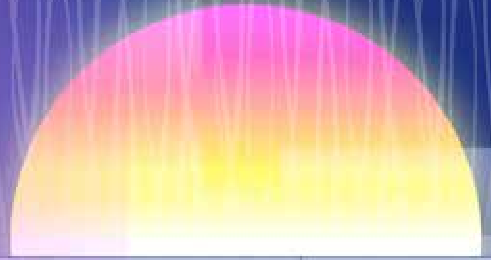
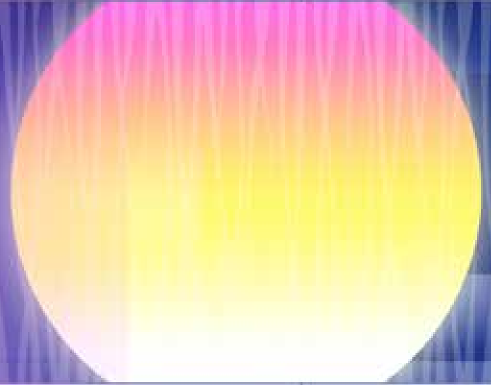

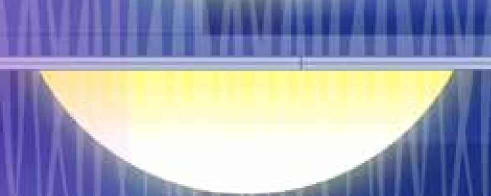
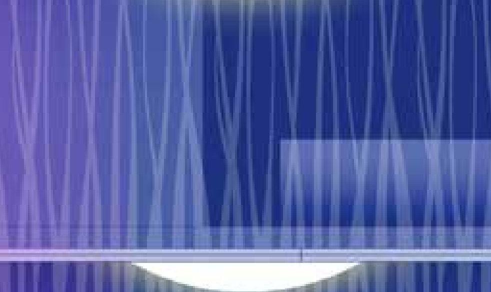

THE MDC CELEBRATES

15 YEARS OF BIMSB

16TH BERLIN SUMMER MEETING

6-7 SEPT. 2023

MAX DELBRÜCK CENTER BIMSB

Discovery for tomorrow's medicine

Berlin Institute for Medical Systems Biology

WELCOME TO THE 16TH BERLIN SUMMER MEETING

15 YEARS OF BIMSB

Berlin Institute
for Medical
Systems Biology

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CONFERENCE VENUE

Wednesday, September 6, 2023
MDC.C, Robert-Rössle-Str. 10, 13125 Berlin

Thursday, September 7, 2023
Langenbeck-Virchow-Haus, Luisenstr. 58/59, 10117 Berlin

ORGANIZATION & CONTACT

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@MDC_Berlin @BIMSB_MDC

WELCOME ADDRESS

Dear friends and colleagues,

2023 marks the 15-year anniversary of the Berlin Institute for Medical Systems Biology (MDC-BIMSB). BIMSB was founded in 2008 as an expansion of the Max Delbrück Center into Medical Systems Biology with a strong focus on understanding how different levels of gene regulation are integrated in cells and driving healthy or pathological phenotypes. Tight integration of data science and experimental molecular biology was necessary to make this vision work. This expansion was initially financed through a grant from the Federal Ministry of Education and Research and the Berlin Senate and then expanded into funding for ~25 groups and a dedicated new building in the center of Berlin. Within 15 years, more than 30 independent group leaders were internationally recruited and hundreds of scientists conducted research projects at MDC-BIMSB.

In 2019, MDC-BIMSB moved from Campus Buch into a specifically designed building in the center of Berlin, on the Life Sciences Campus of the Humboldt University, to further foster close collaborations with Charité – Universitätsmedizin Berlin, Berlin Universities and other local research institutions.

The annual “The Berlin Summer Meeting: Computational and Experimental Biology Meet” brings together scientists at the interface of experimental molecular biology and computational biology and connects young researchers with top-level senior scientists.

For our “The MDC celebrates 15 years of BIMSB” edition of the meeting, we are thrilled to be hosting exceptional international scientists (including a selection of our alumni) who will be presenting exciting new developments in the field of Medical Systems Biology.

The first day of the meeting is being held in the Max Delbrück Communications Center in Buch. The second day will take place at the Langenbeck-Virchow-Haus in Mitte. The event concludes with an evening reception & exhibition in the MDC-BIMSB research building, 5 min walking distance from the meeting venue.

For this anniversary installation of the series, the Scientific Organizing Committee encompasses 31 MDC PIs. We are delighted that you will be joining us for this joyful occasion, and we are looking forward to inspiring science, sparking lively discussions and productive scientific exchange, both following the talks and during the poster session. We thank the BMBF, the Senate of Berlin, and the DFG and our sponsors for funding and support. We are also excited to welcome colleagues from NOVA University Lisbon, with whom we have partnered for building NIMSB (NOVA Institute for Medical Systems Biology) in Lisbon.

Sincerely,

The BSM2023 Scientific Organizing Committee:

Altuna Akalin, Janine Altmüller, Melissa Birol, Marina Chekulaeva, Gaetano Gargiulo, Mina Gouti, Stefanie Grosswendt, Simon Haas, Laleh Haghverdi, Norbert Hübner, Jan Philipp Junker, Dagmar Kainmüller, Michael Kaminski, Stefan Kempa, Markus Landthaler, Leif Ludwig, Darío Lupiáñez, Jakob Metzger, Irmtraud Meyer, Uwe Ohler, Ilaria Piazza, Ana Pombo, Nikolaus Rajewsky, Michael Robson, Agnieszka Rybak-Wolf, Maike Sander, Ashley Sanders, Matthias Selbach, Michael Sigal, Jana Wolf and Robert Zinzen

PROGRAM

DAY 1 WEDNESDAY, SEPTEMBER 6, 2023

MDC.C, Robert-Rössle-Str. 10, 13125 Berlin

9:00 am – 9:15 am Welcome address by **Maike Sander**

SESSION 1 HOST: NIKOLAUS RAJEWSKY

9:15 am – 10:00 am
BERND BODENMILLER
University of Zurich, ETH Zurich, Switzerland
tba

10:00 am – 10:15 am
NORA FRESMANN
MDC-BIMSB, Berlin, Germany
Cause and consequence of intra- and inter-tumor heterogeneity in zebrafish models of neuroblastoma

10:15 am – 11:00 am
HANS CLEVERS
Roche Pharma Research and Early Development
Roche Innovation Center Basel, Switzerland
Organoids to model human disease

11:00 am – 11:30 am **Coffee break**

SESSION 2 HOST: NIKOLAUS RAJEWSKY (MDC-BIMSB Alumni Session)

11:30 am – 11:50 pm
PINAR ÖNAL
Bilkent University, Ankara, Turkey
Evolutionary dynamics of a robust transcriptional network

11:50 am – 12:10 pm
BARIS TURSUN
Universität Hamburg, Germany
A reprogrammed worms perspective on BIMSB

12:10 pm – 12:30 pm
ELENA TORLAI TRIGLIA
Broad Institute, Cambridge, MA, USA
Identifying molecular determinants of cancer phenotypes with edited human cell models

12:30 pm – 12:50 pm

LEA GREGERSEN
University of Copenhagen, Denmark
Oxidative stress triggers a unique and transient dynamic transcriptional response

