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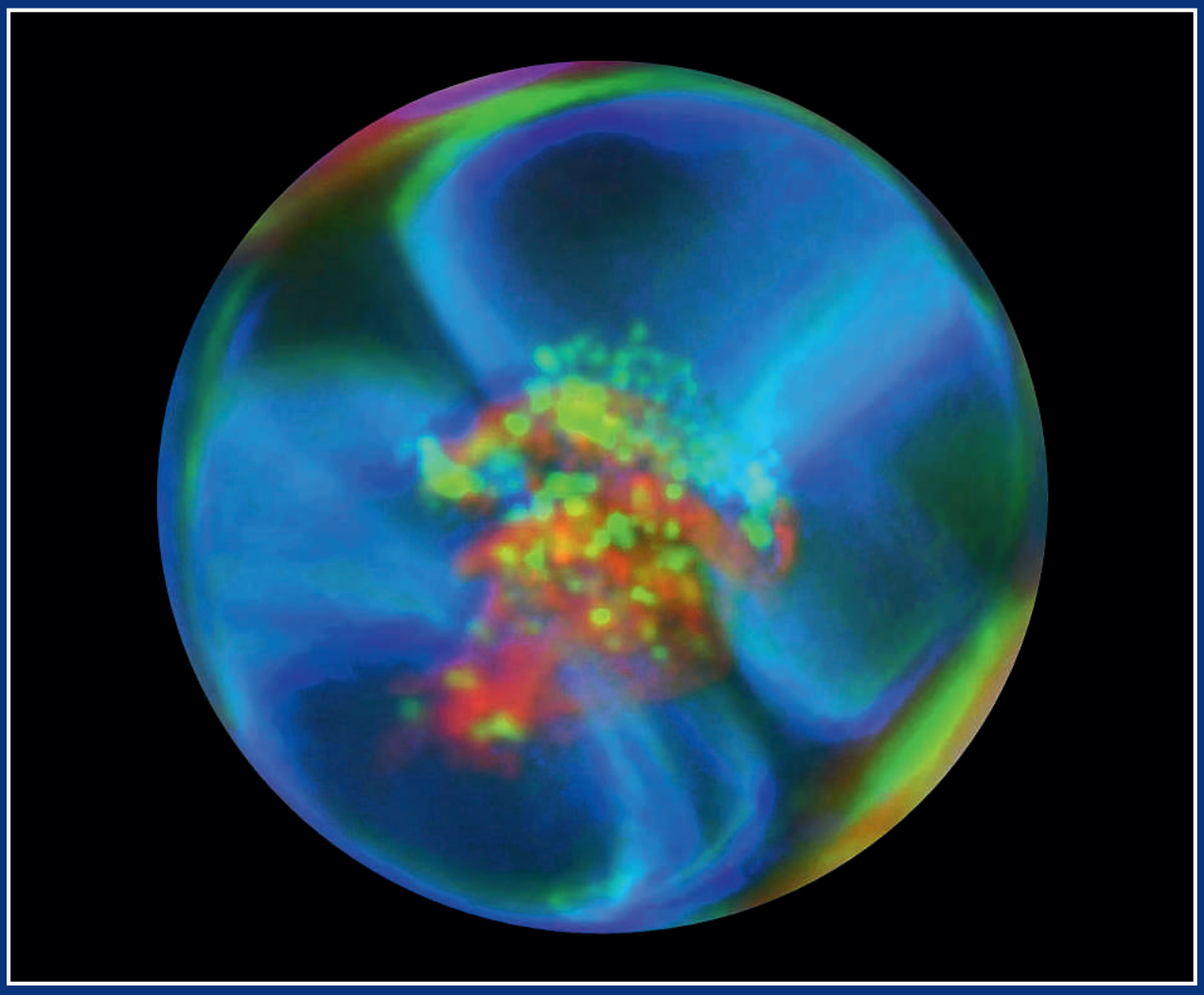
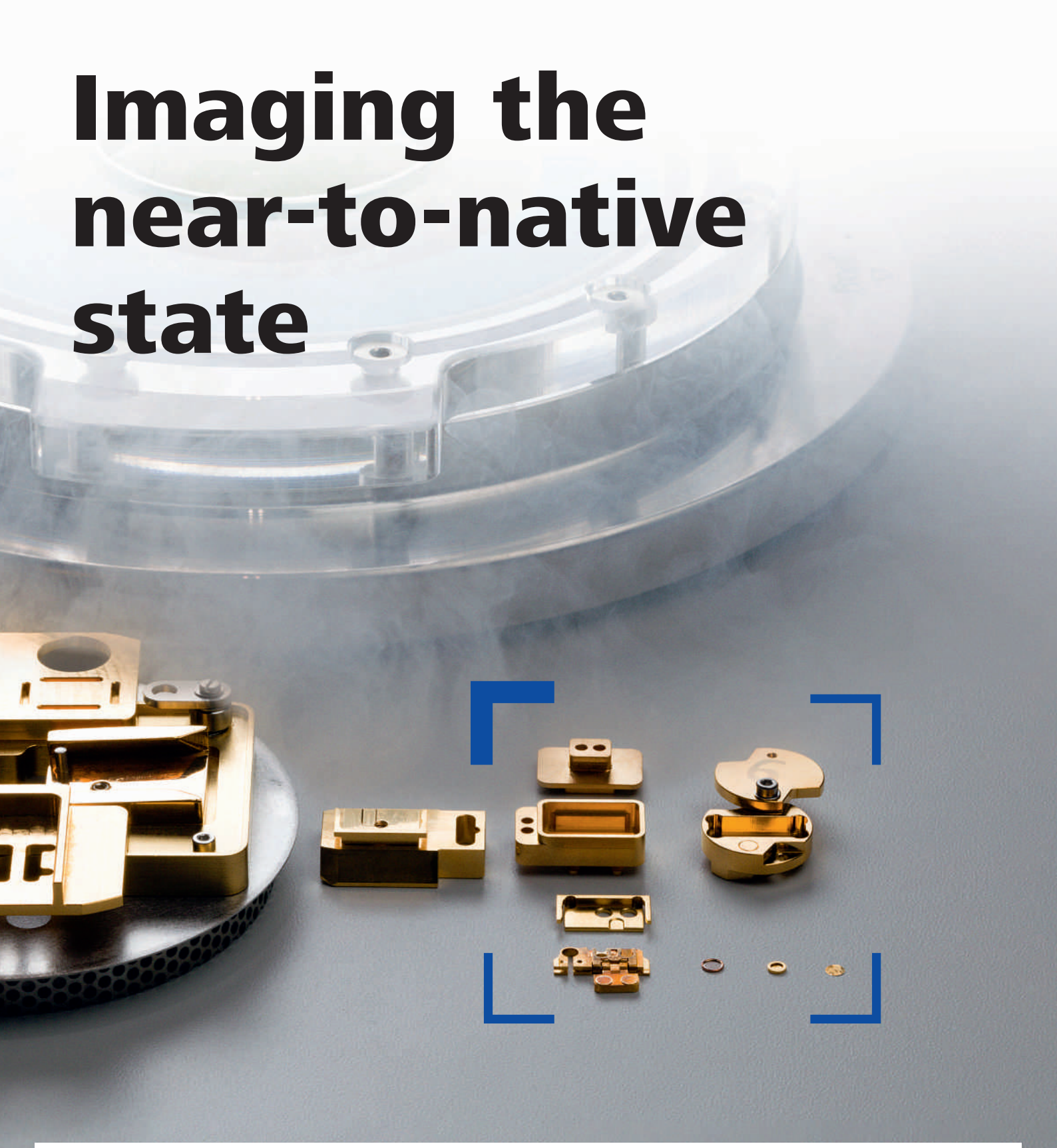


DGZ Prize Winners 2020

DGZ

Imaging the near-to-native state



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Seeing beyond

CONTENT

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Cover Image: HystemC hydrogel droplet (blue autofluorescence) containing Lifeact-GFP expressing cells (green) and fluorescent beads (red). Copyright: Dr. Laura Hockaday Kang, Münster

Dear members and friends of the German Society for Cell Biology!

The ongoing pandemic has greatly affected us all and required a reorganization of our day-to-day activities. In particular, the manner in which we conduct and experience social interactions in our private life and professionally has been completely turned on its head. As a scientific society we depend and build on interactions between our members and these changes have posed significant challenges. However, they have also forced us to face long-standing issues regarding the reach and structure of our society. Following the election in December 2020, the new board with Roland Wedlich-Söldner, Gislene Pereira, Sandra Iden and Ralf Jungmann has started to tackle some of these issues in order to strengthen the DGZ in the years to come:

- **International meeting:** After having to cancel our international meeting in 2020 and with still no end to the pandemic in sight we decided to hold our international conference on "Life in between – The cell biology of interfaces" in a fully digital format from 27th-29th of September 2021 (see call in this issue). In order to highlight outstanding collaborative research, this year's international meeting will be the first to showcase various local and national research consortia that work on interfaces in and between cells. The conference will also strengthen international collaborations with a dedicated session organized by the mechanobiology institute (MBI) in Singapore. Registration to the conference will open on May 10th, so make sure to join us for this exciting event!

- **DGZ web presence:** We are currently working on a complete overhaul of our web presence to offer a central platform for all cell biology research in Germany – and more. We will showcase important new publications, link meeting announcements and offer extended services like seminar series, opportunities for networking and resources for career development.

- **Topical organization:** To further the exchange and collaboration between DGZ members as well as to support junior scientists we will establish thematic working groups that cover major topics of cell biological research.

- **Scientific awards 2021:** Thanks to the generous sponsorship by ibidi the Walther Flemming Award will once again honor groundbreaking research establishing the independence of young scientists. The calls for all five DGZ awards in 2021 can be found in this issue of CellNews.

- **Scientific awards 2020:** Even during the pandemic we have honored achievements in Cell Biology at various career levels. Petra Schwille presented the Zeiss lecture, Chun So received the Nikon young scientist award, Christian Münch received the Binder Innovation prize and Katerina Rohlenova is the recipient of the Werner Risau prize. The award presentations can be found on the DGZ homepage (https://zellbiologie.de/wissenschaftspreise/), and in this issue of CellNews the four winners give us some additional insights into their work.

We hope to return soon to some sort of normalcy - privately and professionally - while trying to change for the better. What can we do better? What kind of services should we aim to offer? Please let us know about your thoughts on these changes and any additional wishes for the DGZ.

Stay safe and we will hopefully be able to meet in person again soon - and together shape the future of cell biology in Germany.

The DGZ!

Life in between – The cell biology of interfaces

DGZ International Meeting

27-29. September 2021, Schloss (virtual) Münster, Germany

Confirmed Speakers

M Affolter, S Barnett, P Bassereau, P Bauer, Y Bellaïche, P Bieling, M Bohnert

K Busch, CJ Chan, Ü Coskun, A del Campo, P Dersch, F Fröhlich

Z Gartner, MI Geli, M Gotta, R Grosse, TY Han, J Hegemann

H Hilbi, A Holle, A Honigmann, B Hoogenboom

C Kleanthous, L Klotz, U Kutay

J Lammerding

M Lecuit

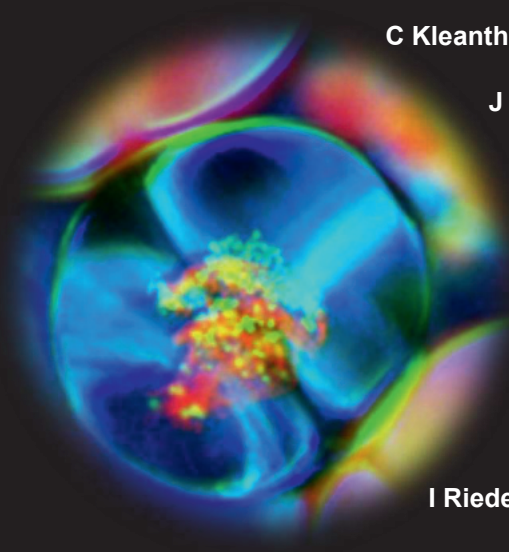
HK Lee

RV Pappu

N Pfanner

S Pfeffer

I Riedel-Kruse, S Rizzoli



A Roux, S Rumpf, G Schmidt, D Schmucker

S Schuck, M Schuldiner, A Tijore, B Trappmann, JP Vincent

D Wachten, H Wang, W Weber, S Wegner, J Young, C Ziegler

https://dgz.orgalution.de/

Carl Zeiss Lecture

How relevant is physiological relevance? A physicist's journey in and out of cell biology

Petra Schwille, MPI of Biochemistry

It's the interactions, stupid!

More than a century of "life sciences", there are still important gaps in our fundamental understanding of what makes life such a special phenomenon and how it could have originated. The secret may lie in its composition, i.e., a very specific carbon-based chemistry, or in the fact that living systems are incredibly complex, because this is how we find existing living system to be, even in their simplest representations. But the physicist's answer would probably be "it's the interactions, stupid". Around the middle of the last century, before the formulation of the central dogma, this was suggested by theorists, such as Erwin Schrödinger in his famous public speculations about life (Schrödinger, 1944) or Alan Turing, who laid the foundation of understanding morphogenesis (Turing, 1952). Theory suggests that life in its most abstract form emerges from particular dynamics and interactions of its constituents that allow to couple energy dissipation to the emergence of structure and function in a fundamentally irreversible process. Reaction features such as nonlinearity and autocatalysis, as well as particular feedback connections appear to be of great importance for the emergence of living systems (Ganti 2003, Gierer and Meinhardt 1972), irrespective of the exact molecular makeup of life's precursors. Importantly, the wiring of processes in cells and organisms existing today, in spite of their huge degree of complexity accumulated over 3.5 billion years of evolution, still follows pretty much the same design features, and quantitatively elucidating their underlying reactions in space and time has remained the ultimate challenge, even if the past decades with their many "omics" have focused largely on the mapping of molecular species and their connections as such. In fact, the enormous technical breakthroughs made in the past years with regard to resolving cellular ultrastructures, and the analytical and data-based revolution, providing the means for an almost complete registry of the genetic and protein content of single cells, disguise the fact that our understanding of reaction dynamics in living cells, at least at comparable precision and comprehensiveness, lags way behind. The cell biologist's favorite tool, the light microscope, has in the past years improved greatly with regard to spatial resolution, but in order to capture molecular diffusion, spontaneous reactions, and molecular transitions in living cells, often lacks the temporal resolution, which is an even greater concern for the highly popular super-resolution microscopy.

Astonishingly, the technology for precise dynamic analysis of spontaneous molecular processes on the level of single biomolecules in the important time regime of microseconds to seconds, Fluorescence Correlation Spectroscopy (FCS), has been around for more than two decades (Schwille 2001), and even found its entry into confocal microscopes of various manufacturers as modular option, but still - or again, after a period of greater attention - ekes out a niche existence among cell biologists. And this is not only because of the less glamorous kind of data it yields, i.e., dry curves, as compared to colorful images. So, what has gone wrong with the biological applications of this powerful dynamic method that it is still not liked and accepted very much?

