschli (1848-1920) and others. For this purpose, the old specimens had to be extensively restored and at the same time presented in a scientifically up-to-date format. >95% of all exhibits have found their new home in the new showcases of the Timeline Evolution exhibition. This major approach was financed by the university with a generous support of the Schmeil foundation.

„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 thousand 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. The aim of 'Timeline Evolution' is therefore to reconstruct biological evolution and biodiversity on the basis of genetic information (DNA). This concept is new in this form and combines the latest molecular and organismic findings. Based on the genomes of almost all large groups of organisms available today, it is possible to illustrate processes of evolution for research and teaching purposes and also to explain them to an interested public in a science-based and understandable way. The vivid exhibition concept therefore not only addresses interrelationships and processes, but also the latest research approaches and findings as well as future issues such as the consequences of climate change on the threatened biological diversity in all habitats.

This integrative concept required the presentation of valuable historical specimens AND the didactic treatment of biological processes using modern media approaches. 'Timeline Evolution' has succeeded in setting innovative standards in the communication of key issues in the field of biology. This has been impressively successful, as reflected in the comments from local and international visitors so far. The support of the Schmeil Foundation was central to the success and added value of the exhibition, as the communication of complex biological processes - such as the molecular mechanisms of evolution - can only be understood through additional information. At the same time, this provides a perspective on the fundamental question of biological evolution and the beginning of life processes in the cosmos, as well as the consequences of the current irreversible loss of biodiversity. The aim of Timeline Evolution is to illustrate such relationships.

The media stations are continuously updated and are to be expanded using further media techniques (e.g., QR code).

Another important aim for COS and the Timeline Evolution is the link to other scientific fields. This is the case in the areas of climate, cosmology, mass extinctions and physics, chemistry and geology, but also in the social and ethical implications for the humanities and social sciences. Such links will also be incorporated into the university‘s collection concept, which is currently being developed.

Based on our scientific concept and continuous impacts, the new exhibition Timeline Evolution was 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. It also received this prestigious award for Timeline evolution in summer 2023.

Figure 1:
The past, present and future of biodiversity are at the core of our exhibition, respresented here by a collection of endangered and extinct species

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 diverse funding organizations (external funding). During the reporting period, the internal funding was increased by the University to support the centre's activities (Figure 1). This increase was driven by hires at the professorial level, or offers to COS PIs by other institutions and the associated negotiations, as well as salary increases. Internal Funding mostly comes in the form of positions for staff, flexible funds are still extremely limited.

The main funding organization for external grants is the Deutsche Forschungsgemeinschaft (DFG) through several different funding instruments such as Collaborative Research Centers (CRCs), Research Units (RUs) 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) and Horizon 2020), the Bundesministerium für Bildung und Forschung (BMBF), Foundations (Klaus Tschira Stiftung, Baden-Württemberg-Stiftung, Lautenschläger-Stiftung, Volkswagen-Stiftung, Alexander v. Humboldt-Stiftung etc.) and others (including industry funding, equipment purchases, scholarships).