12:50 pm – 1:10 pm

MATHIAS MUNSCHAUER
Helmholtz Institute for RNA-based Infection Research, Würzburg, Germany
The host protein SND1 binds SARS-CoV-2 negative-sense RNA and promotes viral RNA synthesis

1:10 pm – 3:00 pm **Lunch & Poster session**

SESSION 3 HOST: MARKUS LANDTHALER

3:00 pm – 3:45 pm

DOMINIC GRÜN
University of Würzburg, Germany
Reconstructing cell fate decision in time and space

3:45 pm – 4:00 pm

TILL SCHWÄMMLE
Max Planck Institute for Molecular Genetics, Berlin, Germany
Zic3 and Otx2 link Xist activation to the formative pluripotency network

4:00 pm – 4:15 pm

LISA BUCHAUER
Charité - Universitätsmedizin Berlin, Germany/ Weizmann Institute of Science, Rehovot, Israel
Spatial discordances between mRNAs and proteins in the intestinal epithelium

4:15 pm – 5:00 pm

GENE YEO
University of California, San Diego, CA, USA
RNA binding protein networks in health and disease

DAY 2 THURSDAY, SEPTEMBER 7, 2023

Langenbeck-Virchow-Haus, Luisenstr. 58/59, 10117 Berlin

SESSION 4 HOST: ANA POMBO

9:00 am – 9:45 am	**PATRICK CRAMER** Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany **New insights into transcription regulation**
9:45 am – 10:00 am	**TANCREDI PENTIMALLI** MDC-BIMSB, Berlin, Germany **3D molecular reconstruction of a human tumor at single-cell resolution reveals invasion dynamics and predicts mechanism-based, personalized therapeutic targets**
10:00 am – 10:15 am	**MIKAELA BEHM** DKFZ, Heidelberg, Germany **An interactive cellular ecosystem blocks epithelial transformation in naked mole-rat**
10:15 am – 11:00 am	**STEPHAN PREIBISCH** Janelia Research Campus, Ashburn, VA, USA **Peta-scale microscopy – challenges, solutions and possibilities**
11:00 am – 11:30 am	**Coffee break**

SESSION 5 HOST: ASHLEY SANDERS

11:30 am – 12:15 pm	**NADA JABADO** McGill University Health Centre, Montreal, Canada **Oncohistone in disease: from cancer to neurodegeneration and beyond**
12:15 pm – 12:30 pm	**MAX TRAUERNICHT** Netherlands Cancer Institute, Amsterdam/ Oncode Institute, Utrecht, the Netherlands **Multiplexed detection of transcription factor activity**

12:30 pm – 12:45 pm	**ANDRÉA WILLEMIN** MDC-BIMSB, Berlin, Germany **A GAM-based approach to explore the contribution of proteins or PTMs to 3D genome folding and cellular state transitions**
12:45 pm – 1:30 pm	**STEIN AERTS** KU Leuven Center for Human Genetics/VIB Center for Brain and Disease Research, Belgium **Single-cell driven enhancer modelling and design**
1:30 pm	**Group photo**
1:30 pm – 3:00 pm	**Lunch break**

SESSION 6 HOST: STEFANIE GROSSWENDT

3:00 pm – 3:45 pm	**AMOS TANAY** Weizmann Institute of Science, Rehovot, Israel **Profiling and modelling the blood to understand disease progression**
3:45 pm – 4:30 pm	**TUULI LAPPALAINEN** New York Genome Center, New York City, NY, USA/KTH Royal Institute of Technology and SciLifeLab, Stockholm, Sweden **Genetic effects on gene expression dosage underlying cellular and physiological phenotypes**
4:30 pm – 5:15 pm	**SARA WICKSTRÖM** Max Planck Institute for Molecular Biomedicine, Münster, Germany **Coordination of cell states and tissue architecture by mechanical forces**
5:15 pm	**Closing remarks**
5:30 pm	**Reception**, MDC-BIMSB, Hannoversche Str. 28, 10115 Berlin (5 min walking distance from the meeting venue)

(IN ORDER OF TALKS IN THE PROGRAM SCHEDULE)

BERND BODENMILLER

University of Zurich, ETH Zurich, Switzerland

NORA FRESMANN

NORA FRESMANN[1,*] , Bastiaan Spanjaard[1], Anton Gauert[2], Luca Brame[2], Pedro Olivares Chauvet[1], Anja Heeren Hagemann[2], and Jan Philipp Junker[1]

[1]Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine Berlin

[2]Department of Paediatrics, Division of Oncology and Haematology, Charité Berlin

*Presenting author

Cause and consequence of intra- and inter-tumor heterogeneity in zebrafish models of neuroblastoma

Neuroblastoma (NB) is a childhood cancer originating from multipotent sympathoadrenal progenitor cells with low survival rates for high-risk patients. As there is an overall paucity of frequently recurring somatic mutations and druggable targets in NB, it is crucial to better understand NB cell plasticity in relation to disease progression and to identify ways to target specific cellular programs. We aim to elucidate mechanisms of cancer progression and metastasis formation by employing single-cell RNA-sequencing (scRNA-seq) with lineage tracing on MYCN-driven tumors from various zebrafish models of NB.

Multiplexed scRNA-seq of 55 primary tumors from transgenic zebrafish expressing either MYCN alone or together with LMO1 under the control of a sympathoadrenal lineage-specific promoter revealed diverse NB and microenvironmental cell populations. Zebrafish NB cells show a largely adrenergic transcriptomic profile, but we further observe sub-states, whose occurrence and frequency differs between tumors, despite the controlled genetic background. CRISPR/Cas9-based single-cell lineage tracing showed that several progenitor cells transform and contribute to tumor growth in these models. The observed transcriptional divergence between NB cell subpopulations within a tumor is mostly independent of the cells' lineage relationship, indicating that NB cells plastically adapt to their niche. To further probe the impact of microenvironmental changes on NB cell states, we transplanted NB cells into zebrafish blastulae with transcriptomic read-out before, shortly after and months after transplantation. We are currently analyzing this data, measuring clonal predisposition to survival in a new niche and associated adaptation mechanisms.

HANS CLEVERS

Roche Pharma Research and Early Development, Roche Innovation Center Basel, Switzerland

Organoids to model human disease

The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. We originally found that Lgr5+ve crypt base columnar cells (CBC) generated all epithelial lineages throughout life, implying that they represent the stem cell of the small intestine and colon. Lgr5 was subsequently found by us to represent an exquisitely specific, yet 'generic' marker for active epithelial stem cells, including in hair follicles, kidney, liver, mammary gland, inner ear, tongue and stomach epithelium.

Single sorted Lgr5+ve stem cells can initiate ever-expanding organoids in the lab. These organoids recapitulate key aspects of the organ from which the stem cells were taken. 3D organoids have been developed for the Lgr5+ve stem cells of human stomach, liver, pancreas, prostate, kidney, breast and many others. Using CRISPR/Cas9 technology, genes can be efficiently modified in these organoids . Organoid technology opens avenues for the study of development, physiology and disease, for drug development and for personalized medicine. In the long run, cultured mini-organs may replace transplant organs from donors and hold promise in gene therapy.