FCS in cell biology – hope and despair

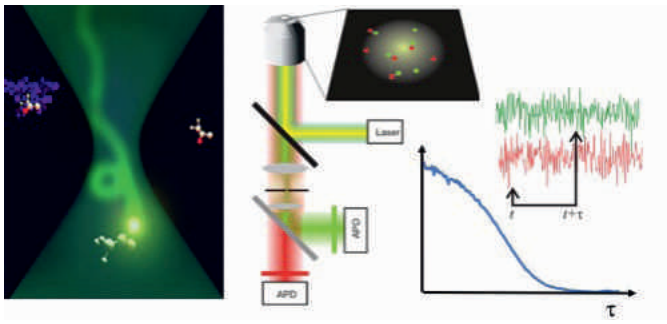


Figure 1: Principles of FCS and FCCS: single molecules can be observed and dynamically analyzed by their diffusion through confocal volume elements, and interactions can be probed by coincident fluctuations in spectrally distinct channels (Bacia et al., 2006)

In order to understand why FCS raised great hopes for a more quantitative and thus, fundamental understanding of molecular dynamics in cells, but also for resolving the question why its application in cell biology can be quite an ordeal, we have to take a quick look at its principles (Figure 1). FCS is mostly performed in a confocal setup, and therefore easy to realize in confocal microscopes. The idea is that the fluorescence signal resulting from fluorescent molecules in a focused laser beam is not integrated into a brightness value that is then scanned in 3D, but rather temporally analyzed. When zooming into the time axis of our fluorescence signal to capture processes on fast time scales

down to the sub-microsecond range, which is possible with the time resolution of point detectors such as PMTs or APDs as built into common confocals, we will no longer integrate over many molecules to yield a single bright spot, but be able to actually see single ones of them buzzing around, i.e., diffusing in and out of our laser focus. Thus, when looking at the intensity signal with this high time resolution, we will see bursts of fluorescence that lead to an overall fluctuating average, and these fluctuations contain the precious information about the spatio-temporal dynamics of our molecules. Temporal (autocorrelation) analysis of these fluctuations reveals the underlying patterns, yielding diffusion coefficients and binding kinetics to larger interaction partners or immobile structures, such as membranes or chromosomes. When shifting to two or more color labeling, interactions between different species of diffusing molecules can be studied by the Cross-Correlation variant (FCCS, Schwille et al. 1997), which analyzes coincident fluctuations in two independent fluorescence channels, such as green and red, pointing to coordinated movement of different species of molecules. This, in turn, provides a much less ambiguous indication that the two molecular species have truly bound each other, as compared to fluorescence colocalization studies that are otherwise used to probe binding. But FCS delivers even more information. The amplitudes of the autocorrelation curves are inversely proportional to the respective number of molecules in the focal spot at any time, such that with the right calibration measurements, local concentrations, as well as the brightness per particle, pointing to potential multimerization, can be derived.

In fact, all of these applications have been reported in the past, many of them even in the living cell or multicellular model organism, such as the zebrafish embryo (Bacia et al. 2006, Yu et al. 2009). Why then is FCS/FCCS not applied much more regularly and frequently? One major problem is at the same time the strength of the technique: its sensitivity to pick up signals from single molecules. In well controlled buffer solutions that can be kept at low concentrations of fluorescently labeled particles, and more importantly, largely free of background fluorescence, this sensitivity can indeed be played at its strength. However, in cells with hardly controllable concentrations and a large pool of unwanted background molecules, many of them autofluorescent, great sensitivity just means that a lot of information is picked up that nobody really wants. It is a bit like putting up a radio astronomy telescope in the mid of Manhattan. Simply reducing the excitation intensity and measuring for a longer time does not necessarily help, as FCS records fluctuations around an equilibrium, and the cell is obviously not in equilibrium at all, i.e., the measurements should ideally not last much more than fractions of minutes. Thus, in spite of the many molecular processes in cells that would greatly benefit from sensitive FCS/FCCS analysis, very few cellular FCS experiments have so far reached the state of widely-applied routines. But even if one truly masters the technique and accomplishes a tight control of the biological setting by sophisticated assay design, which can be a quite painstaking expertise: even then it is all but clear that the dynamic data that one collects indeed displays the level of unambiguity that would be desirable. The reason is that processes in cells are so intricately linked that the standard rationale of a physicochemical measurement: keeping as many reaction parameters as possible constant and varying only few or ideally only one, is doomed to failure. Focusing on a specific cellular process and hoping that all other players in the game will remain silent for the time of measurement is like fleas herding, and although established pharmacological tricks to suppress cellular processes may help to some extent, there will always be a sizeable "basal" level of processes that cannot be suppressed as long as the cell is alive, which a single molecule technique will certainly register.

Make biology simple again?

Having been an FCS/FCCS development and application lab of the first hour, the said problems with applications in "physiological settings" have increasingly bothered us over the many years of collaborations with groups working on different biological systems. It also became obvious that applying our methods in established model organisms, while potentially contributing to the quantitative understanding of these systems, will not reveal their fundamental makeup as living entities. Thus, in the past decade, we proposed and have since been pursuing a fully new approach towards biology, i.e., "bottom-up synthetic biology" (Schwille 2011). The underlying idea is that only a radical simplification and abstraction of a biological cell will allow us to understand the distinctive features of life, because even the simplest life forms on earth have accumulated a huge degree of redundance in order to remain viable in a hostile and competitive environment. Abstracting from this massive and in large parts non-hierarchical complexity of interactions in a living system, otherwise being the hallmark of the physicist's approach, is doomed to failure. Thus, in order to arrive at a self-sustaining minimal system of molecular interactions with the ability to evolve – a minimal living system - we likely need to build it from scratch. Technically speaking, we need to assemble functional modules from the bottom-up until the system emerges basic functions of life. Exactly this has been the focus of our research for more than 20 years now.

In particular, we have focused on the emergence of cell division as a genuinely physicochemical process. With compartmentation being a key facilitator of biological identity, the challenge is to elucidate the mechanistic origin of self-replication of membrane compartments. Like many other groups, we employ Giant Unilamellar Vesicles (GUVs) as bases for protocells, because of their facile deformability and comfortable sizes, allowing to study membrane transformations by light microscopy. For a long time, our - retrospectively slightly blue-eyed - idea has been to functionally reconstitute into them a minimal set of self-positioning and membrane-transforming proteins and identify the conditions under which a spontaneous division process could be engineered. In spite of this overly optimistic approach (given all the unknowns about existing minimal divisomes, even

in microorganisms), a huge breakthrough was made when we accomplished the reconstitution of a minimal self-organizing biological machinery at the basis of bacterial cell division (Loose 2008). These proteins, MinD and MinE from E.coli, are supposed to orchestrate the positioning of the divisome to mid-cell by establishing oscillating concentration gradients on the membrane between the two poles and the center through spatiotemporal self-organization. Here we showed that a set of only these two proteins

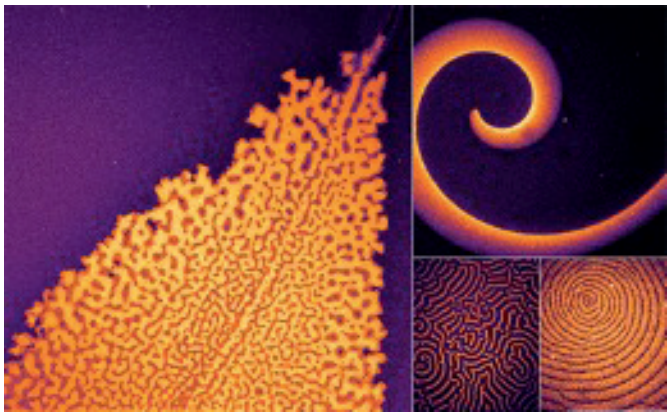


Figure 2: Self-organized patterns resulting from engineered MinDE proteins, dissecting them into minimal functional modules (Glock et al., 2019)

purified from E.coli bacteria, a membrane, and a metabolic energy source yielded emergent behavior of pattern and gradient formation. Intriguingly, the ability of these spatial patterns to act as a spatial cue for other proteins of the bacterial divisome, first and foremost the division-ring forming tubulin homologue FtsZ, could be confirmed in cell-shaped compartments (Zieske and Schwille 2014). In order to arrive at the mechanistic core of this archetypal self-organization reaction, combining the key paradigms of cooperativity and feedback, we invested greatly in molecular biology and mutational analysis of the Min proteins (Kretschmer 2017, Heermann 2020a). Many years of intense biochemical research enabled us to engineer these proteins towards basic and simplistic functional modules, finally being able to define a minimal set of functional features for biological pattern formation (Glock et al., 2019, Figure 2). This led to the recent design of pattern-forming molecules that are much simpler than the wildtype proteins, in which even protein modules can be exchanged for nucleic acid sequences (Heermann 2020b).

When proteins surpass themselves

Although many other functional modules will likely be required to assemble something like a minimal machinery that actively and spontaneously accomplishes large vesicle division, our research on the self-organizing Min proteins yielded a wealth of highly interesting and partly also spectacular observations. This first became apparent when, after many years of intense assay optimization, we accomplished the efficient transfer of functional MinDE into free-standing GUVs (Litschel et al., 2018). Intriguingly, we did not only observe pole-to-pole-oscillations and an also expected set of other oscillation modes induced by the mostly spherical geometry of the vesicles, but also spectacular deformations of osmotically deflated vesicles which resulted in a bouncing and "dancing" behavior of the whole vesicle in accord with the oscillating membrane binding and unbinding of the proteins within. In cases of dramatically deflated vesicles, this went as far "hemi-fission", i.e., almost fully divided vesicles only connected by a small membrane neck. All of this was solely accomplished by the cooperative membrane binding through MinDE self-organization, which itself is based on ATP consumption. Thus, chemical energy provided by ATP was turned into mechanical work of deforming the membrane, a process that could be more clearly visualized by Min proteins pulling and stretching elastic membrane tubes as if they were mechanical springs (Fu et al. 2020). However, an even more unexpected discovery was that this set of molecules also induced a directional active transport of other, functionally unrelated molecules by diffusiophoresis (Ramm et al. 2021). Like the pulling of membrane tubes, this process is mechanical in nature, beautifully evidenced by the fact that the transport process is selective for the size of the molecules on the membranes, which can be used to sort different species of particles on the membrane surface. Again, we observe the conversion of chemical energy into work by the bacterial MinDE – which in complex cells is accomplished by far more complex motor proteins. It is thus fair to state that the E.coli Min proteins, which in their cellular context have a limited well-defined functional role, when subject to minimal cellular topology display a rich set of unexpected, "hidden" functionality far beyond what could be expected.

Relevant or not relevant?

The million-dollar question, and unfortunately the first one that an excited biophysicist when reporting about new and unexpected protein functions will hear from biology-based reviewers, is now: How is this physiologically relevant? Which, in order to prove, would require returning to exactly the complex, unwieldy, and often extremely time-consuming business of cellular measurements that we had hoped to have left behind. With regard to the particular "hidden functions" observed in MinDE proteins as outlined above, it is extremely unlikely that mechanical transformations of cell membranes are to result from the Min protein oscillations in vivo, because the bacterial cell wall – missing in the flexible vesicles – would preclude that. In contrast, the Min proteins' "sweeping" role, i.e. directional transport of other membrane-attached molecules during the oscillation cycles, presumably towards mid-cell, is much more likely to play a role, but will prove quite complicated to be shown convincingly, due to the small size of bacterial cells. In fact, first hints that the effect is at least reproducible on live cell membranes could be demonstrated when transferring the Min proteins into yeast cells (Ramm et al., 2021). But regardless of whether or not the effect plays an important role in bacteria, it should be allowed

to ask back the following provocative question: Is the relevance of a protein function indeed only dependent on whether it is found indispensable in a particular organism? Or is not the fact that a protein displays a particular function in whatever setting a clear indication that this function may become relevant, or may have been relevant, in an evolutionary different situation? Wouldn't it make quite some sense for cells and organisms to "store" unused protein functionalities that may only unfold under very specific conditions, rather than having to develop them from scratch by random genetic mutations under evolutionary pressure? This question can certainly not be addressed easily, but with respect to the more practical goal of constructing a minimal cell from the bottom-up, unexpected functionalities are definitely good news, as they expand the library of functional tasks with a limited set of modules. In this respect, our newly discovered functionalities as displayed in minimal systems, will be able to be used exactly for this. Thus, even if the goal of assembling a minimal self-dividing system is still far from being reached, our approach shows that biology is always ready for a surprise, and sometimes even simplifies tasks, rather than complicating them. That's a great motivation for the next years of minimal cell research and development.

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Curriculum Vitae
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Academic Education

1996	PhD, Braunschweig University of Technology
1993-1996	Doctoral thesis, Max-Planck-Institute for Biophysical Chemistry, Göttingen Supervisor: Prof. Dr. Manfred Eigen
1987-1993	Study of Physics and Philosophy, Universities of Stuttgart and Göttingen

Professional Career

Since 2012	Honorary Professor at the Ludwig-Maximilians-University Munich (Physics Faculty)
Since 2011	Director at the Max Planck Institute of Biochemistry, Martinsried
2002-2012	Full Professor of Biophysics, BIOTEC, University of Technology, Dresden
1999-2002	Group Leader at MPI for Biophysical Chemistry, Göttingen
1997-1999	Postdoctoral Fellow at Cornell University, Ithaca, NY, USA
1996-1997	Postdoctoral Fellow at MPI for Biophysical Chemistry, Göttingen

Awards

2020	Carl Zeiss Lecture Prize, German Society for Cell Biology (DGZ)
2018	Bavarian Maximilian Order for Science and Art
2017	Biophysical Society Fellow
2015	Honorary Fellow of the Royal Microscopical Society
2013	Suffrage Science Award, MRC-CSC, London
2011	Braunschweig Research Prize
2010	Gottfried Wilhelm Leibniz Prize of the German Research Foundation (DFG)
2005	Max Planck Fellow of the MPI for Molecular Cell Biology and Genetics
2004	Philip Morris Research Prize 2004
2003	Young Investigator Award for Biotechnology of the Engelhorn Foundation
2001	Lecturer award by the German Chemical Industry Fund

Nikon Young Scientist Award

Liquid-liquid phase separation organizes microtubule regulatory proteins for acentrosomal spindle assembly in mammalian oocytes

Chun So

Meiosis in mammalian oocytes

In mammals, oocytes are arrested in diplotene stage and are kept in discrete follicles in the ovaries after birth. Throughout the entire reproductive lifespan in females, cyclic hormonal surge triggers the growth of a portion of follicles and the subsequent resumption of meiosis in a portion of oocytes.

To become fertilizable eggs, oocytes have to reduce their chromosomes by half with the help of a specialized microtubule spindle during meiosis. The female meiotic spindle in mammalian oocytes was first examined at ultrastructural level back in 1970s (1). Although the pathways responsible for microtubule nucleation have largely been identified in mammalian oocytes in the past 50 years (2-4), little was known about the assembly of female meiotic spindles, which are of size larger than a somatic cell.

In somatic cells, centrosomes dominate mitotic spindle assembly (5). Centrosomes consist of a pair of centrioles surrounded by the pericentriolar material (PCM), which contains factors for microtubule nucleation and anchoring (5). However, in mouse oocytes, centrioles are lost early after pachytene stage and the PCM persists as acentriolar microtubule organizing centers (aMTOCs) (6). In oocytes from other mammalian species, the PCM is even dispersed, resulting in the complete degeneration of centrosomes (6). Notably, despite the absence of centrosomes, many centrosomal proteins were detected in mammalian oocytes in a recent proteomics study (7). To better understand the functions of these proteins, we decided to first systematically analyze the localization of 70 selected centrosomal and spindle-related proteins in mouse oocytes.

Identification of the LISD: A liquid-like spindle domain

To our surprise, we found that 19 different proteins localized to a previously undescribed domain in mouse oocytes (Fig. 1A). These proteins includes centrosomal proteins (AKAP450, CEP170, and KIZ), centriolar satellite proteins (CEP72, PCM1, and LRRC36), minus-end binding proteins (CAMSAP3 and KANSL3), dynein-related proteins (HOOK3, NDE1, NDEL1, and SPDL1) and proteins that regulate microtubule nucleation and stability (CHC17, chTOG, GTSE1, HAUS6, MCAK, MYO10, and TACC3).