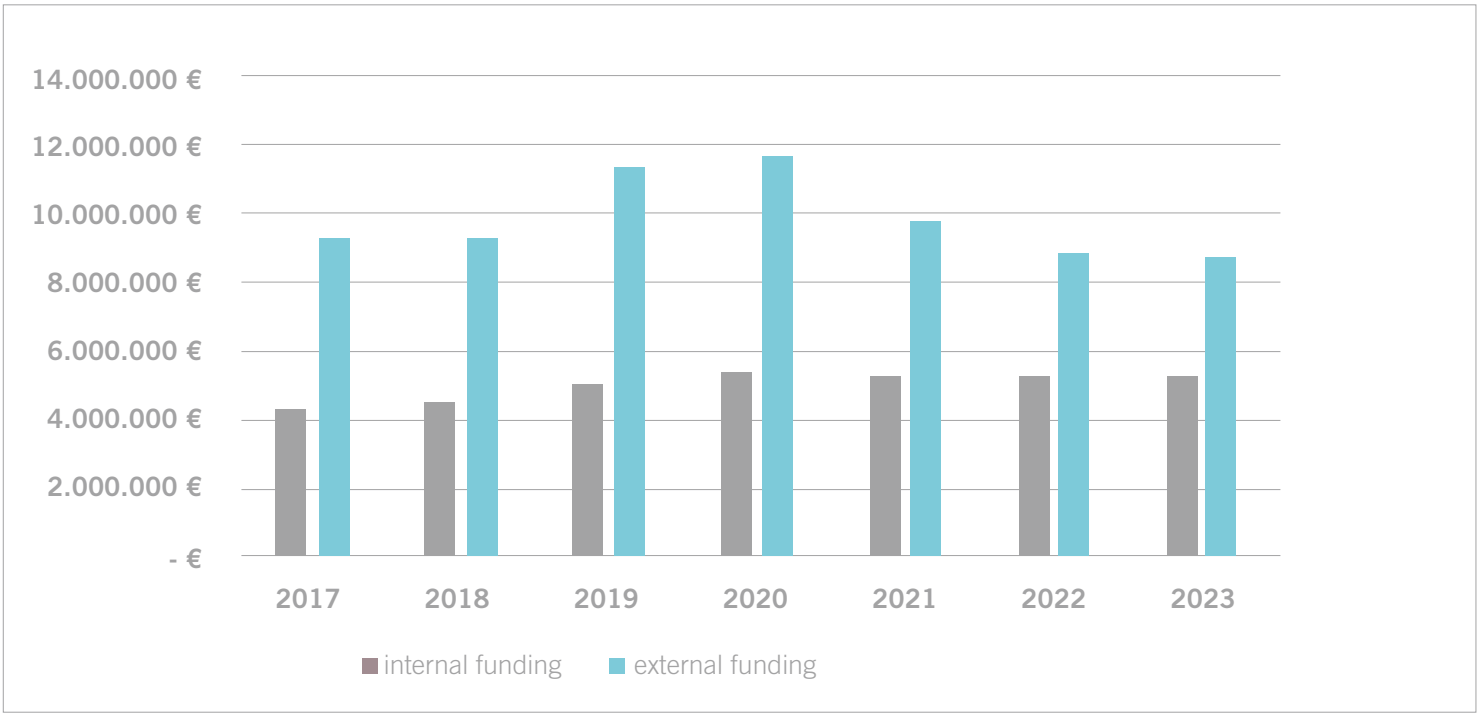

Figure 1:
COS global finances: internal funding versus external funding in € p.a. from 2017 to 2023. Internal funding without individual financial offers of appointment (Berufungszusagen), funds from open positions (Mittelschöpfung) and project-bound state funding (Zweitmittel). Numbers according to the budget of Heidelberg University and SAP expenses.

The ratio between internal and external funding varies between 2,18 (2017), 2,21 (2020) and 1,68 (2022). The most recent decrease of external funding in the years 2022 and 2023 was mainly caused by retirements of COS PIs at the professorial level as well as junior PIs leaving for professorships elsewhere. Another driver was the ending of funding networks with strong COS participation, most prominently the CRC873 and the FOR2581. Conversely, space constraints have limited our ability to hire junior group leaders and COS had to turn away multiple candiates, including two with funded Emmy Noether grants worth more than 3.5 M. We expect that now that all major hires are complete, this trend will reverse. The Emmy Noether Grants to Britta Velten and Lauren Saunders, as well as the ERC grants to Gaspar Jekely and Gilles Storelli will have substantial impact. In addition, with our new colleagues on board, we are in a strong position to apply for new coordinated funding programs, for example a planned CRC on Growth Control and Coordination. Another example of an important ongoing funding initiative is the Cluster of Excellence application GreenRobust, which would represent a substantial boost to the plant sciences at COS.