PINAR ÖNAL

PINAR ÖNAL[1], Eleanor Degen[2], Dilan Akdogan[1], Shelby Blythe[2], Steve Small[3]

[1]Ihsan Dogramaci Bilkent University

[2]Northwestern University

[3]New York University

Evolutionary dynamics of a robust transcriptional network

Changes in the transcription factor sequences are instrumental in the evolution of transcriptional networks when combined with gene duplications. The primary anterior determinant of Drosophila embryo, Bicoid (Bcd), is a Homeodomain (HD) transcription factor that emerged after a gene duplication event together with its sister protein Zen and diverged from its ancestral role mainly through changes in its HD. We have reconstructed the ancestral proteins that Bcd and Zen HD evolved from and have established several transgenic lines expressing the potential ancestral variations as the sole source of Bcd. We propose that these variants with the consecutive and suboptimal but increasing biological activities represent a multistep pathway of evolution of Drosophila Bcd's novel function and interactions. Using these series of Bcd HD alleles, we are currently investigating the in vivo dynamics of evolvable transcriptional interactions and morphologies during Drosophila embryogenesis as well as biochemical and structural details of robust protein-DNA interactions.

BARIS TURSUN

Universität Hamburg, Germany

A reprogrammed worms perspective on BIMSB

Cellular reprogramming holds great therapeutic potential for repairing tissues and organs damaged by diseases or trauma. By reprogramming cells, it becomes possible to restore organ function and correct tissue dysfunction. However, achieving the desired cell type through reprogramming faces challenges due to molecular cell fate-safeguarding mechanisms, limiting or even obstructing the conversion of cell identities. Incomplete reprogramming can undermine the therapeutic potential of reprogrammed cells and may lead to adverse consequences, such as the development of tumors. To ensure the faithful establishment of the target cell identity, it is essential to identify and assess molecular reprogramming barriers. Research at the MDC-BIMSB with the nematode C. elegans as an in vivo model organism contributed to the identification of evolutionarily conserved molecular barriers, thereby contributing vital knowledge to improve reprograming for prospective tissue replacement therapies.

ELENA TORLAI TRIGLIA

Broad Institute, Cambridge, MA, USA

Identifying molecular determinants of cancer phenotypes with edited human cell models

DNA alterations can transform healthy cells into tumors. Cancer patients bear heterogeneous combinations of mutations, which drive the diversity of their disease manifestations. The ability to causally link mutations to their consequences on cell states, and disease-relevant phenotypes, will significantly improve our ability to tailor therapies to each patient. However, for most tumor types, we do not know the precise effects mutations have on cell and tissue behaviors. I will show how we leveraged genome editing, and the fitness advantage provided by cancer-driver mutations, to generate human cell models to identify genotype-to-phenotype connections in cancer (Hodis*, Torlai Triglia* et al., 2022, *co-first authors). Starting from primary healthy human melanocytes, the cell of origin of melanoma, we sequentially disrupted pathways dysregulated in patients: RB, MAPK, telomerase, PI3K/AKT, p53, and Wnt. We characterized these models in vitro and in vivo, using physiological assessment, histopathology, and single-cell RNA-seq, and leveraging computational methods and machine learning. Through these approaches, we linked melanocyte genotypes to gene expression programs, replicative immortality, malignancy, metastatic abilities, and histopathological appearance. We showed that these models approximate defining features of melanoma while allowing experimentally-tractable studies of causal relationships between mutations and disease-relevant phenotypes.

LEA H. GREGERSEN

University of Copenhagen, Denmark

Oxidative stress triggers a unique and transient dynamic transcriptional response

Proper control of transcription is required for accurate gene expression. While it is well known that cellular stress leads to activation of stress response genes, it is less appreciated that cellular stress also can promote a global repression of transcription. In particular, the transcriptional response to oxidative stress is poorly understood. Through transcription activity profiling, we uncover a rapid and widespread reduction of global transcription following oxidative stress. Interestingly, this is governed by mechanisms different from the transcriptional response to heat shock or UV irradiation. By monitoring total RNAPII occupancy we established that transient oxidative stress arrests the progression of RNAPII within few minutes regardless of its position in the gene. Remarkably, upon recovery, nascent transcription activity is rapidly restored, while transcription termination is severally inhibited. Together, our data show that overall transcription dynamics is affected by oxidative stress and promise insight into a unique and transient remodeling of transcription in response to cellular stress.

MATHIAS MUNSCHAUER

Helmholtz Institute for RNA-based Infection Research, Würzburg, Germany

The host protein SND1 binds SARS-CoV-2 negative-sense RNA and promotes viral RNA synthesis

Regulation of viral RNA biogenesis is fundamental to productive SARS-CoV-2 infection. To characterize host RNA-binding proteins involved in this process, we biochemically identified and characterized proteins bound to genomic and subgenomic SARS-CoV-2 RNAs. We find that the host protein SND1 specifically binds to the 5 -end of negative-sense viral RNA and is required for SARS-CoV-2 RNA synthesis. SND1-depleted cells form smaller replication organelles and display diminished virus growth kinetics. We discover that NSP9, a viral RNA-binding protein and direct SND1 interaction partner is covalently linked to the 5 -ends of positive and negative-sense RNAs produced during infection. These linkages occur at replication-transcription initiation sites, consistent with NSP9 priming viral RNA synthesis. Mechanistically, SND1 remodels NSP9 occupancy and alters the covalent linkage of NSP9 to the initiating nucleotides in viral RNA. Our findings implicate NSP9 in the initiation of SARS-CoV-2 RNA synthesis and unravel an unsuspected role of a cellular protein in orchestrating viral RNA production.

DOMINIC GRÜN

Abdelrahman Mahmoud[1], **DOMINIC GRÜN**[1]

[1]Würzburg Institute of Systems Immunology, Max Planck Research Group at the Julius-Maximilians-Universität Würzburg, Germany

Reconstructing cell fate decision in time and space

Diseases can be conceived as deviations from healthy cell state manifolds. Numerous methods leverage the power of single-cell RNA-sequencing for reconstructing cell state trajectories. Such approaches generally rely on sufficient sampling of cell states covering the entire trajectory. Since patients usually undergo treatment only after symptoms have already manifested, clinical samples covering intermediate disease states are generally not available. Reconstruction of missing states on disease trajectories is a major open challenge. To overcome the limitations of current approaches, we developed Cell-DRL, a deep reinforcement learning agent, that generates actions in gene expression space and learns stochastic policies to reconstruct trajectories connecting two anchoring cellular states. We validate CELL-DRL on ground truths scenarios by hiding intermediate states, and demonstrate the capacity to reconstruct multipotent hematopoietic stem cell states connecting distinct lineage-specific progenitors. We showcase the power of Cell-DRL to recover unseen states in healthy as well as disease scenarios at the individual patient level. Furthermore, Cell-DRL predicts a novel human cardiac fibroblast-to-cardiomyocyte trans-differentiation path. We expect that Cell-DRL will be crucial to gain valuable mechanistic insights into the development and progression of diseases at high temporal resolution.