This domain was distinct from aMTOC foci, which form a ring

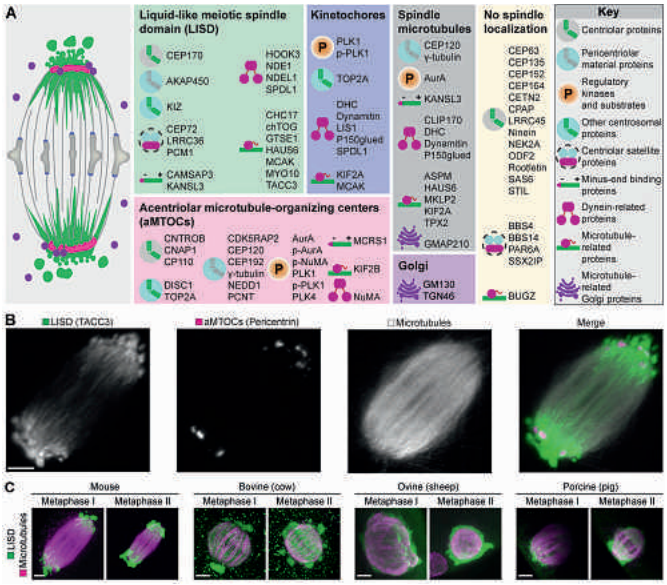

Fig. 1 Identification of a previously unknown spindle domain in mammalian oocytes.
(A) Schematic representation of the mouse metaphase I spindle (scheme; LISD in green, aMTOCs in magenta, kinetochores in blue, Golgi in purple, and spindle microtubules in gray). (B) Immunofluorescence images of a mouse metaphase I spindle. Green, LISD (TACC3); magenta, aMTOCs (pericentrin); gray, microtubules (α-tubulin). (C) Immunofluorescence images of metaphase I and II spindles in mouse, bovine, ovine, and porcine oocytes. Green, LISD (TACC3); magenta, microtubules (α-tubulin). Scale bars, 5 µm.

at the spindle poles (Fig. 1B). It infiltrated a large region of the spindle poles, and formed prominent spherical protrusions that extended well beyond the spindle poles (Fig. 1B). Strikingly, this domain was also present in bovine, ovine and porcine oocytes (Fig. 1C), suggesting that this domain is well conserved in other mammalian species.

To further characterize this domain, we performed live imaging by fluorescently tagging proteins in this domain. Interestingly, we noticed that the spherical protrusions at the spindle poles underwent rapid fusions (Fig. 2A). Because these protrusions existed in a region that is largely devoid of spindle microtubules, we asked whether these protrusions could persist in the

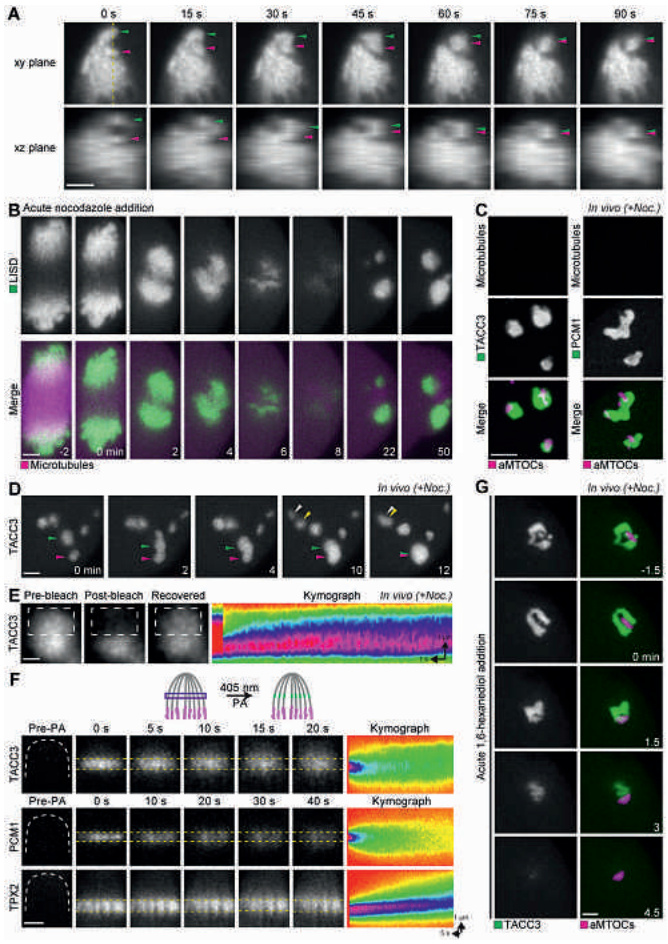

Fig. 2 The LISD forms by phase separation.
(A) Stills from time-lapse movies of mouse late metaphase I oocytes. Gray, LISD (TACC3-mClover3). Yellow lines mark the position of xz planes on the corresponding xy planes. Arrowheads highlight fusing LISD protrusions. (B) Still images from time-lapse movies of acutely nocodazole-treated mouse metaphase I oocytes. Green, LISD (TACC3-mClover3); magenta, microtubules (EB3-3×mCherry). Time is given as minutes after 10 µM nocodazole addition. (C) Immunofluorescence images of spherical condensates in acutely 10 µM nocodazole–treated mouse metaphase I oocytes. Gray, microtubules (α-tubulin); green, spherical condensates (TACC3 or PCM1); magenta, aMTOCs (pericentrin). (D) Still images from time-lapse movies of acutely 10 µM nocodazole–treated mouse metaphase I oocytes. Gray, spherical condensates (TACC3-mClover3). Arrowheads highlight fusing spherical condensates. (E) Partial bleaching of TACC3-mClover3 in a spherical condensate in acutely 10 µM nocodazole–treated mouse metaphase I oocytes. The bleached area is outlined by the dashed box. Scale bar, 1 µm. (F) Photoactivation (PA) of different proteins on mouse metaphase I spindles. Spindle poles are outlined with white dashed lines; photoactivated bars are marked with yellow dashed lines. Time is given as minutes after photoactivation with a 405-nm laser. (G) Still images from time-lapse movies of acutely 1,6-hexanediol-treated mouse metaphase I oocytes pretreated with 10 µM nocodazole. Time is given as minutes after 3.5% 1,6-hexanediol addition. Scale bars, 5 µm unless otherwise specified.

absence of microtubules. When we acutely added nocodazole, a microtubule-depolymerizing drug, the domain initially collapsed but then rapidly re-organized into large blobs (Fig. 2, B and C). Similar to the protrusions at the spindle poles, these blobs are spherical in shape and underwent rapid fusions (Fig. 2D). Because these characteristics were reminiscent of liquid droplets, we hypothesized that the domain was formed by liquid-liquid phase separation, a phenomenon that occurs when the interactions between proteins are more thermodynamically than the interactions between the proteins and the cytosol (8).
To test this hypothesis, we first confirmed that the domain was not surrounded by membranes using volume electron microscopy (data not shown here). We then performed fluorescence recovery after photobleaching and photoactivation experiments to show that proteins within the domain were dynamic and could undergo internal rearrangement (Fig. 2, E and F). To demonstrate the reversibility of the domain, we performed acute nocodazole addition and washout experiments to reversibly assemble and disassemble the LISD and nocodazole-induced blobs (data not shown here). Finally, we showed that the domain was maintained by weak hydrophobic interactions, as reflected by its susceptibility to 1,6-hexanediol treatment (Fig. 2G and data not shown here). Taken together, these results strongly suggest that the domain was formed by liquid-liquid phase separation, and we therefore refer to it as the liquid-like spindle domain (LISD).

AURA kinase activity and TACC3 are essential for LISD assembly in vivo

The LISD was not present in arrested oocytes and was timely assembled upon resumption of meiosis, indicating that LISD assembly was tightly linked with meiotic progression. To understand how the LISD was assembled, we examined the role of several regulatory kinases (AURA, PLK1, and PLK4), which control spindle assembly in multiple systems including oocytes (9-10). Whereas pharmacological inhibition of PLK1 and PLK4 did not affect LISD assembly, inhibition of AURA prevented LISD assembly (data not shown here). Of the 19 LISD proteins, only two proteins (GTSE1 and TACC3) are known mitotic substrates of AURA (9). To test whether AURA promoted LISD assembly via its substrates, we depleted GTSE1 and TACC3. Whereas depletion of GTSE1 had little effect on the LISD, depletion of TACC3 dispersed other LISD proteins into the cytoplasm and prevented LISD assembly (data not shown here). These results suggest that both AURA kinase activity and TACC3 are essential for LISD assembly in vivo.

TACC3 phase-separates via its N terminus in vitro and in vivo

To test whether TACC3 contributed to LISD assembly via phase separation on its own, we purified recombinant TACC3 and examined its behaviors in vitro. Indeed, in the presence of macromolecular crowding agent, which mimics the intracellular environment, TACC3 underwent phase separation in vitro (Fig.

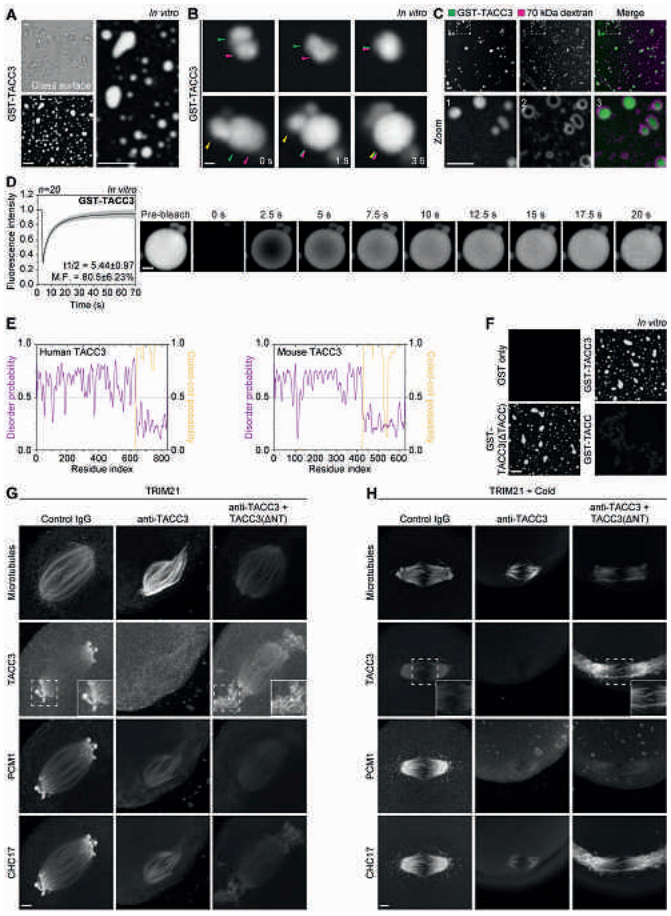

Fig. 3 The N terminus of TACC3 is necessary for phase separation in vitro and in vivo.

(A) Bright-field and fluorescence images of GST-TACC3 droplets in vitro. Inset is the magnification of the region marked by the dashed line box. (B) Still images from time-lapse movies of fusing GST-TACC3 droplets in vitro. Scale bar, 1 µm. Arrowheads highlight fusing GST-TACC3 droplets. (C) Fluorescence images of GST-TACC3 droplets in the presence of 70-kDa dextran in vitro. Green, GST-TACC3; magenta, 70-kDa dextran. Insets are magnifications of regions marked by dashed line boxes. Scale bar, 2.5 µm. (D) FRAP of GST-TACC3 droplets in vitro. Gray, GST-TACC3. The number of analyzed droplets is specified in italics. M.F., mobile fraction. Scale bar, 1 µm. (E) Domain organization of human and mouse TACC3 showing the disordered region [purple; analysis with DisEMBL (100)] and the coiled-coil domain [yellow; analysis with MARCOIL (101)]. (F) Fluorescence images of GST, GST-TACC3, GST-TACC3(ΔTACC), and GST-TACC in vitro. (G and H) Immunofluorescence images of the metaphase I spindle in control, TACC3-depleted, and TACC3(ΔNT)-TACC3–depleted mouse oocytes with and without cold treatment. IgG, immunoglobulin G. Insets are magnifications of regions marked by dashed line boxes. All in vitro assays were performed in pH 6.4 buffer with 150 mM KCl and 12% PEG. Scale bars, 5 µm unless otherwise specified.

3A). TACC3 condensates wet glass surface, underwent fusion and internal rearrangement, and excluded large dextran (Fig. 3, B to D), implying that they were liquid-like.

In silico analysis predicted that TACC3 is a bipartite molecule with a disordered N-terminus and an ordered, coiled-coil C terminus (Fig. 3E). Because both disordered and coiled-coil sequences have been implicated in driving phase separation (11), we tested whether one or both sequence(s) were required for phase separation of TACC3. Whereas TACC3 deleted of the coiled-coil C terminus (TACC3(ΔTACC)) phase-separated just like the full length protein, TACC3 deleted of the disordered N-terminus (TACC) failed to phase-separate but self-organized into network-like structures (Fig. 3F). In line with this finding, although TACC localized to the spindle in TACC3-depleted oocytes, TACC failed to phase-separate and recruit other LISD proteins (Fig. 3, G and H). Instead, it assembled network-like

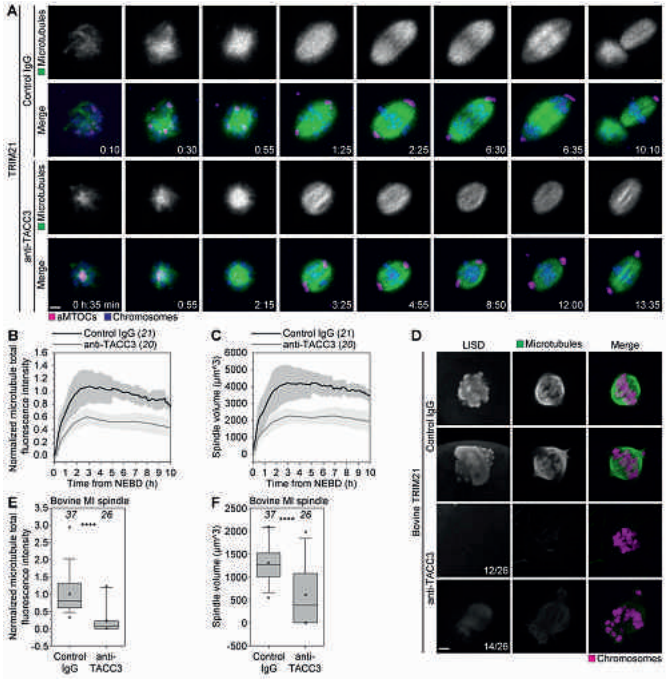

Fig. 4 Microtubule loss and defective spindle assembly in TACC3-depleted oocytes.

(A) Stills from time-lapse movies of control and TACC3-depleted mouse oocytes. Green, microtubules (mClover3-MAP4-MTBD); magenta, aMTOCs (CEP192-mScarlet); blue, chromosomes (H2B-miRFP). Time is given as hours:minutes after NEBD. (B and C) Quantification of total fluorescence intensity of microtubules and spindle volume in control and Tacc3-depleted mouse oocytes. (D) Immunofluorescence images of the metaphase I spindle in control and TACC3-depleted bovine oocytes. Gray, LISD (TACC3); green, microtubules (α-tubulin); magenta, chromosomes (Hoechst). (E and F) Quantification of total fluorescence intensity of microtubules and spindle volume in control and TACC3-depleted bovine metaphase I (MI) oocytes. The number of analyzed oocytes is specified in italics. Error bars (shaded areas) represent SD. Scale bars, 5 µm.

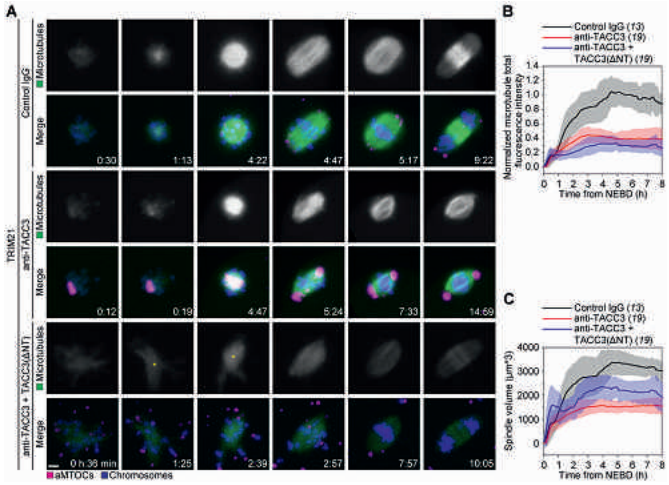

Fig. 5 The N terminus of TACC3 is required for spindle assembly in mouse oocytes.
(A) Still images from time-lapse movies of control, TACC3-depleted, and TACC3(ΔNT)-TACC3–depleted mouse oocytes. Green, microtubules (mClover3-MAP4-MTBD); magenta, aMTOCs (CEP192-mScarlet); blue, chromosomes (H2B-miRFP). Time is given as hours:minutes after NEBD. Yellow asterisks highlight the unfocused spindle intermediate. (B and C) Quantification of total fluorescence intensity of microtubules and spindle volume in control, TACC3-depleted, and TACC3(ΔNT)-TACC3–depleted mouse oocytes. The number of analyzed oocytes is specified in italics. Error bars (shaded areas) represent SD. Scale bars, 5 µm.