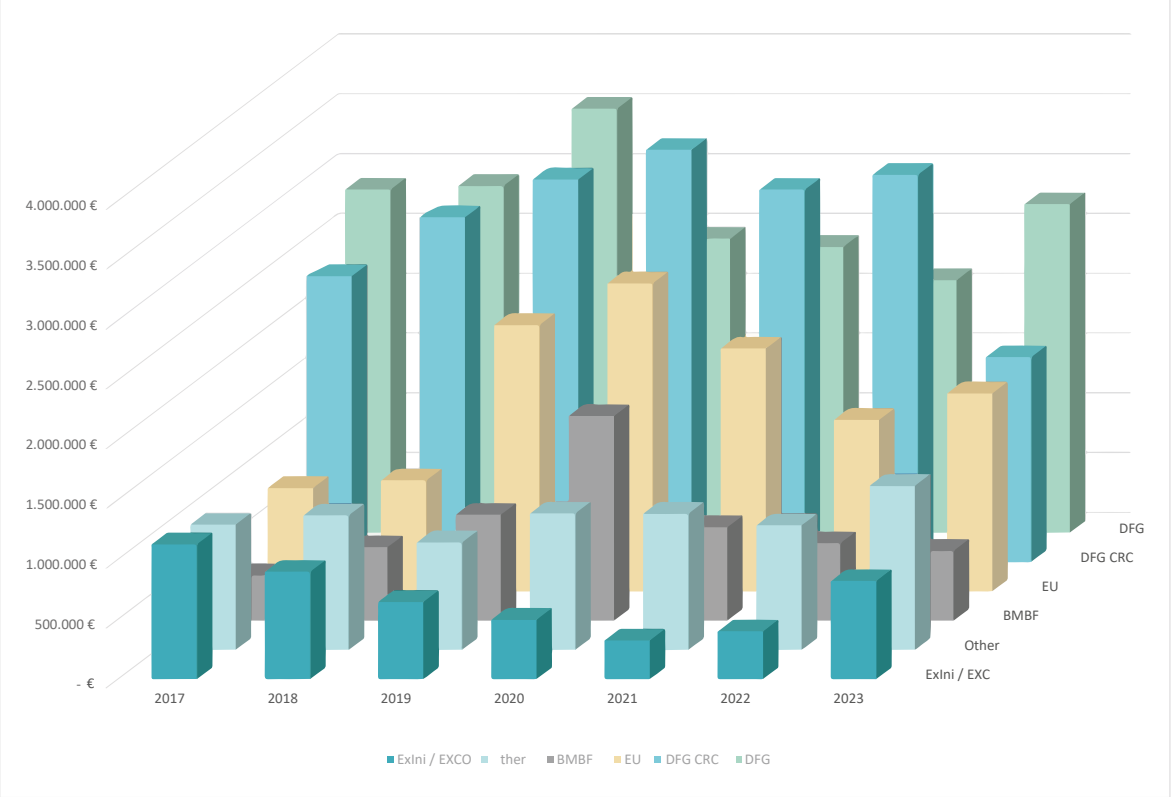

Figure 2:

Detailed analysis of external funding 2017 – 2023 based on SAP expenses. Abbreviations: BMBF Bundeministerium für Bildung und Forschung, CRC Collaborative Research Center, DFG Deutsche Forschungsgemeinschaft, EU European Union, ExIni Exzellenzinitiative des Bundes und der Länder, EXC Exzellenzstrategie des Bundes und der Länder.

A. 2 COS EVENTS

Events: Seminars, Symposia and Public Outreach Activities
Researchers at COS participate in and organize a broad portfolio of 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. In particular, COS has engaged continuously in attracting high school students to the life sciences.

COS Keynote
In order to create a seminar series that would draw attention from a broad base within COS and campus instead of catering to more restricted audiences, the lecture format COS Keynote was started in 2020 with Prof. Dame Caroline Dean as first speaker.

This format was initially planned for with 3-4 COS Keynote lectures per year, replacing the regular COS seminar series, that featured roughly 10 speakers per yaer. For the COS Keynote, high-profile scientists, who are outstanding speakers are selected with the aim to attract scientists working on very diverse systems across the Heidelberg life science community. Their work ideally crosses systematic boundaries or multiple scales of investigation and establishes new concepts of very broad impact.

COS Keynote 2020-2024

Date	Invited Speaker	Title
20.02.2020	Caroline Dean (John Innes Centre, UK)	Antisense-mediated Chromatin Silencing
18.05.2021	Stein Aerts (KU Leuven, Belgium)	From single-cell multi-omics to gene regulatory networks and enhancer logic
23.05.2022	Seth Blackshaw (Johns Hopkins University, US)	Building and rebuilding the retina – one cell at a time
12.01.2023	Irene Miguel-Aliaga (ICL, UK)	The sex and the geometry of inter-organ communication
19.06.2024	Zachary Lippman (CSHL, US)	Dynamic evolution of duplicated genes in shaping plant traits in nature and agriculture

While the COS Keynote turned out to be a very successful format with broad participation, the small number of events sparked the desire to re-establish a more frequent and informal seminar series at COS to increase scientific discussion and to broaden the experience of young researchers. Hence, in parallel to the COS Keynote, the COS seminar series returned from fall of 2024 with a monthly lecture.