TILL SCHWÄMMLE

TILL SCHWÄMMLE[1,*], Gemma Noviello[1], Alexandra Martitz[1], Vivi Feng[1], Jade Scouarnec[1], and Edda Schulz[1]

[1]Max Planck Institute for Molecular Genetics

*Presenting author

Zic3 and Otx2 link Xist activation to the formative pluripotency network

Xist, the master regulator of X-chromosome inactivation (XCI) in mammals, is upregulated in the epiblast during the formative phase of pluripotency, specifically in females. In the past, its regulation has been tightly linked to repression during naive pluripotency. However the identity of activators at the onset of XCI remains unknown. To fill this gap, we perform a comprehensive CRISPR screen targeting transcription factors during the early differentiation of female mouse embryonic stem cells. We uncover two groups of regulators: Priming and boosting factors. Priming factors, including the Xlinked transcription factor ZIC3, are transiently upregulated prior to full Xist activation. Since many of these factors are more highly expressed in XX cells, we suggest this priming step might be necessary to restrict Xist expression to females. Boosting factors, including the key regulator of the epiblast OTX2, drive high transcript levels following Xist activation. Subsequently, we use a series of CRISPR screens targeting individual reporter constructs in order to functionally investigate the effect of priming and boosting factors on the cis-regulatory landscape of Xist. With this study, we provide a systems level view of the trans- and cis-regulatory network that links Xist activation to formative pluripotency and ensures female-specificity.

LISA BUCHAUER

LISA BUCHAUER[1, 2,*], Yotam Harnik[2], Shani Ben-Moshe[2], Inna Averbukh[2], Yishai Levin[2], Alon Savidor[2], Raya Eilam[2], Andreas E. Moor[3], and Shalev Itzkovitz[2]

[1]Charité Universitätsmedizin Berlin

[2]Weizmann Institute of Science

[3]ETH Zürich

*Presenting author

Spatial discordances between mRNAs and proteins in the intestinal epithelium

The use of transcriptomes as reliable proxies for cellular proteomes is controversial. In the small intestine, enterocytes operate for 4 days as they migrate along villi, which are highly graded microenvironments. Spatial transcriptomics have demonstrated profound zonation in enterocyte gene expression, but how this variability translates to protein content is unclear. Here we show that enterocyte proteins and messenger RNAs along the villus axis are zonated, yet often spatially discordant. Using spatial sorting with zonated surface markers, together with a Bayesian approach to infer protein translation and degradation rates from the combined spatial profiles, we find that, while many genes exhibit proteins zonated toward the villus tip, mRNA is zonated toward the villus bottom.

Finally, we demonstrate that space-independent protein synthesis delays can explain many of the mRNA–protein discordances. Our work provides a proteomic spatial blueprint of the intestinal epithelium, highlighting the importance of protein measurements for inferring cell states in tissues that operate outside of steady state.

GENE YEO

University of California, San Diego, CA, USA

RNA binding protein networks in health and disease

PATRICK CRAMER

Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany

New insights into transcription regulation

TANCREDI MASSIMO PENTIMALLI

TANCREDI MASSIMO PENTIMALLI[1,*] , Simon Schallenberg[2], Daniel León-Periñán[1], Ivano Legnini[1,3], Ilan Theurillat[1], Gwendolin Thomas[1], Anastasiya Boltengagen[1], Sonja Fritzsche[4], Jose Nimo[4], Lukas Ruff[5], Gabriel Dernbach[2,5], Philipp Jurmeister[6], Sarah Murphy[7], Mark T Gregory[7], Yan Liang[7], Michelangelo Cordenonsi[8], Stefano Piccolo[8,9], Fabian Coscia[4],Andrew Woehler[10], Nikos Karaiskos[1], Frederick Klauschen[2, 5], and Nikolaus Rajewsky[1]

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[2]Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität Berlin, Berlin, Germany

[3]Human Technopole, Milan, Italy

[4]Max-Delbrück-Centrum for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany

[5]Aignostics GmbH, Berlin, Germany

[6]Ludwig-Maximilians-Universität, Munich, Germany

[7]NanoString® Technologies, Inc., Seattle, WA, USA

[8]University of Padua, Padua, Italy

[9]IFOM ETS, the AIRC Institute of Molecular Oncology, Milan, Italy

[10]Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine,

*Presenting author

3D molecular reconstruction of a human tumor at single-cell resolution reveals invasion dynamics and predicts mechanism-based, personalized therapeutic targets

Solid tumors are complex, three-dimensional (3D) tissues shaped by the cross-talk between malignant, stromal and immune cells. However, while cells live and interact in 3D cellular neighborhoods, histology and spatial omics mostly focus on 2D tissue sections. Here we present the first 3D molecular reconstruction of a human tumor (non-small cell lung cancer) by combining the in situ quantification of 960 cancer-related genes across ~340,000 cells (CosMx) with measurements of tissue-mechanical components.

The unbiased analysis of 3D cellular neighborhoods revealed the organisation of the tumor microenvironment in 10 distinct tumor, stromal, and immune multicellular niches. Multicellular niches enabled the identification of tumor cells infiltrating beyond the tumor surface, revealing the spatio-temporal dynamics of tumor invasion. Interestingly, pro-invasive epithelial-to-mesenchymal (EMT)

already occurred in one region at the tumor surface, where myofibroblasts and M2 macrophages specifically co-localized with pre-invasive tumor cells and their combined molecular signature predicted poor patient survival. On the other hand, cytotoxic T-cells did not infiltrate this niche but colocalized with inhibitory dendritic and regulatory T cells. Leveraging the sensitive detection of receptor and ligand expression, we systematically scored cell-cell interactions in 3D neighborhoods and identified which signalling axes orchestrated tumor invasion, T cell recruitment and immune escape.

Compared to 2D, 3D neighborhoods improved the characterization of immune niches by identifying dendritic niches, capturing the 3D extension of T-cell niches and boosting the quantification of nichespecific cell-cell interactions, including druggable immune checkpoints.

Overall, we provide the proof-of-principle for the computational reconstruction and systematic molecular exploration of 3D cellular neighborhoods, leading to the identification of targetable molecular mechanisms active in the patient under study. We envision that 3D molecular pathology will inform large-scale clinical studies aimed at assessing the benefit of mechanism-based, personalized, combination therapies in the immuno-oncology era.

MIKAELA BEHM

MIKAELA BEHM[1,*], Pablo Baeza Centurión[1], Luca Penso-Dolfin[1], Veronica Busa[1], Sylvain Delaunay[1], Francisco Javier Botey Bataller[1], Nick Hirschmüller[1], Marie-Luise Koch[1], Stefania Del Prete[1], Daniela Sohn[1], Celine Reifenberg[1], Meike Schopp[1], Fritjof Lammers[1], Llorenç Solé-Boldo[1], Jeleana Dutton[2], Sabine Begall[3], Walid Khaled[2], Ewan St. J. Smith[2], Duncan Odom[1], Michaela Frye[1], and Angela Goncalves[1]

[1]DKFZ

[2]University of Cambridge

[3]University of Duisburg- Essen

*Presenting author

An interactive cellular ecosystem blocks epithelial transformation in naked mole-rat

The mechanisms underlying cancer resistance in long-lived organisms are largely unexplored. Here, we report how naked mole-rats (Heterocephalus glaber) resist tumorigenesis when exposed to potent skin carcinogens. Single-cell transcriptional analysis of homeostatic full-thickness naked molerat skin revealed higher epidermal turn-over, a distinct composition of dermal extracellular matrix (ECM) and fewer resident T-cells, when compared to mouse and human. Carcinogenic treatment of skin generated the expected mutational landscape, but unlike in the mouse, failed to stimulate Tcells or generate tumours. Instead, naked mole-rat accelerated epidermal turnover to shed mutant cells. At the same time, the ECM was extensively remodelled via enhanced anti-inflammatory communication among fibroblasts and between fibroblasts and epidermal cells, creating a tumoursuppressive microenvironment. We demonstrate that, in contrast to mouse, naked mole-rat dermal fibroblasts lack intrinsic tumour-promoting capabilities in vitro and in vivo. Our results showcase that the cellular ecosystem of naked mole-rat skin actively suppresses epithelial transformation.