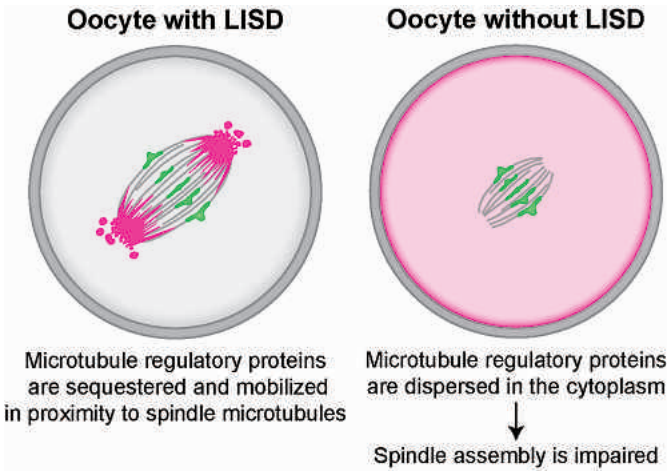

Fig. 6 Phase Separation of Microtubule Regulatory Proteins Promotes Acentrosomal Spindle Assembly in Mammalian Oocytes.
In oocytes, centrioles degenerate during early oogenesis, and spindles (in gray) are assembled in the absence of centrosomes. Mammalian oocytes sequester microtubule regulatory factors in the liquid-like spindle domain (LISD; in magenta) via phase separation, and thereby enrich and mobilize them in proximity to spindle microtubules. In the absence of the LISD, microtubule regulatory factors are dispersed in the large cytoplasmic volume, thus impairing spindle assembly.

structures similar to what we obtained in vitro (Fig. 3, F to H), further reassuring the physiological relevance of our in vitro assay conditions.

The LISD promotes acentrosomal spindle formation by sequestering microtubule regulatory factors

To understand the functions of the LISD, we ablated the LISD via depleting TACC3 and inhibiting AURA. Depletion of TACC3 in mouse oocytes caused severe reduction in both the total microtubule intensity and spindle volume, to about half of the values in control oocytes (Fig. 4, A to C). In addition, aMTOCs were no longer scattered around the spindle poles but coalesced into two single foci (Fig. 4A). Similar phenotypes were observed in AURA-inhibited oocytes (data not shown here). Likewise, TACC3 depletion caused severe microtubule loss as well as spindle assembly defects in bovine oocytes (Fig. 4, D to F), suggesting that the LISD was essential for acentrosomal spindle assembly in mammalian oocytes.
In mitotic systems, TACC3 depletion causes a minor loss of spindle microtubules (12-13), but results in misaligned chromosomes and metaphase arrest (13-17). To uncover LISD-specific functions, we took advantage of the N-terminus-deleted TACC3 (TACC3(ΔNT)) which neither phase-separated in vitro nor restored LISD assembly in vivo (Fig. 3, G and H). Although expression of TACC3(ΔNT) in TACC3-depleted oocytes fully rescued chromosome-related defects and partially rescued the reduction in spindle volume, it failed to rescue the reduction in total microtubule intensity (Fig. 5, A to C). This suggest that the effect on microtubule assembly was LISD-specific.
Liquid-liquid phase separation is a powerful intracellular organizing principle. One of its functions is the selective enrichment of proteins to promote reactions or storage (8). Recent studies in mitotic systems proposed that phase separation of Xenopus microtubule-binding protein BuGZ, regulatory kinase PLK4, and the Caenorhabditis elegans centrosomal protein SPD-5 promote microtubule nucleation in centrosomal spindles via enriching tubulin dimers (18-20). However, the LISD and nocodazole-induced blobs did not significantly enriched tubulin dimers (data not shown here). Given that many of the LISD proteins are microtubule regulatory factors and were dispersed in the cytoplasm in the absence of the LISD, we asked whether the LISD promotes microtubule assembly via modulating microtubule dynamics instead. Indeed, microtubule growth rates were significantly decreased, and their overall turnover was significantly increased in TACC3-depleted oocytes (data not shown here). This suggest that microtubule dynamics were altered in the absence of the LISD.

The proposed model

Our data suggest a model whereby the LISD sequesters microtubule regulatory factors and mobilizes them in a dynamic manner in proximity to spindle microtubules (Fig. 6). In the absence of the LISD, the low cytoplasmic concentration of microtubule

regulatory factors is unlikely to be sufficient for microtubule assembly, thus destabilizing microtubules within the female meiotic spindle (Fig. 6).

Although overexpression of TACC3 gives large blobs that can associate with the mitotic spindle (21), endogenous level of TACC3 does not seem to support phase separation in mitotic cells, regardless of the presence or absence of centrosomes (22-23). The LISD is thus likely a meiosis-specific mechanism. Instead of enriching tubulin dimers as in somatic cells, such mechanism may serve as an alternative way to promote spindle assembly in large cells such as oocytes, where tubulin is unlikely limiting (24-25).

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BINDER Innovation Prize

Proteomics of dynamic cell changes upon stress

Christian Münch

Summary

The intracellular environment is in a constant flux to adjust to changing conditions and perturbation by stresses. To be able to cope with stress conditions, cells contain a large number of dedicated response pathways that change cellular function in an attempt to overcome stress. Stress responses are highly spatio-temporally controlled to quickly respond to stimulation across compartments and the whole cell. Consequently, the study of stress responses requires methods that are global and time-resolved to examine these dynamic effects. We developed novel proteomics approaches that allow to measure protein translation rates at high temporal precision to reflect these rapidly occurring changes. This approach allowed us to comprehensively study the two main cellular stress hubs controlling translation – the integrated stress response and mTOR. We revealed that the protein sets, whose translation rates are controlled by these pathways, largely overlapped and that their translation was instead guided by intrinsic properties. With the outbreak of COVID-19, we then applied this new translation proteomics method to define host cell responses to SARS-CoV-2 infection and revealed novel therapeutic targets.

Dynamic stress responses

Cells contain thousands of different proteins that form an intricate network across the cellular compartments. Within this dynamic system, proteins forms complexes, interact, process or modify each other, and carry out their enzymatic function to allow cellular activity. Protein homeostasis (proteostasis), the balanced control of protein translation, translocation, processing, folding, modification and degradation, is essential to maintain protein function and cell health(Gloge et al., 2014; Kim et al., 2013). All these aspects of proteostasis are tightly controlled in the different cellular compartments(Pilla et al., 2017) and the cell as a whole. At the same time, cells constantly face different intra- and extra-cellular sources of perturbation that cause stress on the cellular system and imbalance proteostasis. These stresses include protein misfolding, such as observed in neurodegenerative diseases or poisoning, genetic modification in cancer, infection, starvation, fever, and many more. In an attempt to restore proteostasis, cells contain a number of different stress response pathways across their different compartments, including the integrated stress response (ISR) and heat shock response in the cytosol, the unfolded protein response in the endoplasmic reticulum (ER), and the mitochondrial unfolded protein response (mtUPR) in mitochondria(Münch, 2018; Pakos-Zebrucka et al., 2016; Walter and Ron, 2011). These responses try to maintain proteins in their folded and active state to restore cell health and avoid toxicity. To do so, these stress responses activate highly dynamic processes and attempt to increase the folding capacity by activating transcriptional programs to increase factors such as chaperones, while also decreasing the folding load by inducing proteases and attenuating translation (Münch, 2018). Strikingly, these stress responses are rapid, carrying out their function within minutes to hours, and contain built-in switches that shift the response pattern to more disruptive processes, such as degradation of cellular compartments by autophagy or activation of apoptosis, when activated for a prolonged timespan (Figure 1). These properties cause a number of challenges for research: i) To be able to study the signaling and immediate outcomes, rapid stress inducers are required. As a consequence, compounds are predominantly used to induce

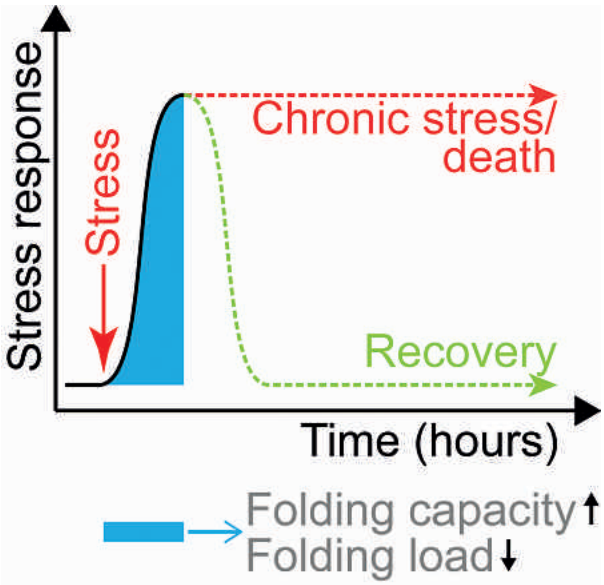

Figure 1. Dynamics of folding stress responses. Protein misfolding activates transient responses that attempt to increase folding capacity and decrease folding load via modulation of transcription and translation attenuation. Recovery occurs within hours. Prolonged stress causes chronic stress with compensatory effects and activation of alternative pathways including apoptosis. From Münch, BMC Biol 2016.

stress. ii) Methods are required that allow monitoring the protein changes that occur within a tight temporal frame across large parts of the proteome and that exhibit a small dynamic range. In my laboratory, we aim at monitoring the dynamics of cellular stress responses to gain a better understanding of the underlying signaling and functional consequences. We focus on system-wide approaches, such as proteomics, to be able to monitor the cell-wide consequences of stress. This allows us to elucidate effects in the different compartments as well as the interaction and co-activation of different stress responses to bring about the cellular changes required to overcome stress and strengthen proteostasis in compartments not yet affected by stress. Our studied sources of stress are predominantly protein misfolding and infection (i.e. host cell responses) with a major focus on their effects on mitochondria.

Translation proteomics

Stress responses typically affect translation. The key regulators are two central cellular signaling hubs – the ISR and mTOR. During the ISR one (or more) of four known kinases phosphorylates eukaryotic initiation factor 2 alpha (eIF2α) upon stresses such as ER protein misfolding, infection, or amino acid deficiency. Phosphorylated eIF2α limits general translation initiation and, via selective translation of transcription factors, leads to extensive rearrangements in transcription (Pakos-Zebrucka et al., 2016). Inhibition of mTOR, induced by a number of signaling pathways, starvation, and metabolic imbalance, attenuates translation through a lack of phosphorylation of EIF4E binding protein 1 and 2, activates autophagy, and alters the transcriptome (Appenzeller-Herzog and Hall, 2012; Sonenberg and Hinnebusch, 2009). The ISR and mTOR occupy key roles in the regulation of translation upon stress. However, their specific translation targets were unclear. The main limitations were that these pathways, responding to numerous cellular perturbations, are highly sensitive to the experimental method applied and cause extensive translation changes. Ribosome profiling has been the method of choice to monitor translation based on next generation sequencing of mRNA fragments protected by bound ribosomes. However, upon conditions of extensive global translation effects, such as achieved by the ISR and mTOR, ribosome profiling suffers a strong normalization bias(Chen et al., 2015; McGlincy and Ingolia, 2017) that has so far prevented its use to define the translatomes under ISR or mTOR regulation. Proteomics approaches have focused on the incorporation of the non-natural amino acid azidohomoalanine or puromycin, which cause defects in tRNA charging and thus ISR activation or in translation itself, respectively (Kiick et al., 2002). Thus, both approaches showed limited capability to monitor ISR or mTOR translation regulation.

An alternative approach is the addition of isotope-labeled amino acids (pulse-SILAC labeling) to mark newly synthesized proteins(Schwanhäusser et al., 2009). These amino acids are indistinguishable from their non-isotope-labeled counterparts, since they harbor naturally occurring isotopes. Consequently, pulse-SILAC labeling does not perturb cells or activate stress responses. However, the turnover and thus translation rate of proteins is low with a median protein half-live of 46 hours (Schwanhäusser et al., 2011). Thus, within the first few hours, the heavy labeled signal, which reflects newly synthesized peptides, is of very low intensity (only several %) when compared to the light signal of the "old" protein in acute pulse-SILAC measurements. Therefore, pulse-SILAC approaches have been very limited in their capability to monitor acute translation changes with sufficient accuracy and depth (Bagert et al., 2014). As peptides containing SILAC amino acids cannot be enriched, we tested alternative approaches to allow acute translatome measurements. We specifically increased the signal of the heavy peptides to be able to efficiently detect these in a method we

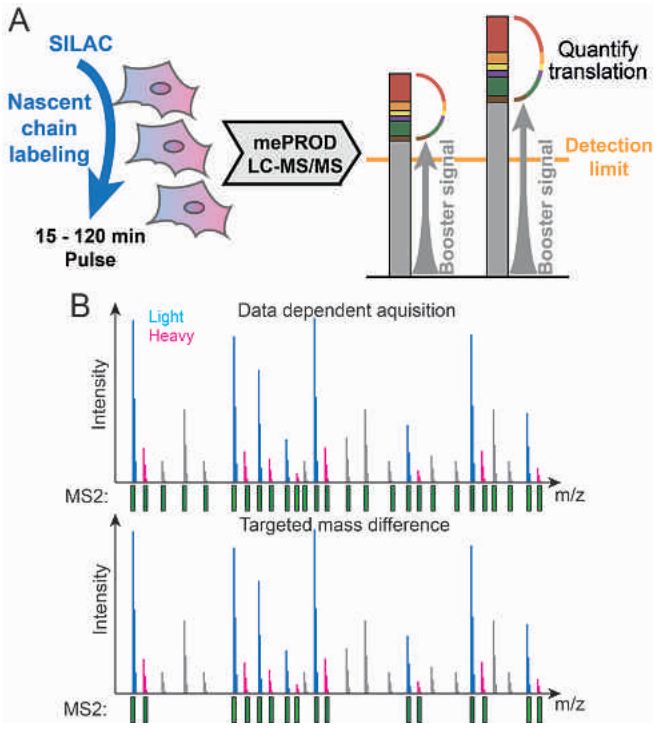

Figure 2. Multiplexed enhanced protein Dynamics (mePROD) proteomics. A, Depiction of the mePROD method that allows to monitor globally protein translation by proteomics. Isotope-labelled amino acids (i.e. SILAC amino acids) are incorporated into newly synthesized proteins. Using standard approaches, the intensity of the newly synthesized proteins is too low to be detected. The mePROD method includes sample multiplexing and a booster signal that allows passing the detection limit to quantify translation of thousands of proteins. B, The use of instrument logic approaches further increases the sensitivity of the mePROD method. Using regular data dependent acquisition, all peaks are selected for a secondary measurement in the mass spectrometer (indicated by green rectangles, top). Using instrument logic for targeted mass difference selection, only the desired light and heavy peaks are selected for further measurement (bottom). Adapted from Klann et al., Mol Cell 2020 (A) and from Klann and Münch, Anal Chem 2020 (B).