Seminars and Seminar Series at COS
In addition to seminars by external speakers, there is an internal platform for PhD students and postdocs to present and discuss their work. Since 2014 PhD students and postdocs organize and participate in a weekly internal seminar solely for the COS community, the "COS PhD and postdoc seminar" re-named "COS talk" in 2018. During the pandemic, the seminar was temporarily changed to an online format and proved to be an important platform not only for scientific exchange but also for social interactions during the weeks of strict lockdown. Since summer 2022, the COS talks are back to in person meetings. To enhance the learning experience for participants every week, PIs are assigned to specific dates to provide structured feedback to PhD students and postdocs on their presentations. COS faculty is in close exchange with the PhD and postdoc community about the seminar to ensure that the format of the talks serves best the needs of postdocs and PhD students and provides a stimulating experience for all COS members.

Symposia at COS
COS Symposia are organized on a biannual basis reflecting a topic selected by the research group leaders of COS. They represent important crystallization points for our scientific development and have become a trademark event on campus. Renown experts from inside and outside COS are invited, short talks selected from abstracts as well as poster sessions provide a platform for PhD students and postdocs to present their projects. COS symposia have received generous financial support by the Klaus Tschira Foundation, HBIGS, GfE, EMBO, eurofins, elife and Nikon as well as from several CRCs and the flagship initiative Engineering Molecular Systems from Heidelberg University.

During the reporting period, two symposia were held.

7th International COS Symposium
Date: October 13 & 14, 2022
Title: Building Functionality - The Relevance of Form Across Biological Scales

Session 1: Architecture of cells & tissues

Julie Gray (University of Sheffield, UK)	Stomata: Form and function of plant pores
Yohanns Bellaïche (Institut Curie, France)	Regulation of epithelial tissue morphogenesis

Open Lecture

Manu Prakash (Stanford University, USA)	Curiosity-Driven Science

Session 2: Organization of molecules and organelles

Edith Heard (EMBL Heidelberg, Germany)	Epigenetic mechanisms in development and disease: insights from the X chromosome
Mónica Bettencourt-Dias (Gulbenkian Institute, Portugal)	Centrioles in development, evolution and disease: tiny organelles, multiple and critical functions

Session 3: Cellular and tissue mechanics

Verena Ruprecht (CRG, Spain)	Morphodynamic cell and tissue plasticity in development
Gáspár Jékely (University of Exeter, UK)	Organismic biology in Platynereis: linking genes, brains and bodies to the environment

Session 4: Engineering biological systems

Kerstin Goepfrich (MPI Medical Research, Germany)	Synthetic cells: Building functionality with DNA nanotechnology
Linnea Hesse (Freiburg University, Germany)	Taking a looking into living material systems: how plants can inspire technology

Session 5: Organismal form

Toshihiko Fujimori (NIBB, Japan)	Trans-Scale polarity formation in the mouse oviduct
Edwige Moyroud (University of Cambridge, UK)	One-size-fits-all? Understanding how flowering plants build communication devices on their petals

8th International COS Symposium
Date: July 22 & 23, 2024
Title: Life in Context - Organismal sensing and adaptation in the natural environment

Session 1

Thomas Richards, Luis Galindo Gonzalez – Oxford University, UK	How eukaryotic cellular complexity arose and diversified
Rosa Lozano-Durán – ZMBP Tübingen, Germany	Manipulation of plant development by Geminiviruses
Magdalena Julkowska - Boyce Thompson Institute Ithaca, USA	Stress-induced changes in plant architecture

Session 2

Hanh Vu (EMBL Heidelberg, Germany)	Control principles defining animal body size
Mitsuyasu Hasebe (NIBB, Japan)	The evolution of novel traits in land plants
Janna Nawroth (TUM Munich, Germany)	Physics-based mechanisms of animal locomotion, feeding, defense and interaction in aquatic environments
Flash talks by selected poster abstracts	

Session 3

Nicole Dubilier (MPI Bremen, Germany)	Biology and ecology of associations between bacteria and eukaryotes
James Saenz (B CUBE, TU Dresden, Germany) (short talk)	Minimal cells as models for membrane sensing and adaptation
Francesco Licausi (Oxford University, UK)	Adaptations to oxygen fluctuations throughout plant evolution
Nóra Szabó (Eötvös Loránd University Budapest, Hungary) (short talk)	The development of the labyrinth organ in the air-breathing paradise fish (*Macropodus opercularis*)