STEPHAN PREIBISCH

HHMI, Janelia Research Campus, Ashburn, VA, USA

Peta-scale microscopy – challenges, solutions and possibilities

Modern developmental biology increasingly relies on large, high-resolution datasets that for instance enable connectomics studies or allow to investigate molecular mechanisms in the context of entire organs and organisms. Creating such datasets requires powerful microscopy setups combined with state-of-the-art computational methods for data handling, reconstruction, and analysis. In this talk, we will cover two large-scale projects that highlight the challenges, solutions, and possibilities for the emerging field of peta-scale microscopy.

Focused ion beam scanning electron microscopy (FIB-SEM) can be used to image very large samples such as an entire Drosophila central nervous system (CNS) at an isotropic resolution of 8nm, as necessary for the reconstruction of the connectome, the map of all neural connections. We have pioneered a pipeline that performs image stitching using distributed global optimization based on machine learning and computer vision techniques to assemble a complete dataset of the Drosophila CNS. Following neuron segmentation using flood-filling machine learning networks, the final connectome is extracted using substantial manual proofreading. Currently, we are adapting the pipeline for novel Multi-SEMs developed by the Hess lab to eventually scale up to the mouse connectome, for which manual proofreading of segmentation results is likely to be the biggest challenge. We will thus highlight our current efforts at developing graph neural network-based approaches to significantly reduce manual proofreading effort.

Lightsheet microscopy is another emerging technology that now enables imaging of very large, fixed samples such as adult mouse brains at single-cell and single-molecule resolution. Previously, we developed the BigStitcher software that efficiently handles and interactively reconstructs large lightsheet acquisitions up to the terabyte range. However, new types of image acquisitions use modes such as stage-scanning lightsheet microscopy on expanded tissues of mice, monkey and eventually human samples, and thus pose new challenges in terms of dataset size and the complexity of the reconstruction process. We will present our newest solutions for efficiently handling, aligning, and interactively visualizing, as well as performing accurate single-molecule spot detection in peta-scale lightsheet datasets.

NADA JABADO

McGill University Health Centre, Montreal, Canada

Oncohistone in disease: from cancer to neurodegeneration and beyond

Since the discovery of recurrent mutations in histone H3 variants in pediatric brain tumours, so-called 'oncohistones' have been identified in various cancers. While their mechanism of action remains under active investigation, several studies have shed light on how they promote genome-wide epigenetic perturbations. These findings converge on altered post-translational modifications on two key lysine (K) residues of the H3 tail, K27 and K36, which regulate several cellular processes, including those linked to cell differentiation during development. We will review how these oncohistones affect the methylation of cognate residues, but also disrupt the distribution of opposing chromatin marks, creating genome-wide epigenetic changes which participate in the oncogenic process. Ultimately, tumorigenesis is promoted through the maintenance of a progenitor state at the expense of differentiation in defined cellular and developmental contexts. As these epigenetic disruptions are reversible, improved understanding of oncohistone pathogenicity can result in needed alternative therapies.

MAX TRAUERNICHT

MAX TRAUERNICHT[1, 2,*] , Teodora Filipovska[1, 2,] and Bas van Steensel[1, 2]

[1]Netherlands Cancer Institute

[2]Oncode Institute

*Presenting author

Multiplexed detection of transcription factor activity

Transcription factors (TFs) are proteins that control the activity of the ~25,000 genes in our genome by binding to specific DNA sequences nearby these genes. Typically, in any given cell type, dozens of TFs act in concert to control the expression of the genome. Despite their considerable importance in determining cell identity and their pivotal role in numerous disorders including cancer, we currently lack simple tools to directly measure the activity of many TFs in parallel. If we would know the precise activity of all TFs in any given cell type, we might be able to understand how TFs interpret incoming signals and mediate downstream changes in gene expression over time, which is crucial to conceptualize regulatory mechanisms underlying cell differentiation or pathologies.

Here, we use massively parallel reporter assays (MPRAs) to systematically optimize barcoded transcriptional reporters for 90 TFs. By probing thousands of *in silico* designed TF reporters, we have systematically identified and optimized critical design features. We show that many TFs require an optimized spacer length between TF binding sites, and that the reporter activity depends on the choice of minimal promoter and the distance between TF binding sites and minimal promoter. Moreover, we have extensively characterized TF reporter sensitivity and specificity by testing the reporter libraries across nine different cell types and upon down- or upregulation of most TFs and stimulation of dozens of signaling pathways. We have identified highly sensitive and specific reporters for at least 50 TFs, many of which are superior to commercially available TF reporters. Next, we aim to use this collection of barcoded TF reporters for accurate multiplexed detection of TF activity cascades during gastrulation and from single cells to characterize TF-mediated gene regulatory mechanisms during differentiation.

ANDRÉA WILLEMIN

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A GAM-based approach to explore the contribution of proteins or PTMs to 3D genome folding and cellular state transitions

Cell function and identity are controlled by fine-tuned gene regulatory networks, involving signalling cascades and transcription factors (TFs) that are tightly and dynamically interlinked with the threedimensional (3D) architecture of the genome at multiple length scales. A variety of important mechanisms have emerged, including chromatin contacts, loop extrusion, phase separation and association of genomic regions with specific nuclear microenvironments, that are orchestrated by various regulatory proteins and RNAs. Despite these advances, we still lack a quantitative understanding of how different molecular players cooperate to drive chromatin folding and their direct implications for gene regulation, their dynamics over time and cell type specificity. We have developed Genome Architecture Mapping (GAM), a high-throughput technique that quantifies multiple aspects of 3D genome folding by sequencing genomic DNA from ultrathin cryosections of cells or tissue samples. Importantly, RNAs and proteins are well preserved within the well-fixed thin cryosections used for GAM, enabling their simultaneous quantification with genomic DNA at single-cell resolution. Here, we develop Ab-GAM, a novel version of the GAM technology that simultaneously quantifies relative concentrations of regulatory proteins and/or their posttranslational modifications (PTMs) and maps 3D genome structure. To combine epitope (protein/PTM) measurements with GAM, we use immunodetection by sequencing, where oligoconjugated antibodies recognizing target epitopes are applied to cryosections followed by laser microdissection of single cellular slices, and parallel single-cell detection of genomic DNA and antibody-derived barcoded oligonucleotides. We show that antibody conjugates access and specifically recognize their targets inside GAM-grade slices, and that epitope levels can be quantified by converting antibody binding into DNA reads through sequencing of the oligos. Using Ab-GAM, we currently study

the dynamic changes in genome structure that accompany cell state transitions in populations of mouse embryonic stem cells, where changes in abundance of pluripotency TFs and PTMs of signalling proteins define functionally distinct cell states.