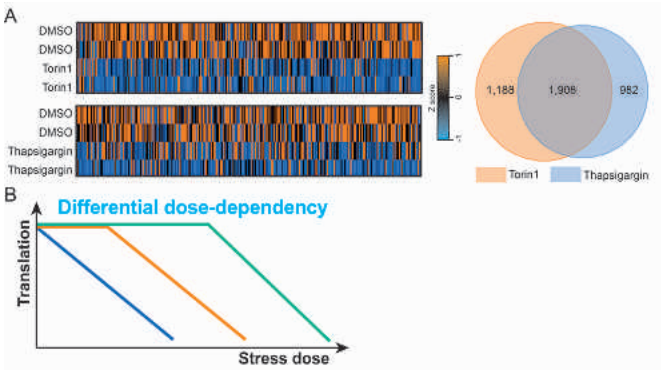

Figure 3. Translatome profiles of stress response pathways. A, Analysis of protein translation changes using mePROD proteomics during inhibition of mTOR with Torin 1 or activation of the integrated stress response by thapsigargin (left). Venn diagram of proteins with reduced translation rates (right). B, Translation rates of individual proteins are largely defined by intrinsic properties with different sensitivities to global translation attenuation. From Klann et al., Mol Cell 2020.

termed multiplexed enhanced protein dynamics (mePROD) proteomics (Klann et al., 2020). There, we multiplexed samples using tandem mass tags (TMT). These tags label every peptide in solution and currently allow to pool up to 16 samples into a single mass spectrometry run (Thompson et al., 2019). During fragmentation of peptides in the mass spectrometer, it becomes possible to correctly quantify the amounts of every peptide that originated from the individual samples. The strength of mePROD lies in the combination of pulse-SILAC with TMT and one special feature of the latter: TMT tags are isobaric that is of the same mass. Thus, it was possible to add fully heavy-labeled peptides to boost only the signal of the newly synthesized (heavy) peptides (Figure 2a). Using this approach, we were able to increase the accuracy and number of peptides detected to be able to monitor translation from cells after minutes of labeling (Klann and Tascher, 2020). The relative ease of the method, following standard proteomics protocols, allowed the throughput required to measure changes in translation over time and across conditions. To further improve accuracy and increase the sensitivity so that it is compatible with the low amounts of material available from clinical samples (often < 100,000 cells), we included machine logic approaches on the mass spectrometer. This method analyses the first mass spectrometry scan to identify the signals of interest only (i.e. heavy and light peptide pairs) for their further analysis (Figure 2b) thereby using the measurement time more efficiently. As a result, we obtained an ~3-fold increase in identifications without compromising accuracy (Klann and Münch, 2020). The development and improvement of mePROD proteomics provided us with the tools to quantitatively and globally measure changes in translation after stress with high temporal resolution.

Translation regulation during cell stress

To understand the functional consequences of the ISR and mTOR, it is crucial to understand how these stress pathways change translation. While these pathways share similar behavior *in vivo*, they had been assumed to control distinct sets of proteins (Wengrod and Gardner, 2015). We applied the mePROD method to monitor conditions that cause activation of the ISR or inhibition of mTOR. Monitoring acute translation changes across thousands of proteins, we made two striking observations: 1) we found extensive overlap in proteins regulated by the ISR and mTOR in accordance with both pathways mainly controlling cap-dependent translation (Figure 3a). 2) Analyzing the behavior of individual proteins across a range of stress conditions revealed that translation rates of proteins were predominantly defined by intrinsic properties that are directly related to the observed impact on global translation. Independent of the source of translation attenuation (stresses or chemical translation inhibition), some proteins stopped to be translated at very mild inhibition of global translation, other proteins only responded in their individual translation rates upon much more severe global translation attenuation. One set of mainly housekeeping proteins was largely insensitive to translation inhibition (Figure 3b). Together, these findings showed that translation control by the ISR and mTOR overlap and that there are common translational programs to respond to the range of stresses driving these pathways.

SARS-CoV-2

Infection is a form of cellular stress that poses very similar technical challenges as do purely intracellular stress responses. Host

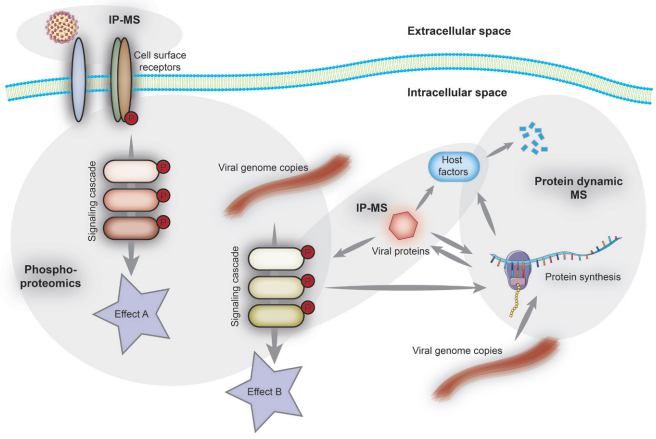

Figure 4. Proteomics to monitor dynamic host cell responses to viral infection. Viruses induce multi-layered responses in cells, driven by interaction of viral particles, RNA or DNA, and proteins with the host cell. Proteomics offer a number of tools to quantify changes in signalling, protein-protein interactions, and protein translation/degradation on a global level. Molecular systems medicine approaches are required to integrate molecular and global data and deduce cell-wide changes part of cellular responses and phenotypes. From Klann et al., Adv Virus Res 2021.

cell responses are often extensive and causing highly dynamic changes across the proteome. Here, proteomics offers a wide toolset to monitor these different levels of regulation with the required temporal resolution (Figure 4). One key difficulty is the analysis of the resulting complex and multi-layered data obtained. Systems biology approaches are ideal to integrate these different datasets and to extract pathways important for viral replication (Klann et al., 2021). Having had just published our new mePROD proteomics method to measure translation, with the outbreak of SARS-CoV-2, we applied this method to gain some first insight into the host cell responses to SARS-CoV-2 infection in cell infection models (Bojkova et al., 2020). We determined the host cell changes upon identification and importantly revealed a number of pathways crucial for SARS-CoV-2 replication (Figure 5a). Blocking these pathways pharmacologically prevented virus replication in cells. Two key targets identified – ribavirin (nucleotide biosynthesis) and 2-deoxyglucose (glycolysis) – have already been tested in clinical or preclinical studies for COVID-19. We next carried out phospho-proteomics to examine cell-wide signaling events after SARS-CoV-2 infection (Klann et al., 2020b). These analyses revealed extensive changes across cells. Computational integration of the protein abundance and phosphorylation data with available networks of FDA-approved drugs and their targets revealed that activation of growth factor receptor signaling is central during SARS-CoV-2 infection and that there are a number of available drugs to block this pathway, including dexamethasone (Figure 5b). We tested a number of molecules blocking different steps of growth factor receptor signaling and were able to prevent SARS-CoV-2 replication in cells (Klann et al., 2020b). In summary, we described the host cell responses after SARS-CoV-2 using several proteomics approaches. This exemplified the power of translation proteomics to monitor time-resolved changes upon cellular perturbation and revealed several candidates for the development of COVID-19 treatments.

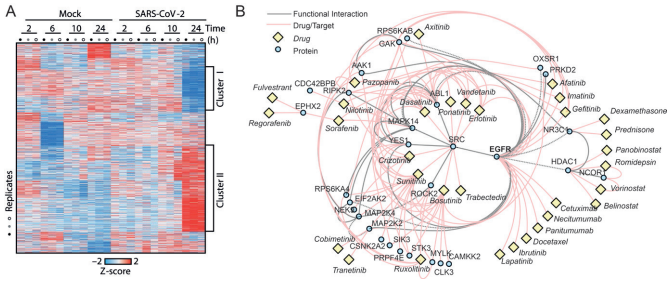

Figure 5. Computational networks to identify therapeutic approaches for SARS-CoV-2. A, Proteome changes in mock- or SARS-CoV-2 infected cells over time. Cluster I and cluster II indicate host cell proteins decreased or increased during infection, respectively. Based on cluster II, candidate drugs inhibiting enriched cellular functions were selected and tested for their potential to prevent SARS-CoV-2 replication in cells. B, Computational overlay of proteins with significantly increased phosphorylation and a drug-target network of FDA-approved drugs. The network was filtered for connected drug-target protein networks to identify potential drugs preventing SARS-CoV-2 replication. From Bojkova, Klann, Koch et al., Nature 2020 (A) and Klann, Bojkova et al., Mol Cell 2020 (B).

Outlook

Stress responses are at the forefront of numerous diseases, including neurodegenerative diseases and the tumor microenvironment. Yet, our understanding of the various outcomes and integration of stress responses remains limited. One major restriction has been the lack of comprehensive, multi-layered, and cell-wide analyses across different stresses to identifying their functional outcomes and integration. In particular, the high spatio-temporal control of stress responses necessitates detailed and precise analyses. Molecular systems biology approaches will help us to inch closer towards a molecular and global understanding stress responses and their interactions that bring about the changes observed in cells. This will be crucial to better understand the role of these responses in disease and the potential for a well-define modulation to increase cellular health. To gain this knowledge, we will continue to extend the use of systems approaches to study stress responses and to contribute to developing new methods that allow obtaining the required multilevel information.

Acknowledgement

I would like to thank all the current and former members of the Münch laboratory for their fantastic work and commitment to their projects and the laboratory. It is a daily joy to see how the projects evolve and every member of the laboratory grows. Special thanks go to Kevin Klann, who was and is on the forefront of the projects described here and has contributed tremendously to the success of the laboratory. My deepest gratitude also goes to the people who have supported me throughout the years, particularly my PhD and postdoc supervisors Anne Bertolotti and Wade Harper, but also Ulrich Hartl, Stefan Jentsch, Steve Gygi, and Ivan Dikic. In addition, the many great collaborators, who made science especially joyful and allowed to expand our research. I would also like to thank the Institute of Biochemistry II at Goethe University Frankfurt, which offers a great environment for my laboratory, and the generous support by the European Research Council under the European Union's Seventh Framework Programme (ERC StG 803565), CRC1177 on Selective Autophagy and the Emmy Noether Programme of the Deutsche Forschungsgemeinschaft (DFG, MU 4216/1-1), HMWK LOEWE Center for Cell and Gene Therapy, Frankfurt Cancer Institute, Cardio-Pulmonary Institute, the Johanna Quandt Young Academy Science Funding Award, Aventis Foundation Bridge Award, and the Goethe Corona Fonds.

About the author

Christian Münch studied biochemistry at the University of Tübingen, including a one year laboratory rotation at the Max Planck Institute of Biochemistry in Munich. From 2008-2011, he carried out his PhD work on protein misfolding in amyotrophic lateral sclerosis with Anne Bertolotti at the MRC Laboratory of Molecular Biology and University of Cambridge (UK). In 2012, he joined Wade Harper's laboratory at Harvard Medical School (USA) with an EMBO long-term fellowship to work on the mitochondrial unfolded protein response and proteomics. End of 2016, he started his independent laboratory at the Institute of Biochemistry II at Goethe University Frankfurt – Medical School. He is recipient of an ERC Starting Grant and the Emmy Noether Program and won the British Neuroscience Association Postgraduate Award, the Johanna Quandt Academy Science Funding Award and the Aventis Foundation Bridge Award for his work.

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Werner Risau Prize

Endothelial metabolic transcriptome plasticity in pathological angiogenesis

Katerina Rohlenova, Ph.D.

Targeting the metabolism of endothelial cells (ECs) is a promising strategy to block pathological blood vessel growth, or angiogenesis, for the treatment of diseases like cancer. Understanding the landscape of metabolic gene expression at the single-cell level will aid in identifying novel angiogenic targets. We surveyed thousands of ECs in pre-clinical models of age-related macular degeneration and lung cancer and identified genes and metabolic pathways that are congruently upregulated across diseases and tissues during angiogenesis. Using an integrated analysis, we generated a list of prioritized metabolic candidates and validated the importance of two candidates, SQLE and ALDH18A1, in pathological angiogenesis, supporting their potential as therapeutic targets.

EC metabolism regulates angiogenesis and is an emerging target for anti-angiogenic therapy (AAT) in cancer and wet age-related macular degeneration (AMD) (Eelen et al., 2018). The design of new AATs by targeting EC metabolism would benefit from a better understanding of individual EC metabolism, but it was unknown if ECs express a heterogeneous metabolic gene signature and how single ECs reprogram their metabolic transcriptome signature when forming new vessels in disease. However, metabolomics is insufficiently sensitive to determine single EC metabolism. Since we previously documented that changes in metabolic gene expression signatures at the bulk population level can be predictive of changes in metabolism in ECs (Bruning et al., 2018; Cantelmo et al., 2016; Kalucka et al., 2018; Vandekeere et al., 2018), we analyzed the metabolic transcriptome of ECs at the single cell level.

During vessel sprouting, a navigating tip EC leads the way, while proliferating stalk cells elongate the vessel sprout (Potente et al., 2011); once newly formed vessels become perfused, ECs adopt a quiescent phalanx phenotype (Welti et al., 2013). ECs rely on metabolic reprogramming when switching from quiescence to vessel sprouting (Eelen et al., 2018; Li et al., 2019; Sawada and Arany, 2017; Yu et al., 2018). In tumors, bulk metabolic gene expression profiling identified metabolic targets in tumor ECs (Cantelmo et al., 2016). AMD is a common blinding disease of elderly people, characterized by ocular neovascularization. Laser-induced choroid neovascularization (CNV) is a preclinical model of AMD (Ambati and Fowler, 2012). Since angiogenic ECs in AMD/CNV have not been studied at the single cell level, we used scRNA-seq to profile their (metabolic) transcriptome heterogeneity.

Anti-VEGF drugs are used for the treatment of cancer and AMD, but resistance limits their efficacy (Jain, 2014; Yang et al., 2016). Hence, there is an unmet clinical need to identify novel angiogenic targets. ScRNA-seq is a powerful technology to identify such candidates, but an outstanding challenge is to prioritize targets for further clinical translation. We thus used a strategy, starting from scRNA-seq and complemented with orthogonal techniques, to prioritize metabolic targets that control angiogenesis.

Identification and characterization of CNV-ECs by scRNA-seq

To model CNV in mice, we laser-induced CNV lesions and micro dissected choroids 7 days later, using choroids from healthy mice as controls (6 mice per sample, in triplicate) (Figure 1A,B). Single cell suspensions, enriched for CD45-/CD31+ ECs (Cantelmo et al., 2016) were subjected to scRNA-seq. After quality filtering, batch correction and in silico EC selection, graph-based clustering was performed to group a total of 28,337 ECs according to their gene expression profile. Clusters were annotated based on marker genes and results were visualized using t-distributed stochastic neighbor embedding (t-SNE) (Figure 1C,D).

Choroidal ECs (CECs) from control mice were indistinguishable from healthy peripheral CECs from lasered mice and clustered together (Figure 1C-F). However, we detected a new separate population in lasered mice, not present in healthy CECs, representing CNV-ECs (Figure 1D). In CECs, we identified previously unknown sublineages of the classical arterial, capillary and venous EC phenotypes and two putative lymphatic EC phenotypes (Figure 1E-I). Angiogenic CNV-ECs were distinct from normal CECs and included proliferating ECs and tip ECs, but we also found 3 previously unknown phenotypes that expressed signatures associated with transitioning from pcv to angiogenic EC phenotypes, and immature and maturing (neophalanx) neovasculature (Figure 1J).

Metabolic transcriptome reprogramming during pathological vessel sprouting

We wondered if ECs underwent a differentiation trajectory during vessel sprouting and if EC differentiation was associated with metabolic transcriptome changes. Trajectory inference analysis predicted that the hierarchy of angiogenic phenotypes resulted from differentiation of activated pcv CECs to transitioning CNV-ECs, then to immature CNV-ECs, which thereafter differentiated to tip cells and finally to more mature neophalanx CNV-ECs (Figure 2A). This prediction extends previous morphological evidence that neovessels may originate from pcvs (Folkman, 1982). Since pcv CECs expressed a previously validated signature of resident endothelial stem cells (ESCs), our analysis provides further suggestion that ESCs might contribute to new vessel sprouting, as previously established by lineage tracing (Corey et al., 2016; Manavski et al., 2018; McDonald et al., 2018; Mondor et al., 2016; Red-Horse et al., 2010; Wakabayashi et al., 2018; Wakabayashi et al., 2013).