Session 4

Kazuo Inaba - Shimoda Marine Station Japan	Structure, function and evolution of cilia and flagella in marine organisms
Tonni Grube-Andersen – MPIZ Cologne, Germany	Plant root communication with the environment
Jiyan Qi – Northwestern Poly-technical University, Xian, China) (short talk)	Single-cell transcriptome atlas in developing multicellular glandular trichomes

Session 5

Josefa Gonzalez - CSIC Barcelona, Spain	Organism adaptation to the environment – a comprehensive view in the fly
Flora Vincent – EMBL, Heidelberg, Germany	Diversity and impact of marine microbial interactions across different biological scales

The EMBO Lecture

Liam Dolan (GMI, Austria)	Early land plant evolution

Bertalanffy Lecture Series
The Bertalanffy Lecture Series [1] 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 regularly attracts more than 200 students from high schools in Heidelberg as well as other cities and resonates deeply 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.

Date	Invited Speaker	Title
14./15.10.2021	Elaine Ostrander	Tip to tail: How to construct a dog & Genetics of morphology in the domestic dog
10./11.11.2022	Alfonso Martinez Arias	Cells are us & Stem cell models of mammalian embryogenesis: Hype or hope?
29./30.06.2023	Angela Nieto	30 years travelling with cells & Cell plasticity trajectories in development and adult disease
29.02./ 01.03.2024	Detlev Arendt	The origin of the nervous system - decision-making in early animals & Building bilateral brains: Innovations in molecular machinery, neuron types and circuits
18./19.06.2024	Zachary Lippman	Tomatoes in space! & Dynamic evolution of duplicated genes in shaping plant traits in nature and agriculture (COS Keynote)

The Bertalanffy Lecture is joined by several other measures and formats to attract high school students and the interested public to the life sciences.

– **Bertalanffy Practical**
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.

– **Science goes to school**
Since 2016, COS PhD students visit biology courses in schools and talk about their research project. This way, high school students gain insight and PhD students train their communication and presentation skills.

1 https://www.cos.uni-heidelberg.de/en/centre-for-organismal-studies-heidelberg/public-outreach

2 http://www.stuetzpunktschulen.de

– **Bertalanffy Interview**
Since 2023, lecturers of the Bertalanffy program are also invited for an interview[3] and thus providing further insights for high school students.

– **wissenschaft.leben**
During the pandemic, the digital format "wissenschaft.leben" was initiated. It aims at explaining basic research to the public[4] and offers both discussion rounds and podcasts on Heidelberg University's

Event	Invited Speaker	Title
Panel discussion	Claudia Denkinger, Andreas Welker, Michael Knop	Universität und Gesundheitsamt: Mit Tests gemeinsam gegen Corona
Panel discussion	Barbara Mittler, Johanna Stachel, Ekkehart Reimer	Wissenschaft – ein Team-Sport?
Panel discussion	Beatrice Lugger, Albrecht Schütte, Marius Fletschinger	Wie erreicht die Wissenschaft die Gesellschaft?
Panel discussion	Bernhard Eitel	Mit Vollgas durch den Nebel – 16 Jahre im Maschinenraum der Exzellenz in Heidelberg
Podcast, episode 1	Annika Guse Sebastian Rupp	Alles schon im Kopf drin, das ist der erste Schritt
Podcast, episode 2	Steffen Lemke Sylvia Urbansky	Kommt einem vielleicht komisch vor
Podcast, episode 3	Thomas Greb Theresa Schlamp	Ich wollte das eigentlich geheim halten
Podcast, episode 4	Rüdiger Hell Laura Armbruster	Und da hat eine Schnecke meine Tabakpflanze angefressen
Podcast, episode 5	Heike Lindner Michael Raissig	Aber es geht ja weder um Leben und Tod
Podcast, episode 6	Alexis Maizel Michael Stitz	Ein kleines bisschen Probleme mit Autorität
Podcast, episode 7	Melanie Krebs Roland Gromes	Man muss sich die Information natürlich dann auch irgendwann durchlesen
Podcast, episode 8	Jochen Wittbrodt Christina Schlagheck	Möglicherweise ist Kaffee ein guter Katalysator
Podcast, episode 9	Jan Lohmann Denis Janocha	... bald ein ganzer Mensch
Podcast, episode 10	Ingrid Lohmann Katrin Domsch	Der tollste Beruf, den man eigentlich machen kann

3 https://bertalanffy-live.de/bertalanffy-lecture/

4 https://bertalanffy-live.de/wissenschaft-leben/

– **Bertalanffy Explorer Day**[5]
  This new format is targeted to high school students aiming for an apprenticeship and explains the infrastructure of a research institution with a broad range of jobs from lab technician, mechanic, electrician, administrator or systems administrator.