STEIN AERTS

KU Leuven Center for Human Genetics/VIB Center Disease Research, Belgium

Single-cell driven enhancer modelling and design

The combination of scRNA-seq and scATAC-seq allows building gene-regulatory atlases of any tissue and species. I will present several new computational strategies that exploit single-cell multi-omics data: (1) to model genomic enhancers using topic modelling and convolutional neural networks; and (2) to derive "enhancer-GRNs" (eGRN) with key transcription factors, genomic enhancers, and predicted target genes per cell type. I will discuss the results of several case studies where we applied these strategies, including the Drosophila brain, human melanoma, the mouse liver, and the evolution of cell types in the vertebrate telencephalon. Finally, I will discuss how enhancer models based on deep learning can be exploited to design synthetic enhancers for Drosophila and human cell types.

AMOS TANAY

Weizmann Institute of Science, Rehovot, Israel

Profiling and modelling the blood to understand disease progression

TUULI LAPPALAINEN

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Genetic effects on gene expression dosage underlying cellular and physiological phenotypes

Detailed characterization of molecular and cellular effects of genetic variants is essential for understanding biological processes that underlie genetic associations to disease. A particularly scalable approach has been linking genetic variants to effects in the transcriptome, which is amenable for scalable measurements in human populations and in model systems, including at the single cell level. Here, I will describe recent advances in our long-term work to characterize genetic associations to the transcriptome and other molecular traits, as well as our recent work on CRISPR-based perturbation of gene expression levels in cellular models. Altogether, integrating insights from these diverse approaches uncovers functional genetic architecture of human traits and the molecular and cellular mechanisms that mediate these effects.

SARA A. WICKSTRÖM

Max Planck Institute for Molecular Biomedicine, Muenster, Germany

Coordination of cell states and tissue architecture by mechanical forces

ABSTRACT: The structure of tissues is tightly linked to their function. During formation of functional organs, large-scale changes in tissue elongation, stretching, compression, folding/buckling, and budding impact the shape, position, packing, and contractility state of cells. Conversely, changes in single cell contractility, shape and position locally alter tissue organization and mechanics. Thus, forces function as important ques that are transmitted to the nucleus to coordinate gene expression programs to control cell states. On the other hand, excessive mechanical stresses have the potential to damage cells and tissues. In my presentation I will discuss our recent research on how cells use the nucleus and the nuclear envelope/chromatin interface to sense mechanical forces and how these mechanosignals are integrated with biochemical inputs to alter cell states and to generate and maintain tissue architecture.

POSTER ABSTRACTS

LOGICAL MODELING OF SIGNALING MECHANISMS IN EARLY NERVOUS SYSTEM DEVELOPMENT

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Nervous system development is a process by which neural stem cells originate from the embryonic neuroectoderm and specialize into diverse neuronal and glial cell types. The generation of distinct neural cell types is tightly regulated and governed by the interpretation of spatial and temporal regulatory signals. Neurogenesis in the Drosophila embryo has been a valuable model in understanding mechanisms of neural development, as most developmental mechanisms such as neuroectoderm patterning are highly conserved. Although specific markers of differentiating neural stem cells have been studied, the underlying signaling mechanisms driving cell type specification remain poorly understood. This project investigates the regulatory mechanisms contributing to cellular diversity by developing a logical model of the main signaling pathways and marker genes in Drosophila. Designing the model as an in silico representation of nervous system development in the Drosophila embryo enabled comprehensive exploration of the regulatory mechanisms in wild-type and perturbed conditions. Here we utilize single-cell RNA-seq analysis of early delaminating neuroblasts to identify unique neuroblast identities and their specific gene e pression patterns. By capturing the transcriptomic landscape of individual cells, this analysis has provided remarkable insights into cell type-specific regulatory networks. I now attempt to integrate the cell type-specific regulons — identified by the data-driven regulatory network reconstruction method SCENIC — into the current logical model enabling further exploration of the inferred interactions.

ARE EXON-EXON JUNCTIONS EMERGING CIS-ELEMENTS TO RECRUIT REGULATORY RNA BINDING PROTEINS?

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Our current understanding of splice site recognition, spliceosome assembly and splicing catalysis mainly relies on in vitro splicing assays that employ in vitro transcription to produce simple, usually single intron pre-mRNA substrates. However, more recent studies have shown that splicing vastly occurs co-transcriptionally from yeast to mammalian cells. This means that actual pre-mRNA substrates in vivo are constantly changing: Not only is the nascent pre-mRNA growing in length, novel sequence elements emerge at the exon-exon junctions as exons are ligated together. Here we address the question whether splicing generates new cis-elements at exon-exon junctions through co-transcriptional splicing. These new sequence elements would be in a position to specifically recruit regulatory splicing factors to positively or negatively affect downstream splicing events. This hypothesis was tested via in vitro pulldown of a "post-splicing" SRSF7 RNA after incubation with nuclear extract. The protein interactome of the SRSF7 exonic junctions was determined via mass spectrometry and compared to the interactome of control SRSF7 RNAs lacking exon-exon junctions, revealing a distinct proteome of the "post-spliced" RNA and identifying candidate "feed forward" splicing factors. Complementary to these experiments, an in-silico analysis screening human exonexon junctions identified motifs for RNA binding proteins genome-wide. We are furthermore determining the consequences of exon-exon junction occupancy on the efficiency of downstream splicing, utilizing custom protocols for nascent RNA analysis. Splicing is a crucial part of gene expression and aberrations are often linked to disease. Our study helps to understand fundamental mechanism underlying its co-transcriptional regulation.

NEUROMUSCULAR ORGANOIDS AS A UNIQUE MODEL TO STUDY NEURO-MUSCULAR INJURY AND REGENERATION IN VITRO

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Spinal cord motor neurons innervate skeletal muscles and instruct body movement through formation of functional neuromuscular junctions (NMJs). The skeletal muscles and axons have remarkable capacities for regeneration after injury due to cross-talk between diverse cell types. Damage to muscle fibers and axons trigger an inflammatory response that activates satellite cells to exit quiescence, proliferate, and enter the myogenic differentiation program. In parallel, glial cells guide regenerating axons to their target myofibers and aid them in reforming NMJs. Still, the skeletal muscle loses its regenerative abilities in cases such as severe wounds, myopathies, and degenerative diseases. While significant progress has been made in recent years to engineer skeletal muscle tissue in vitro, no in vitro human injury models have been described which include myofibers, motor neurons and, by extension, functional NMJs. Here we show that our iPSC-derived neuromuscular organoid (NMO) offers a unique and dynamic model for studying neuromuscular development and the mechanisms of neuromuscular injury and regeneration. NMOs are characterized by the simultaneous development of spinal cord neurons and skeletal muscles that self-organize and form functional NMJs. Mechanical injury in NMOs is achieved by severing the muscle fibers and axons. Analysis of NMOs two days post injury revealed activation and proliferation of satellite cells, indicated by significantly increased numbers of PAX7+ and MYOD1+ cells, marking the initiation of myogenic regeneration. Additionally, injury resulted in a significant reduction in the number and size of NMJs. Strikingly, after two weeks the NMJs recovered to levels comparable to the pre-injury state. Moreover, in response to injury, macrophages (IBA-1+ cells) appeared in the muscle region of the NMOs. Collectively, our data illustrates that iPSC-derived NMOs can be used as a robust model to study neuromuscular injury. This model also opens up the opportunity to investigate the impaired muscle healing mechanisms in various myopathies.