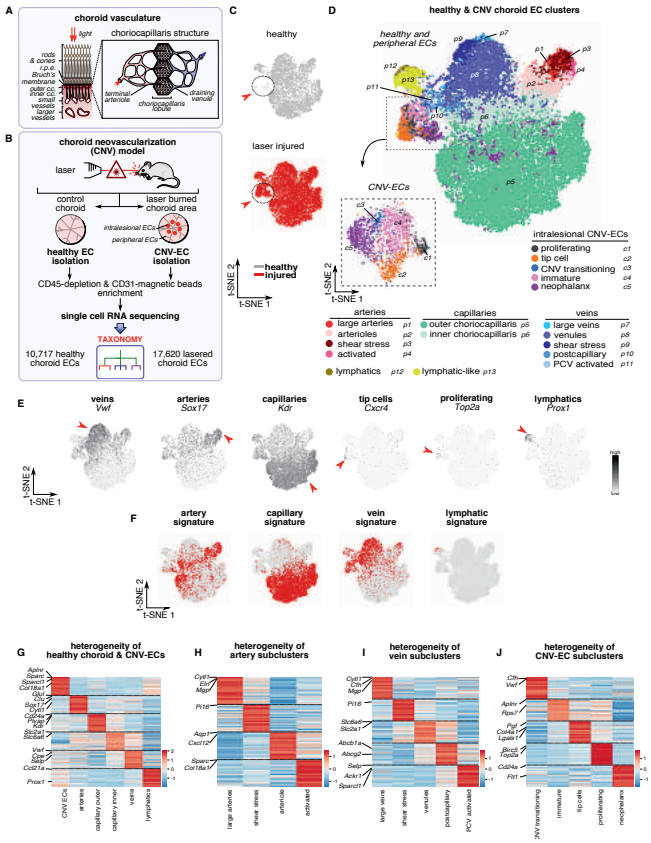

Figure 1: Heterogeneity of choroid NEC and CNV-EC phenotypes

(A) Schematic overview of the choroidal vasculature. **(B)** Schematic overview of the study design. **(C)** t-SNE plot, color-coded for the sample type. ECs isolated from healthy choroid are depicted in grey (upper); ECs isolated from laser-injured choroids in red (bottom). Red arrowhead and dotted circles indicate an emerging population of laser-injured specific CNV-EC phenotypes. **(D)** t-SNE visualization of EC subpopulations in healthy and laser-injured choroids. CNV-EC phenotypes (red arrowhead in panel C) are boxed in the global t-SNE plot and shown in a separate t-SNE plot on the lower left. Subclusters of peripheral (p) and intralesional CNV-ECs (c) are numbered. **(E,F)** t-SNE plots, color-coded for the expression of the indicated marker genes (E) or gene sets (F). Red arrowheads indicate cells with high expression of the indicated marker gene. SCALE: white/grey is low expression, black (gene) or red (gene sets) is high gene expression. **(G–J)** Heatmap of gene expression levels of the top 50 marker genes for broad categories of EC phenotypes (G), artery subclusters (H), vein subclusters (I) and CNV-EC subclusters (J). In this and all further heatmaps depicting marker genes, colors represent row-wise scaled gene expression with a mean of 0 and a standard deviation of 1 (z-scores). ABBREVIATIONS: c.c., choriocapillaris; r.p.e., retinal pigment epithelium.

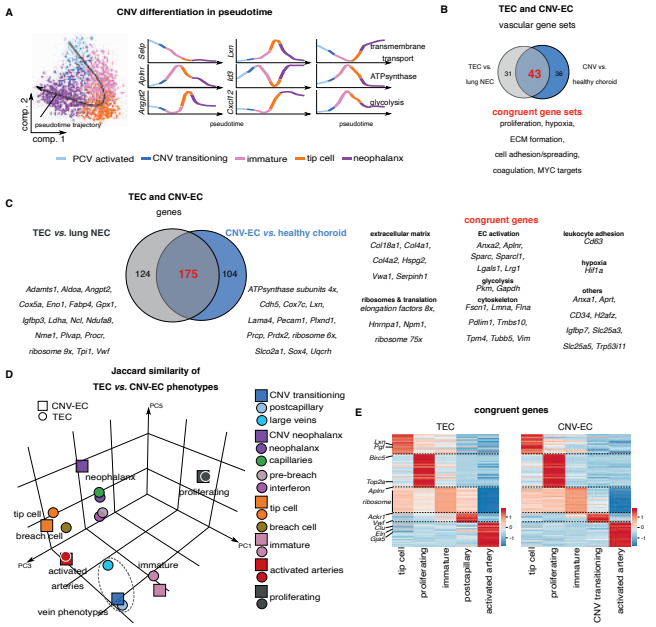

Figure 2: Gene expression signatures in CNV and TEC subtypes

(A) Pseudotime trajectory of the indicated CNV-EC phenotypes (LEFT) and Loess regression-smoothened gene expression of the indicated genes and metabolic gene sets in pseudotime (RIGHT). **(B)** Vascular gene sets upregulated in TECs (vs. lung NECs) and in CNV ECs (vs. choroid CECs). Gene sets congruently upregulated in TECs and CNV-ECs are summarized on the right. **(C)** Genes upregulated in TECs vs. NECs and choroid CECs vs. CNV-ECs. A selection of genes upregulated in lung TECs vs. NECs (LEFT) and in CNV-ECs vs. choroid CECs (RIGHT) are listed. Genes, congruently upregulated in TECs and CNV-ECs, are listed on the right. Genes encoding ribosomal proteins are not listed individually, instead their total number is displayed. **(D)** Three-dimensional principal component analysis (PCA) on the pairwise Jaccard similarity coefficients of marker gene sets between subpopulations in TECs and CNV-ECs. Squares denote CNV-EC phenotypes, circles denote TEC phenotypes. Note, equivalents of breach, pre-breach and interferon TEC phenotypes were not present in CNV-ECs. **(E)** Heatmap of expression levels of congruent genes in TEC and CNV-EC phenotypes (all genes analyzed). NOTE: the TEC and CNV-EC heatmaps show the same set of congruent genes. ABBREVIATIONS: Comp, component; PC, principal component.

Interestingly, when focusing on metabolic genes and pathways, we noted that membrane transport, ATP synthase and glycolysis gene signatures were dynamically regulated during differentiation from quiescent vein to angiogenic ECs (Figure 2A). Maximal differences in metabolic gene expression of central carbon metabolism were observed in the most angiogenic EC phenotypes (immature and tip ECs), possibly suggesting that these ECs had higher metabolic demands to execute their biological functions (Figure 2A).

Next, we explored whether metabolic transcriptome reprogramming was specific to CNV-ECs or a more general hallmark of the angiogenic switch in pathological angiogenesis (such as in tumors), as this would address a fundamental question in vascular biology whether vessels in different tissues and diseases form via similar or different mechanisms. We therefore explored to which extent CNV and tumors contained similar EC phenotypes, and whether they expressed congruent genes.

We analyzed a publicly available dataset of murine lung tumor ECs (TECs) (Goveia et al., 2020) , which comprised largely similar EC phenotypes as CNV-ECs. We explored whether similar EC phenotypes could be detected in these diseases, and whether they expressed congruent genes. First, we performed differential gene expression and gene set enrichment analysis to determine which processes were upregulated in CNV-ECs and TECs versus CECs and NECs, respectively (Figure 2B,C). Gene sets associated with proliferation, hypoxia signaling, and extracellular matrix formation were commonly upregulated (Figure 2B). The commonly upregulated genes were involved in extracellular matrix remodeling, cytoskeleton, glycolysis, EC activation and others. Second, to determine whether the same EC subpopulations were present in CNV-ECs and TECs, we used the Jaccard similarity index to score the similarity of marker gene sets of all EC subpopulations, and observed that marker gene sets across CNV-ECs and TECs were relatively similar for several EC subpopulations (Figure 2D). Further, TECs and CNV-ECs of the same phenotype expressed congruent marker genes (Figure 2E).

We next focused on metabolic genes. Proliferating ECs in both disease models upregulated the expression of genes involved in one carbon metabolism, nucleotide synthesis, tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS) (Figure 3A). In contrast, glycolytic gene expression was upregulated in proliferating, tip and immature ECs in tumors, and was elevated in CNV in proliferating ECs, but less in tip and immature ECs (Figure 3B). The metabolic gene expression signatures between the different TEC phenotypes were more outspoken, possibly reflecting the harsh nutrient-deprived micro-environment in tumors and the fact that TECs grow in an uncontrolled, non-resolving manner.

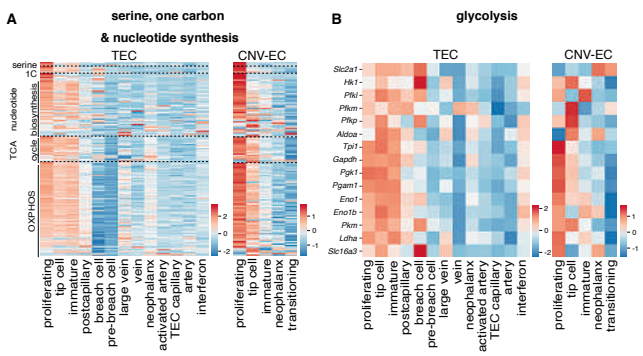

Figure 3: Metabolic heterogeneity in TECs and CNV-ECs

(A) Heatmap of the gene expression levels of the indicated metabolic pathways in TEC and CNV-EC subpopulations. Genes were grouped according to metabolic pathways and ordered so that the most discriminative genes for proliferating ECs are depicted first. **(B)** Heatmap of gene expression levels of the indicated glycolytic genes in TEC and CNV-EC subpopulations.

Metabolic angiogenic target identification

Since OXPHOS and glycolysis are validated metabolic angiogenic targets (Cantelmo et al., 2016; De Bock et al., 2013; Diebold et al., 2019), we designed an integrated analysis to identify other previously unrecognized angiogenic candidates, regulating EC metabolism. We thus performed differential gene expression analysis to determine which metabolic genes and gene sets were commonly upregulated in TECs and CNV-ECs, versus normal ECs. Pathway mapping of gene transcripts involved in central carbon metabolism confirmed that CNV-ECs (and similarly TECs compared to NECs) upregulated transcripts of metabolic pathways in biomass synthesis (Figure 4A,B).

A metabolic gene set enrichment analysis, combined with a congruency analysis, revealed that genes involved in central carbon metabolism (e.g. glycolysis, OXPHOS, nucleotide biosynthesis TCA cycle), were among the most upregulated in TECs and CNV-ECs, compared to NECs and peripheral ECs (Figure 4C-E). Notably, transcripts of genes involved in collagen synthesis were also highly upregulated in angiogenic ECs in both diseases (Figure 4E). Thus, angiogenic ECs have at least two prominent metabolic gene expression signatures, i.e. that of biomass production to support proliferation and of collagen biosynthesis for extracellular matrix remodeling.

Given that changes in transcript levels of metabolic genes alone may not relate to changes in metabolic fluxes, we used genome scale metabolic models (GEMs) to in silico prioritize metabolic candidates. GEMs are mathematical representations of a network of active metabolic pathways (Kim and Lun, 2014; Ryu et al., 2015) and represent computational tools to predict the importance of metabolic reactions for biological responses (Pagliarini et al., 2016; Thiele and Palsson, 2010). We constructed a CEC-tailored GEM and optimized it for biomass or collagen production using two distinct EC-specific objective functions (Figure 5A). Using the CEC-tailored GEM, we identified genes essential for biomass synthesis, involved in glycolysis, TCA cycle, pentose phosphate pathway, OXPHOS, fatty acid oxidation, nucleotide synthesis and salvage, cholesterol biosynthesis, sphingolipid metabolism and amino acid metabolism. The roles

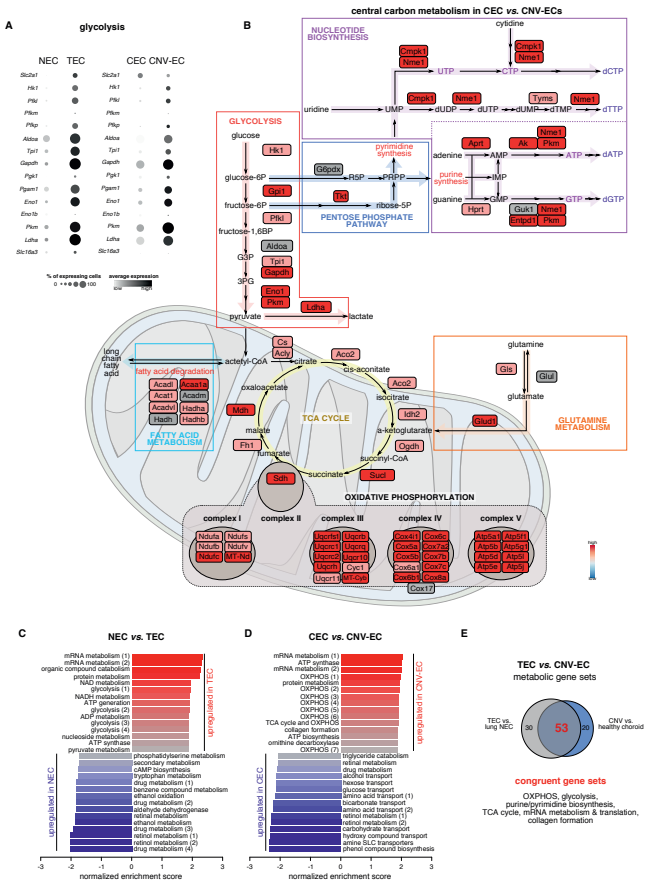

Figure 4: Global metabolic reprogramming in pathological angiogenesis
(A) Dot plot heatmap of the indicated glycolytic genes. The dot size corresponds to the fraction of cells that have higher than average activity of the indicated genes. SCALE: white/grey is low expression, black is high gene expression. **(B)** Map of upregulated central carbon metabolic pathways in CNV-ECs vs. peripheral CECs. Blue color indicates downregulated expression, red upregulated expression and grey unchanged expression. **(C,D)** Metabolic gene set enrichment analysis in NEC vs TECs (C) and CEC vs CNV-ECs (D) ($q < 0.25$ for all gene sets). Numbers between parentheses indicate alternative gene sets pertaining to the same biological function or signaling pathway. **(E)** Metabolic gene sets upregulated in TECs (vs. lung NECs) and in CNV-ECs (vs. choroid CECs). Congruent upregulated metabolic pathways are listed underneath.

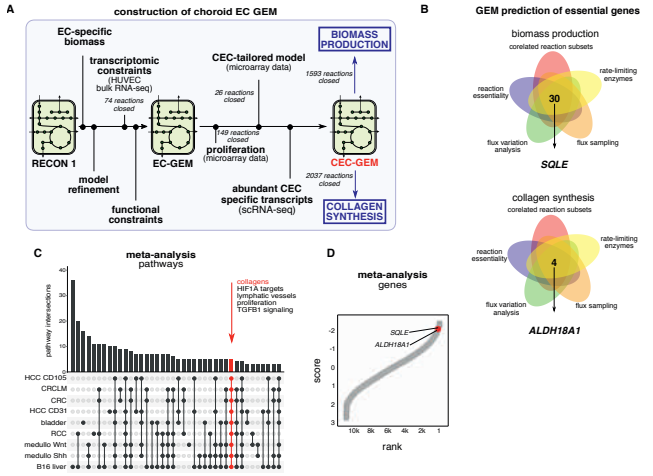

Figure 5: Metabolic target prediction
(A) Schematic representation of GEM reconstruction. **(B)** Venn diagrams indicating metabolic genes that encode rate-limiting enzymes and are predicted to be essential for biomass production (TOP) and collagen biosynthesis (BOTTOM) by four different methods (See STAR Methods for details). **(C)** Upset plot visualization of the results of a differential gene set variation enrichment meta-analysis of nine bulk transcriptomics datasets, showing the number of genes that were higher expressed in TECs than NECs isolated from the indicated tumor type. The bar graph represents the number of gene sets, detected in the tumor type(s) indicated by the dot plot panel below. Five gene sets (displayed on the figure; involved in the displayed processes) were consistently higher expressed in TECs than NECs (red bar graph and intersection). ABBREVIATIONS: HCC, hepatocellular carcinoma; CRCLM, colorectal cancer liver metastasis; CRC, colorectal cancer; medullo Wnt, Wnt driven medulloblastoma; medullo Shh, sonic hedgehog driven medulloblastoma; RCC, renal cell carcinoma. **(D)** Gene expression meta-analysis of the nine NEC versus TEC datasets shown in (C). The S-curve has 10,850 dots, representing genes that were detected in all nine datasets. X-axis: rank numbers from 1 to 10,850 (consistently overexpressed genes in TECs have a low rank number, consistently downregulated genes have a high rank number); Y-axis: the scaled meta-analysis score (consistently overexpressed genes in TECs have a low meta-analysis score, consistently downregulated genes have a high meta-analysis score). SQLE and ALDH18A1 are shown as red dots and listed on the left.

of some of these pathways in EC proliferation have been established (De Bock et al., 2013; Diebold et al., 2019; Huang et al., 2017; Schoors et al., 2015; Vandekeere et al., 2018), thus validating the predictive potential of the CEC-tailored GEM. However, a possible role of cholesterol synthesis in EC growth has only minimally been studied, without conclusive results. Further, consistent with previous reports (Phang, 2019), the CEC-tailored GEM predicted the essentiality of proline biosynthesis for collagen production.