The Botanic Garden with its "Grüne Schule"[6] continues to be very active in public outreach: offering guided tours and courses itself and participating in the "Junge Universität" of Heidelberg University[7].

In addition to the here mentioned scientific and outreach programs, researchers at COS participate in many other initiatives on campus, notably the KinderUni[8] and the courses of the "Tschira Jugendakademie"[9].

5 https://bertalanffy-live.de/bertalanffy-explorer-day/

6 https://grueneschule.cos.uni-heidelberg.de/index.php

7 https://www.uni-heidelberg.de/de/studium/lebenslanges-lernen/junge-uni

8 https://www.uni-heidelberg.de/de/heischool/kinderuni-digital

9 https://www.tschira-jugendakademie.info

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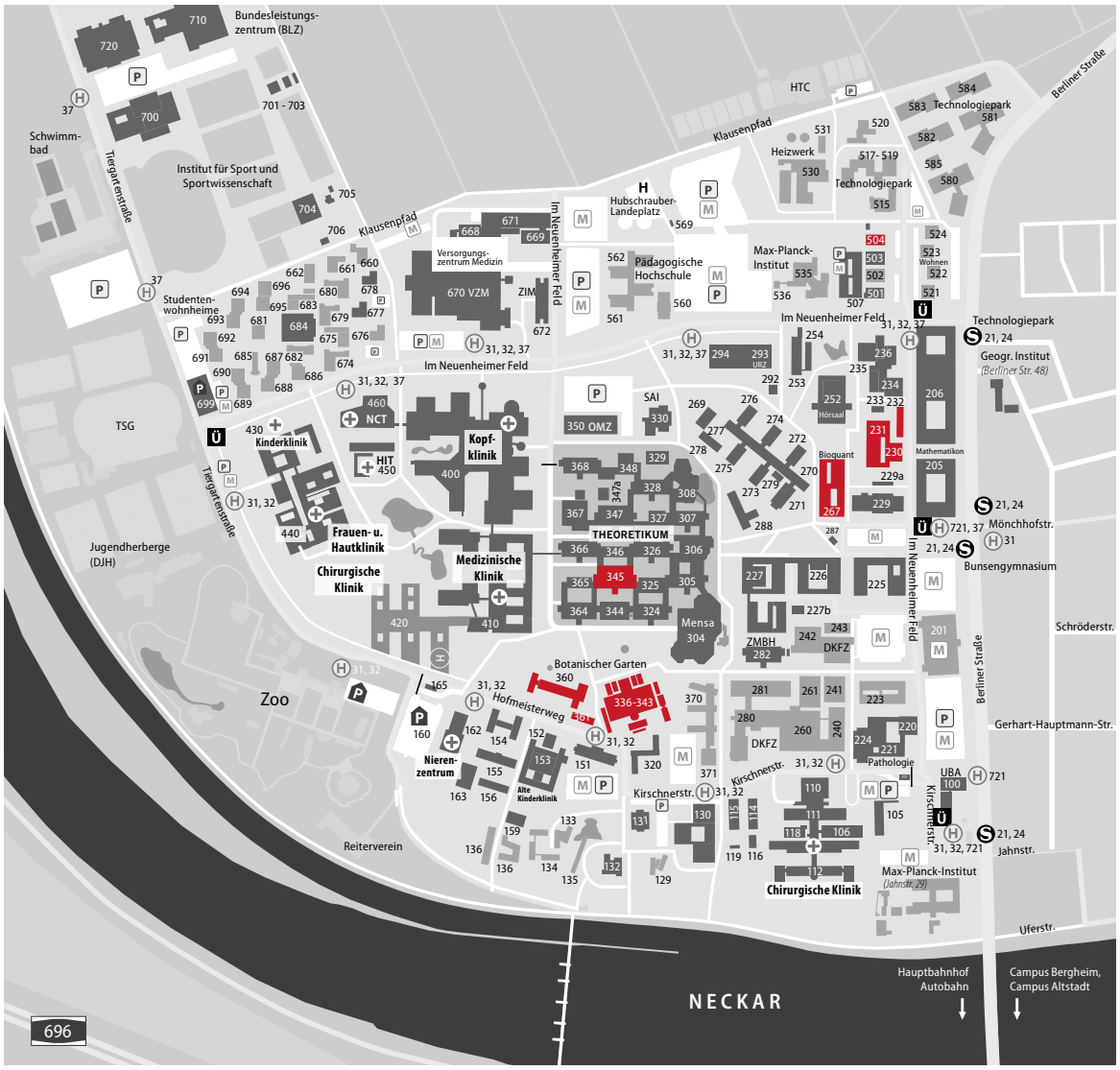