CARDIOVASCULAR DISEASE BIOMARKERS DERIVED FROM CIRCULATING CELL-FREE DNA METHYLATION

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Acute coronary syndrome (ACS) remains a major cause of worldwide mortality. The syndrome occurs when blood flow to the heart muscle is decreased or blocked, causing muscle tissues to die or malfunction. There are three main types of ACS: Non-ST-elevation myocardial infarction, ST-elevation myocardial infarction, and unstable angina. The treatment depends on the type of ACS, and this is decided by a combination of clinical findings, such as electrocardiogram and plasma biomarkers. Circulating cell-free DNA (ccfDNA) is proposed as an additional marker for ACS since the damaged tissues can release DNA to the bloodstream. We used ccfDNA methylation profiles for differentiating between the ACS types and provided computational tools to repeat similar analysis for other diseases. We leveraged cell type specificity of DNA methylation to deconvolute the ccfDNA cell types of origin and to find methylation-based biomarkers that stratify patients. We identified hundreds of methylation markers associated with ACS types and validated them in an independent cohort. Many such markers were associated with genes involved in cardiovascular conditions and inflammation. ccfDNA methylation showed promise as a non-invasive diagnostic for acute coronary events. These methods are not limited to acute events, and may be used for chronic cardiovascular diseases as well.

MIR-7 CONTROLS GLUTAMATERGIC TRANSMISSION AND NEURONAL CONNECTIVITY IN A CDR1ASDEPENDENT MANNER

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The circular RNA (circRNA) Cdr1as is conserved across mammals and highly expressed in neurons, where it directly interacts with microRNA miR-7. However, the biological function of this interaction is unknown. Here, using primary cortical murine neurons, we demonstrate that stimulating neurons by sustained depolarization rapidly induced two-fold transcriptional up-regulation of Cdr1as and strong post-transcriptional stabilization of miR-7. Cdr1as loss caused doubling of glutamate release from stimulated synapses and increased frequency and duration of local neuronal bursts.

Moreover, periodicity of neuronal networks was increased and synchronicity was impaired. Strikingly, these effects were reverted by sustained expression of miR-7 which also cleared Cdr1as molecules from neuronal projections. Consistently, without Cdr1as, transcriptomic changes caused by miR-7 overexpression were stronger (including miR-7-targets down-regulation) and enriched in secretion/synaptic plasticity pathways. Altogether, our results suggest that in cortical neurons Cdr1as buffers miR-7 activity to control glutamatergic excitatory transmission and neuronal connectivity important for long-lasting synaptic adaptations.

ACCELERATING HIGH-THROUGHPUT CHARACTERIZATION OF REGULATORY VARIANTS WITH DEEP LEARNING

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The need for functional characterization of genetic variants residing in the non-coding genome has given rise to high throughput assays such as Massively Parallel Reporter Assays (MPRAs). As part of the NIH/NHGRI Impact of Genomic Variation on Function (IGVF) consortium, we measure the activities of regulatory elements and variants and validate their potential role in human diseases.

Currently, we characterize >170,000 regulatory variants and test their activity in diverse human celltypes using MPRA. It will help us to overcome a shortage of quantitative effect readouts, mostly limited to specific loci, or standing variation. The resulting dataset will provide an unbiased reference for training computational models on allelic variant effects, rather than only on element-level activity. For this purpose, we designed our MPRA library using a deep learning model with convolutional neural network (CNN) architecture and derived a set of genome-wide regulatory variants. Our selection of variants included high or low predicted activity as well as tissue-specific or agnostic predicted effects, by our model which was trained initially on DNA sequences of active and inactive chromatin accessibility from diverse human cell-types.

Assessing model predictions, we confirmed that rare variants have a higher predicted effect compared to common variants (MAF≥5%). With a library complexity of 40M constructs in our initial MPRA in HepG2, we observed high reproducibility between three replicates. Training our CNN model on functional activity for HepG2 yielded 30% improvement in variant effect prediction as compared to the state-of-the-art sequence based DNN models. We aim to further integrate additional functional data with the predictions and MPRAs in HEK293T, WTC11, and K562 cells to gain insight into tissue-specific effects. We believe that the models and datasets generated here will be an important puzzle piece in the genome-wide interpretation of functional variants.

ESTABLISHMENT OF EX-VIVO SLAM-SEQ FOR SINGLE-CELL AND SPATIAL TRANSCRIPTOMICS

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Gene expression is a process that is spatio-temporally regulated in tissue homeostasis, development, and disease. RNA sequencing (RNA-Seq) provides a multidimensional view of spatial and temporal organization, which is crucial in defining cellular biological functions, locations, and communication. Single-cell RNA-Seq (scRNA-Seq) quantifies the expression of thousands of genes from dissociated cells. Recently, Spatial Transcriptomics (ST) platforms provide visualization of expression directly in situ. While these methods allow for phenotyping cell-type spatial heterogeneity, they lack temporal resolution. Metabolic labeling-based RNA-Sequencing approaches (e.g. SLAM-Seq) could overcome these limitations.

In the past SLAM-Seq has rarely been used in more complex biological setups, such as ex vivo tissue cultures or animals. We now have established ex-vivo cultures of different organs, such as adult mouse brain and human primary colorectal cancer samples, for metabolic labeling of RNA with 4SU. Bulk-SLAM-Seq revealed that the approach worked in principle, with tissues showing different conversion rate distributions, likely reflecting different transcription dynamics. By optimizing these steps, we will plan to use SLAM-culture with scRNA-Seq and ST workflows effectively, to offer spatial and temporal mRNA expression information in the aforementioned tested tissues.

AUTOSOMAL DOMINANT TUBULO-INTERSTITIAL KIDNEY DISEASE

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Autosomal Dominant Tubulointerstitial Kidney disease (ADTKD) caused by pathogenic variants in Uromodulin (UMOD) is the 3rd most common cause of monogenic kidney disease in adults[1]. Dialysis and kidney transplantation, which are the only therapeutic options for subsequent end-stage-renaldisease, are associated with adverse health effects. Hence there is an urgent need for treatment alternatives. Recently developed CRISPR/Cas base editors[2], through high efficacy and easy programmability, represent a promising novel treatment strategy, as illustrated by currently ongoing clinical trials[3]. This project aims to establish renal base editing for the treatment of ADTKD. By employing base editing in engineered HEK293T reporter cell lines, we achieved successful correction of pathogenic UMOD variants. Systematic screening of suitable gRNAs and base editors resulted in editing efficiencies of up to ~75%. Additionally, we targeted UMOD splice sites for protein knockdown. Here, we observed a wide variety of editing efficiencies up to ~50 %. We will further validate our approach in primary mouse cells and evaluate its effectiveness in disease prevention and reversion in-vivo. To this end, we demonstrated local renal delivery of Adeno Associated Viruses in a reporter mouse model. We anticipate base editing to serve as an effective and innovative therapeutic approach for renal monogenic diseases.