We next performed an integrated analysis to identify functionally relevant metabolic angiogenic targets in CNV-ECs. Specifically, we focused on the subset of rate-limiting single gene encoded metabolic enzymes, predicted by GEM to be essential for biomass production or collagen biosynthesis, and selected candidates that were higher expressed in CNV-ECs than CECs (Figure 5B). Second, we reasoned that genes that were conserved across models, diseases and species, represent robust targets. We thus filtered for genes consistently upregulated also in tumor angiogenesis. Finally, unbiased meta-analysis across 9 different

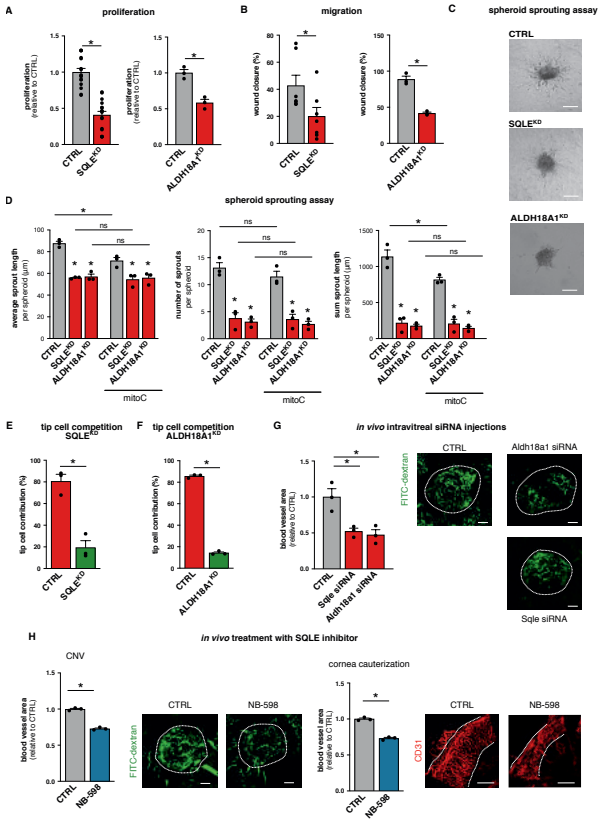

Figure 6: Metabolic target validation

(A) 3H-thymidine incorporation in DNA assay upon SQLE (mean ± SEM, n=16, *p<0.05, unpaired two-tailed t-test) or ALDH18A1 (mean ± SEM, n=3, *p<0.05, unpaired two-tailed t-test) silencing (KD denotes shRNA knockdown). **(B)** Scratch wound migration assay with control and SQLE (mean ± SEM, n=7, *p<0.05, unpaired two-tailed t-test) or Aldh18a1 (mean ± SEM, n=3, *p<0.05, unpaired two-tailed t-test) silenced ECs. **(C)** Bright field photographs of control, ALDH18A1KD and SQLEKD EC spheroids. Scale bar, 100 µm. **(D)** Morphometric quantification of the number of sprouts, average and cumulative sprout length for control, SQLEKD and ALDH18A1KD spheroids with or without MitoC treatment (mean ± SEM, n=3 for all parameters, *p<0.05, unpaired two-tailed t-test). **(E,F)** Mosaic EC spheroid competition of CTRL (red), SQLEKD **(E)** or ALDH18A1KD (F) (green) ECs. Quantification of the fraction of tip cells with the indicated genotype. Scale bar, 50µm. Data are mean ± SEM; n=3; *p < 0.05 by unpaired two-tailed t-test. **(G)** Quantification of CNV blood vessel area in mice treated with control siRNA (CTRL) or siRNA against murine Sqle or Aldh18a1. Representative images are shown on the right. Scale bars, 75 µm. Data are mean ± SEM; n=3 independent experiments each using six mice per group; *p < 0.05; unpaired, two-tailed t-test. **(H)** Quantification of CNV (LEFT) and corneal angiogenesis upon corneal cauterization-induced injury (RIGHT) in mice treated with vehicle (CTRL) or NB-598 (an SQLE blocker). Representative micrographs are shown on the right. Scale bars, 75 µm (CNV) and 500 µm (cornea). Data are mean ± SEM; n=3 independent experiments each using six mice per group; *p < 0.05; unpaired, two-tailed t-test.

murine and human datasets revealed that Aldh18a1, encoding pyrroline-5-carboxylate-synthase (P5CS), the rate-controlling enzyme of proline and collagen biosynthesis and Sqle encoding squalene monooxygenase, a rate-limiting enzyme in cholesterol biosynthesis (Cerqueira et al., 2016) were among the most consistently induced genes in TECs (Figure 5C,D).

To functionally validate the role of ALDH18A1 and SQLE in vessel sprouting, we silenced these genes, which impaired EC proliferation and migration, vessel sprouting (Figure 6A-D), and EC tip cell competitivity (Figure 6E,F). In vivo intraocular treatment with siRNAs against Sqle and Aldh18a1 inhibited neovascularization of laser-induced choroid (Figure 6G). Suggesting potential therapeutic relevance, treatment with NB-598, a pharmacological inhibitor of SQLE reduced both corneal angiogenesis and CNV (Figure 6H).

Discussion

The CEC/CNV-EC taxonomy, which we used together with a previously in-house constructed lung NEC/TEC taxonomy (Goveia et al., 2020) allowed us to characterize metabolic gene expression heterogeneity in sprouting ECs. We detected a complex metabolic transcriptome heterogeneity that is presumably required for EC phenotypes to execute their specialized functions in different vascular compartments. However, given that the micro-environment can influence cellular metabolism (Muir et al., 2018), part of the heterogeneity can possibly also be attributed to different environmental signals.

The EC taxonomies in the eye and lung offered integrated comparison of EC phenotypes. Angiogenic ECs in CNV and tumors seemed to form and maintain neovessels by developing similar EC phenotypes, including proliferating, tip, immature and neo-phalanx ECs. Across diseases and tissues, angiogenic ECs congruently upregulated the expression of non-metabolic marker genes, as well as metabolic genes. Hence, blood vessels seem to sprout by inducing the differentiation of largely similar EC phenotypes across diseases and tissues. Tumors have been named "non-healing wounds" and angiogenesis of tumors may thus build on similar principles as a CNV wound (Dvorak, 1986).

We explored whether metabolic signatures of individual ECs could be utilized for the discovery of metabolic genes. Hypothesizing that genes that are upregulated across diseases represent robust candidates, we performed an integrated multi-layered approach combining scRNA-seq of ECs from different tissues/diseases, congruency transcriptome analysis, genome-scale metabolic modeling and cross-species meta-analysis to identify conserved angiogenic metabolic targets, i.e. Sqle and Aldh18A. Functional validation revealed that silencing of these targets impaired vessel sprouting in vitro and inhibited pathological ocular angiogenesis in vivo. While it was not our primary goal to develop new AAT strategies, rather to provide proof-of-principle of the integrated approach, the identified metabolic targets might nonetheless deserve further attention, though an EC-selective drug delivery approach would then be desirable.

Finally, we acknowledge limitations of our study. The inferred biological role and topographical localization in the vasculature of each EC phenotype require additional functional validation. Transcript levels and GEM modeling do not fully capture the complexity of metabolism. However, gene signatures and GEM modeling have been proven to be predictive of the metabolic flux changes in ECs (Bruning et al., 2018; Cantelmo et al., 2016; Kalucka et al., 2018; McGarrity et al., 2018; Patella et al., 2015; Vandekeere et al., 2018). With excitement, we await future development of new technology to quantify EC metabolism at the single cell level. The potential of using an integrated analysis to overcome technical limitations in metabolic target prioritization is demonstrated by the validation of the functional role of the selected candidates (Sqle, Aldh18A) in vessel sprouting in vivo.
Our data can be explored online at https://www.vibcancer.be/software-tools/Murine_ECTax and via the publicly available added-value database EndoDB (Khan et al., 2019). Our in-house software BIOMEX for omics data interpretation and visualization is available at https://www.vibcancer.be/software-tools/BIOMEX (Taverna et al., 2020).

Acknowledgement

I would like to thank my supervisor and mentor Prof. Peter Carmeliet for the enormous support and inspiration I enjoyed during the time I spent as a postdoctoral researcher in his laboratory. It was a truly exciting period of my scientific career, and it prepared me both scientifically and personally for the next step in the transition to an independent investigator. I also want to acknowledge all my co-authors who contributed to this study. This multi-disciplinary work would not be possible without the combined effort of all team members with their unique expertise. Special thanks go to each of my co-first authors, who were responsible for the specific aspects of the study, such as the computational analysis, in vivo experiments and generation of the genome-scale metabolic model. Finally, I am deeply honoured that our work was recognized by the prestigious Werner Risau prize and the DGZ and feel proud to be a part of the endothelial biology community.
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Education

2016	**Ph.D.** in Immunology, Faculty of Science, Charles University in Prague, Czechia.
2010	**Master's with honors** in Biology, Faculty of Science, Charles University in Prague, Czechia
2008	**Bachelor's in biology**, Faculty of Science, Charles University in Prague, Czechia.

Professional experience

From 2020	**Junior group leader**, Institute of Biotechnology, BIOCEV, Vestec, Czechia
2017	**Postdoctoral researcher**, Prof. Peter Carmeliet, Center for Cancer Biology, VIB-KU Leuven, Leuven, Belgium.
2016	**Postdoctoral researcher**, Prof. Jiri Neuzil, Institute of Biotechnology, BIOCEV, Vestec, Czechia.
2010	**Ph.D. student**, Prof. Jiri Neuzil, Institute of Biotechnology, Prague, Czechia.

Fellowships and awards

2021	**MSCA Individual fellowship**, European Commission.
2020	**Werner Risau prize**. German Society of Cell Biology, DGZ, Germany
2017	**Postdoctoral fellowship**, Fonds voor Wetenschappelijk Onderzoek, FWO. Belgium
2015	**Best poster**, EMBO workshop Mitochondria Apoptosis Cancer, Frankfurt am Main, Germany
2011	**Student research grant**, Charles University Grant Agency, Czechia

Research interest and activity

Cancer metabolism: I was introduced to cancer metabolism during my Ph.D., which focused on characterization of a novel anticancer compound MitoTam that was developed in the laboratory of Prof. Neuzil. I identified the mechanism of action of MitoTam, showing that it accumulates in mitochondria of cancer cells, where it acts as an inhibitor of respiratory complex I, and as an inducer of reactive oxygen species *(Antioxid Redox Signal (2017)*. MitoTam recently completed a phase Ib clinical trial in terminal cancer patients. The treatment is well tolerated and led to tumor stabilization in >50% of patients and tumor regression in 2 patients, demonstrating its high translational potential. In addition, I participated in a paradigm-shifting study that identified de novo pyrimidine synthesis as the essential function of mitochondrial respiration in cancer *(Cell Metab, 2019)*.

Transcriptomics heterogeneity in endothelial cells (ECs) as a target of novel (anti-angiogenic) therapy. My PhD focused on cancer cells' bioenergetic needs. However, I have been aware that cancer is an extremely complex disease, with stromal cells and blood vessels contributing substantially to tumor properties. Hence during my postdoc, I aimed to extend my focus beyond cancer cells, and gain expertise in the multimodal interactions within the tumor. With this aim I joined the laboratory of Prof. Peter Carmeliet, where I focused on EC heterogeneity in pathological angiogenesis using the powerful technology of single cell omics. In this project we identified and validated (i) new angiogenic targets in a study of >100 cancer patients *(Cancer Cell, 2020)* and (ii) conserved metabolic targets in ocular neovascularization and lung cancer (*Cell Metab, 2020; Werner Risau prize)*. I also contributed to studies uncovering organ specific EC heterogeneity *(Cell, 2020; J Am Soc Nephrol, 2020)*, and 2 bioinformatics projects: a database of bulk EC transcriptomics data and an in-house data mining software BIOMEX *(Nucleic Acid Res, 2019 & 2020)*. As an independent PI, I want to build on my expertise in single cell omics and cancer metabolism and use transgenic models of perturbed metabolism to map the metabolic landscape *in vivo* for basic biological understanding and to identify new therapeutic targets in cancer.

10 main publications

- Taverna F, Goveia J, Karakach TK, Khan S, Rohlenova K, Treps L, Subramanian A, Schoonjans L, Dewerchin M, Eelen G, Carmeliet *P. BIOMEX: an interactive workflow for (single cell) omics data interpretation and visualization.* **Nucleic Acid Res.** 2020 May 11. gkaa332.
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WALTHER FLEMMING AWARD 2021

The German Society for Cell Biology (DGZ) and ibidi offer the "Walther Flemming Award" for excellent research in cell biology. The award consists of a financial contribution of EUR 3000 and is given to senior postdoctoral researchers and early career group leaders for recent work that defines their emerging independent research profile.

Candidates need to be members of the DGZ and can either be nominated or apply directly for the prize.

Applications should be submitted in a single pdf file and consist of cover letter, CV and copies of 1-3 publications that document the relevant work of the applicant. Applications will be reviewed by a dedicated award committee of the DGZ.

Please send your application by e-mail to the DGZ office:
dgz@dkfz.de

Deadline: June 30, 2021

NIKON YOUNG SCIENTIST AWARD 2021

The German Society for Cell Biology (DGZ) and Nikon GmbH (Business Unit: Microscope Solutions) annually offer the "Nikon Young Scientist Award" for excellent research in cell biology by PhD students or young postdoctoral researchers within 3 years after graduating (an extension of up to 2 years will be granted for periods of parental leave). The awardee will receive a financial contribution of EUR 1500.

Candidates need to be members of the DGZ and can either be nominated or apply directly for the prize.

Applications should be submitted in a single pdf file and consist of cover letter, CV and copies of publications that document the work of the applicant. Applications will be reviewed by a dedicated award committee of the DGZ.

Please send your application by e-mail to the DGZ office:
dgz@dkfz.de

Deadline: June 30, 2021

BINDER INNOVATION PRIZE 2021

The BINDER Innovation Prize is sponsored by BINDER GmbH in Tuttlingen since 1998 and annually awarded by the German Society for Cell Biology (DGZ). The award is given for outstanding contributions to cell biology and consists of a financial contribution of EUR 4000. It is aimed at junior investigators that have already established and developed their own research profile. Candidates need to be members of the DGZ and can either be nominated or apply directly for the prize.

Applications should be submitted in pdf format and consist of cover letter, CV, a research profile and copies of three selected first/last author publications. Applications will be reviewed by a dedicated award committee of the DGZ.