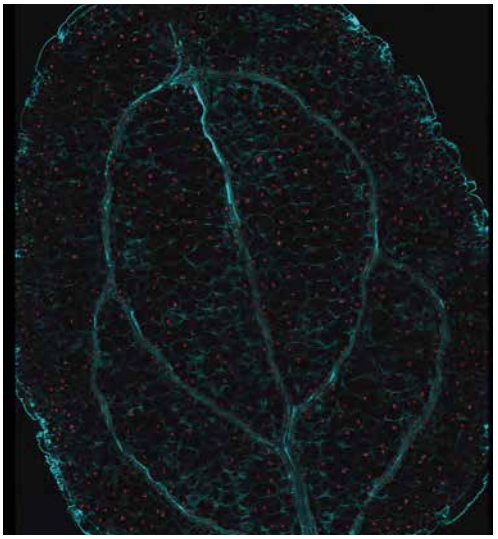

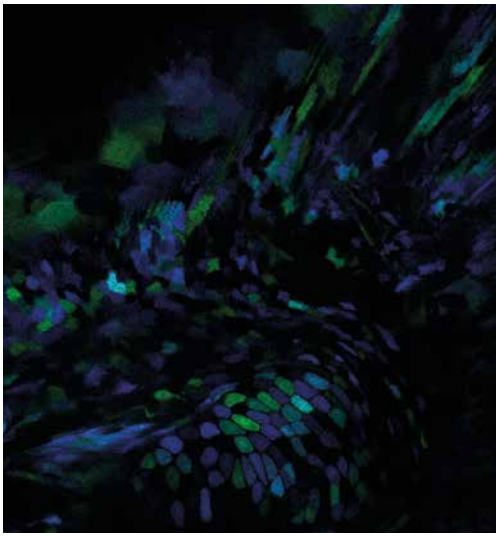
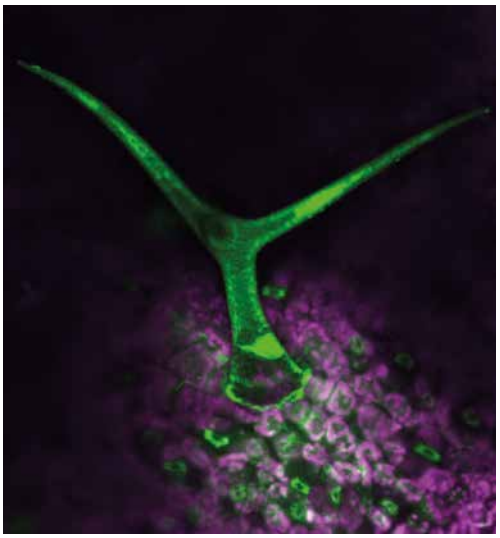



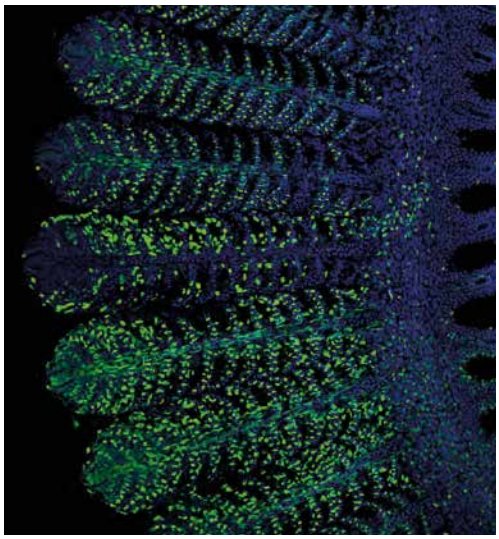

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