Please send your application by e-mail to the DGZ office: **dgz@dkfz.de**

Deadline: June 30, 2021

WERNER RISAU PRIZE 2021

for Outstanding Studies in Endothelial Cell Biology

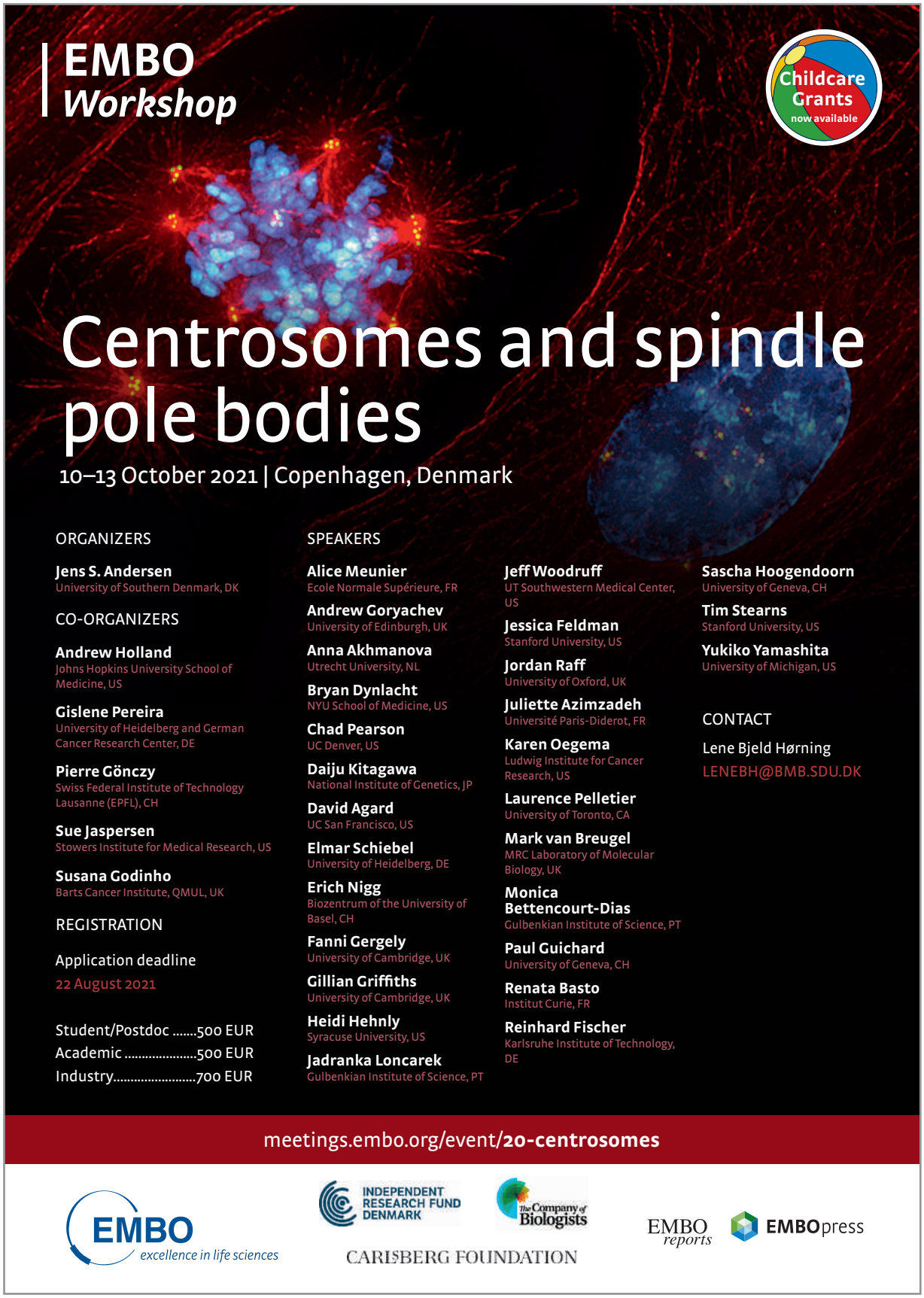
The German Society for Cell Biology (DGZ) and the Werner-Risau-Prize Committee annually award the "Werner-Risau Prize for outstanding studies in endothelial cell biology" to a candidate within the first five years after obtaining their PhD or MD (an extension of up to 2 years will be granted for periods of parental leave). The prize will be awarded for an article already published or in press. The awardee will receive a financial contribution of EUR 4000.

For details visit: http://www.werner-risau-prize.org

Applications should be submitted in a single pdf file and consist of cover letter, CV and a copy of the relevant publication.

Please send your application to the Werner Risau Prize Committee at:
hugo.marti@physiologie.uni-heidelberg.de

Deadline: June 30, 2021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24th Meeting on Signal Transduction

STS

organized by the

Signal Transduction Society (STS)

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Organizational team: Ingo Schmitz (Bochum), Klaudia Giehl (Gießen),
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The pandemic situation permitting, the Meeting will be held on site with an appropriate hygiene & safety concept.

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Scan for further information

Early Bird Registration Deadline: September 30, 2021
Abstract Submission Deadline: October 10, 2021

Protokoll der Mitgliederversammlung 2020 der Deutschen Gesellschaft für Zellbiologie e.V.

Versammlungsleiter und Protokollführer: Prof. Dr. Oliver Gruß, Präsident

Die Mitgliederversammlung fand am 15.12.2020, 16.00 Uhr online über zoom statt.
Alle Mitglieder waren rechtzeitig über email eingeladen und über die Tagesordnung informiert worden und konnten sich über den kommunizierten link zuschalten.

Tagesordnung:

1. Bestätigung des Protokolls der letzten Sitzung
2. Jahresbericht des Präsidenten mit anschließender Diskussion
3. Geschäfts- und Kassenbericht (Bericht über das abgelaufene Kalenderjahr)
4. Bericht der beiden Rechnungsprüfer
5. Entlastung des Vorstandes
6. Bekanntgabe des Wahlergebnisses
7. Besondere Kommissionen
8. Sonstiges / Diskussion

1. Bestätigung des Protokolls der letzten Sitzung

Das Protokoll der Mitgliederversammlung 2019 (Tübingen, 26.09.2019) war in CellNews 4/2019 veröffentlicht worden und wird bestätigt.

2. Jahresbericht des Präsidenten

Oliver Gruß berichtet über die Aktivitäten der DGZ in 2020. Die Mitgliederzahl ist durch 56 Abgänge bei nur 8 Neumitgliedern auf 763 gefallen (819 im September 2019). Die aktuellen Mitglieder sollen helfen neue Mitglieder für die Gesellschaft zu gewinnen.
Das für September 2020 in Münster geplante International Meeting der DGZ wurde mit gleichlautender Thematik „The Cell Biology of Interfaces" auf den 27.-29.09.2021 verschoben. Die meisten Sprecher haben diesen Termin bereits bestätigt. Die zugesagte Förderung durch die DFG besteht weiter. Roland Wedlich-Söldner und das Organisationsteam vor Ort werden zusammen mit den lokalen SFBs versuchen, das Meeting in Präsenz durchzuführen.
Am 10.11.2020 verlieh die DGZ den Nikon Young Scientist Award (Nick So), den Werner-Risau-Preis (Katerina Rohlenova), den Binder-Innovationspreis (Christian Münch) und die Carl-Zeiss-Lecture (Petra Schwille). Die Preisträgerinnen und Preisträger hielten online-Vorträge, die von den Mitgliedern in einer gemeinsamen zoom-Konferenz verfolgt wurden. Die Vorträge sind auch über einen link auf der homepage der DGZ (https://zellbiologie.de/wissenschaftspreise/) abrufbar.

3. Geschäfts -und Kassenbericht

Oliver Gruß berichtet über die Einnahmen und Ausgaben der DGZ. Mitgliedsbeiträge und Werbe-Einnahmen aus der CellNews stehen als größte Posten den Ausgaben für den Betrieb des Büros, Sponsoring von Preisen und Veranstaltungen und Kosten für das International Meeting in Tübingen (u.a. Posterpreise) gegenüber. Das tatsächliche Guthaben ist mit ca. 100.000 € (71.000 € DGZ, 30.000 € Werner-Risau-Preis) im Vergleich zum Vorjahr (72.000 €) annähernd konstant geblieben.

4. Bericht der beiden Rechnungsprüfer

Die Rechnungsprüfer Julia Groß und Ralph Gräf haben die Bücher am 10.03.2020 in Anwesenheit von Sabine Reichel-Klingmann und Oliver Gruß in Göttingen geprüft und bestätigen die Prüfung.

5. Entlastung des Vorstandes

Der Vorstand wird über geheime online-Abstimmung (Umfrage-toll in zoom) mit 43 von 44 Stimmen (eine Enthaltung) entlastet.

6. Bekanntgabe der Wahlergebnisse

Die Wahlergebnisse für die Wahl zum Vorstand, dem Beirat und der Kassenprüfer werden bekanntgegeben:

Präsident: Roland Wedlich-Söldner (Münster), 126 Ja-Stimmen, 5 Nein-Stimmen, 12 Enthaltungen.

Vize-Präsidentin: Gislene Pereira (Heidelberg), 125 Ja-Stimmen, 5 Nein-Stimmen, 13 Enthaltungen.

Geschäftsführerin: Sandra Iden (Saarbrücken), 125 Ja-Stimmen, 4 Nein-Stimmen, 14 Enthaltungen.

Vize-Geschäftsführer: Ralf Jungmann (München), 118 Ja-Stimmen, 6 Nein-Stimmen, 19 Enthaltungen.

Beiratsmitglied: Carien Niessen (Köln), 128 Ja-Stimmen, 6 Nein-Stimmen, 9 Enthaltungen.

Beiratsmitglied: Simone Reber (Berlin), 126 Ja-Stimmen, 4 Nein-Stimmen, 13 Enthaltungen.

Beiratsmitglied: Anne Straube (Warwick, UK), 123 Ja-Stimmen, 3 Nein-Stimmen, 17 Enthaltungen.

Kassenprüferin: Julia Groß (Göttingen), 129 Ja-Stimmen, 1 Nein-Stimme, 13 Enthaltungen.

Kassenprüfer: Ralph Gräf (Potsdam), 133 Ja-Stimmen, 2 Nein-Stimmen, 8 Enthaltungen.

Auf Nachfrage haben die Gewählten ihr Amt angenommen.

7. Besondere Kommissionen

Als Preis-Jury stehen neben dem bisherigen Jury-Mitglied Walter Witke (Bonn), Zuzana Storchová (Kaiserslautern) und Alf Honigmann (Dresden) zur Verfügung.

8. Sonstiges / Offene Diskussion

Man diskutiert das Zeitfenster des International Meetings im Herbst entgegen der langjährigen Tradition eines Frühjahrsmeetings. Die Planung für Herbst 2021 steht, ließe aber die Möglichkeit eines nächsten International Meetings im Herbst 2022 oder im Frühjahr 2023 offen. Keine der Optionen soll für die Planung ausgeschlossen werden.

Anlässlich des nahenden Brexits wird nach Interaktionen mit der englischen Gesellschaft für Zellbiologie gefragt. Eine Unterstützung der englischen community über verstärkte Interaktionen z.B. in gemeinsamen Meetings oder Einbindung von Personen aus UK in die DGZ wird ausdrücklich begrüßt. Die DGZ sollte ein Signal setzen gegen die Schaffung neuer politischer Grenzen, die jetzt nach dem Brexit zu befürchten sind.

BILANZ 2019
EINNAHMEN / AUSGABEN

Einnahmen	EUR	**Ausgaben**	EUR
Mitgliedsbeiträge (abzgl. Retouren)	38.460,00	Bankkosten	752,61
Spenden, Preisgelder	15.000,00	Retoure Mitgliedsbeiträge	400,00
Zinsen	12,50	Reisekosten	441,60
Cell News, Homepage	11.364,50	Spenden, Preisgelder	21.300,00
(Werbeanzeigen, Firmen-Links)		Cell News	2.194,78
DGZ-Tagungen	214,00	DGZ-Tagungen	4.604,14
Überträge	212.987,95	Bürokosten/Gehalt Sekr. (2015-2019)	174.129,51
Sonstige	72,93	Büromaterial, Homepage	
		Überträge	212.987,95
		Sonstige	4.662,11
Summe der Einnahmen:	278.111,88	Summe der Ausgaben:	421.472,70
Guthaben am 31.12.2018:	245.243,39	**Guthaben am 31.12.2019:**	**101.882,57**
Guthaben DGZ:	209.989,61	**Guthaben DGZ:**	**71.046,66**
Werner Risau Preis:	35.253,78	**Werner Risau Preis:**	**30.835,91**

Die Einnahmen und Ausgaben wurden am 19.03.2019 von den beiden Kassenprüfern Julia Groß und Ralph Gräf geprüft und für richtig befunden.

MINUTES OF THE DGZ MEMBER MEETING 2020

The general meeting took place on December 15, 2020, 4 p.m. online via zoom. All members were invited in good time via email and could connect via the communicated link.

Agenda:

1. Confirmation of the minutes of the last meeting
2. Annual report of the President
3. Business and financial report (report on the past calendar year)
4. Report of the two auditors
5. Discharge of the board of directors
6. Announcement of the election results
7. Special Commissions
8. Miscellaneous / Open discussion

1. Confirmation of the minutes of the last meeting

The minutes of the general meeting 2019 (Tübingen, September 26th, 2019) were published in CellNews 4/2019 and are confirmed.

2. Annual report of the President

Oliver Gruß reports on the activities of the DGZ in 2020. The number of members has fallen to 763 by 56 departures with only 8 new members (819 in September 2019). The current members should help to win new members for the society.
The DGZ International Meeting planned for September 2020 in Münster has been postponed to September 27-29, 2021 with the same topic "The Cell Biology of Interfaces". Most speakers have already confirmed this date. The promised funding by the DFG continues. Roland Wedlich-Söldner and the organization team on site will try to hold the meeting in person together with the local SFBs.
On November 10th, 2020 the DGZ presented the Nikon Young Scientist Award (Nick So), the Werner Risau Prize (Katerina Rohlenova), the Binder Innovation Prize (Christian Münch) and the Carl Zeiss Lecture (Petra Schwille). The award winners gave online lectures that were followed by the members in a joint zoom conference. The lectures can also be accessed via a link on the DGZ homepage (https://zellbiologie.de/wissenschaftpreise/).

3. Business and cash report

Oliver Gruß reports on the income and expenses of the DGZ. Membership fees and advertising income from CellNews are the largest items compared to expenses for running the office, sponsoring prizes and events, and costs for the International Meeting in Tübingen (including poster prizes). The actual credit at around € 100,000 (€ 71,000 DGZ, € 30,000 Werner Risau Prize) has remained almost constant compared to the previous year (€ 72,000).

4. Report of the two auditors

The auditors Julia Groß and Ralph Gräf checked the books on March 10, 2020 in the presence of Sabine Reichel-Klingmann and Oliver Gruß in Göttingen and confirmed the check.

5. Discharge of the board of directors

The board of directors is discharged with 43 of 44 votes (one abstention) via secret online voting (survey-toll in zoom).

6. Announcement of the election results

The election results for the election to the board of directors, the advisory board and the cash auditors are announced:

President: Roland Wedlich-Söldner (Münster), yes: 126, no: 5, abstention: 12

Vice-President: Gislene Pereira (Heidelberg), yes: 125, no: 5, abstention: 13

Chief Executive Officer: Sandra Iden (Saarbrücken), yes: 125, no: 4, abstention: 14

Vice Chief Executive Officer: Ralf Jungmann (Munich), yes: 118, no: 6, abstention: 19

Advisory Board member: Carien Niessen (Cologne), yes: 128, no: 6, abstention: 9

Advisory Board member: Simone Reber (Berlin), yes: 126, no: 4, abstention: 13

Advisory Board member: Anne Straube (Warwick, UK), yes: 123, no: 3, abstention: 17

Auditor: Julia Groß (Göttingen), yes: 129, no: 1, abstention: 13

Auditor: Ralph Gräf (Potsdam). yes: 133, no: 2, abstention: 8

7. Special commissions

In addition to the previous jury member, Walter Witke (Bonn), Zuzana Storchová (Kaiserslautern) and Alf Honigmann (Dresden) join the award jury.

8. Miscellaneous / Open discussion

The time window of the International Meeting in autumn is being discussed, contrary to the long-standing tradition of a spring meeting. Planning for autumn 2021 is in place but leaves the possibility of the next international meeting open in autumn 2022 or spring 2023. None of the options should be excluded for planning. On the occasion of the approaching Brexit, interactions with the English Society for Cell Biology will be asked. Support for the English community through increased interactions, for example in joint meetings or the involvement of people from the UK in the DGZ, is expressly welcomed. The DGZ should send a signal against the creation of new political borders, which are now to be feared after Brexit.

Impressum

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