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FUNCTIONAL GENETIC CHARACTERIZATION OF PRONEURAL-TO-MESENCHYMAL TRANSITION IN GLIOBLASTOMA

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Glioblastoma (GBM) is the most common and lethal primary brain tumor, with cellular heterogeneity being an enabling feature of its aggressiveness. GBM cell states span across two opposites, proneural and mesenchymal, associated with neural identity and astrocytic inflammatory response, respectively. However, the mechanisms governing proneural-to-mesenchymal transitions (PMT) remain unclear. Here, we employ a cellular model that undergoes PMT in relevant pathophysiological contexts, integrating cell state-specific synthetic reporters, genome-scale functional genetic screens and pathway analysis. Using genome-wide CRISPR-knockout screens, we identify both positive and negative regulators of PMT on a single gene level. Conversely, the convergence of CRISPR-activation screens and patients datasets allowed to identify known and novel bona fide GBM phenotypic drivers. Importantly, pathway analysis of the genome-wide screens led to the discovery of pharmacologic treatments that modulate GBM cell identity and response to its standard of care - concomitant Temozolomide and ionizing radiation treatments in vitro. Overall, our data provide proof of concept for the combination of advanced cell models for PMT, synthetic genetic tracing, and CRISPR/Cas9 screens to connect genetic and pharmacologic perturbations to cell fate decisions underpinning tumor heterogeneity and resistance to treatments.

POLARITYJAM: AN IMAGE ANALYSIS TOOLBOX FOR CELL POLARITY, JUNCTION AND MORPHOLOGY QUANTIFICATION

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Cellular polarity is important in many biological processes, from development to wound healing and angiogenesis. Fundamental processes of living cells such as cell migration, cell division and morphogenesis depend on prior polarisation and breaking of spatial symmetry. Spatial reorganisation of the plasma membrane, cytoskeleton, cell-cell junction or organelles is required to establish an axis of polarity with a clear direction, i.e. 'front and back', to guide directed processes. These processes require cells to adapt and respond to multiple and often conflicting cues from the environment.

We have developed the PolarityJaM package (https://polarityjam.readthedocs.io/) which aims to provide the user with a modern, versatile means of performing reproducible exploratory image analysis. The analysis can be roughly divided into three parts: (multi-channel) cell instance segmentation, feature extraction and exploratory analysis. Multi-channel segmentation is performed using Cellpose or Segment Anything Model (SAM); the user can specify a pre-trained model or provide their own. Based on the instance segmentation, single-cell features are extracted, including junctional properties, cell and organelle orientation or directed signalling gradients. The extracted features are used to automatically generate a range of publication-ready plots to visualise phenotypes such as collective orientation, tissue-wide size and shape variation.

At this stage of the pipeline, tabulated features per cell exist and can be uploaded to an R shiny application (www.polarityjam.com) where periodic features such as cell polarity can be measured and visualised. Circular statistics of cell polarity, including means and confidence intervals, comparative circular statistics, circular-linear and circular-circular correlation analysis, and spatial statistics can be calculated in the R shiny app. Metadata from all steps is saved with the visualisation output. The PolarityJaM package integrates these analyses in a reproducible way with a level of documentation that allows the user to analyse their data accurately and efficiently.

CRISPR-BASED DIAGNOSTICS

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CRISPR-based diagnostics enable sensitive and specific detection of nucleic acid-based biomarkers, and these methods have mainly been applied to detect pathogens. We show the potential of CRISPR-diagnostics for genotyping, by developing a multiplexed assay to detect two variants that occur in the APOL1 gene. These variants, termed G1 and G2, are found in individuals of recent African ancestry and result in an increased risk for kidney disease and a shortened allograft survival in kidney transplantation. Our assay discriminates between six APOL1 genotypes in a single reaction below 1 hour. We further adapted the assay for a lateral-flow based readout enabling greater pointof-care applicability.

UNDERSTANDING THE LINK BETWEEN ASD AND SLEEP DISTURBANCES BY THEIR EPIGENOMES

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Autism Spectrum Disorder (ASD) is often associated with various sleep problems which are positively correlated with the severity of ASD traits. However, the precise biological mechanisms,and whether the sleep problems contribute to the aetiology or the consequences of ASD remain elusive. Previous studies have revealed altered global levels of HDACs and HATs and changes in acetylations (H3K9ac, H4K12ac) upon sleep deprivation (SD). However, how ASD and SD affect epigenetic marks in CA1 remainsunclear.

We aim to quantitatively assess the global levels of epigenetic marks, including H3K9ac, H4K12ac, H3K27me3, and H3K27ac, through immunofluorescence and imaging, in pyramidal glutamatergic neurons of the CA1 in the murine hippocampus. To study the link between ASD and sleep problems, we use the Shank3ΔC mouse, a unique model for ASD that recapitulates the sleep disturbances observed in ASD patients, and compare it with sleep deprived mice (SD), with sleep deprived Shank3ΔC mutant mice and with a wild type control mice.

By integrating these results with multimodal data (GAM, scRNA and scATAC), we aim to gain a global understanding on the role and the effects of SD in ASD.

SUPER-ENHANCERS AND NEURONAL SIGNALLING GENES ASSOCIATED WITH ADDICTION FORM COMPLEX CHROMATIN INTERACTIONS IN DOPAMINERGIC NEURONS

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Chromatin organisation is a major contributor to regulation of cellular specificity and function. Different aspects of chromatin organisation change dynamically during cellular differentiation, in response to environment, and are often disrupted in a plethora of complex disorders. Nonetheless, the interplay between chromatin state, gene expression, and chromatin contacts, especially higher-order interactions or conformations, remains poorly understood, especially in post-mitotic cells such as neurons.

Genome Architecture Mapping (GAM) is a unique technology that allows mapping of complex chromatin interactions from intact nuclei, avoiding harsh chromatin extraction. Application of GAM in mouse embryonic stem cells (ESCs) identified higher-order chromatin interactions to be enriched for super-enhancers (SEs) and/or highly transcribed regions, suggesting a role of these interactions in regulation of gene expression. Here, we aimed to investigate further the role of complex chromatin interactions in post-mitotic cells by applying GAM in terminally differentiated dopaminergic neurons (DNs). We find that the most prominent three-way interactions occur predominantly between celltype specific SEs and/or expressed genes in both ESCs and DNs, but less frequently between SEs that are common to the two cell types. Three-way interactions found specifically in DNs involved neuronal signalling genes commonly affected in neurological disorders, suggesting a role of these interactions in disease mechanisms and regulation of neuronal activity.

Taken together, our work advances the understanding of the role of chromatin organization in regulation of gene expression in mouse ESCs and DNs, and provides a meaningful resource for further functional studies with potential for clinical applications.

EXTENSIVE 3D FOLDING VARIABILITY BETWEEN HOMOLOGOUS CHROMOSOMES IN MAMMALIAN CELLS

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Genetic variation associated with disease is often heterozygous and deregulates gene expression programs in the homologous chromosome where they reside. Understanding how heterozygous variants influence gene regulation through altered 3D genome folding remains challenging due to inefficient recovery of single nucleotide polymorphisms (SNPs) in ligation-based chromosome conformation capture methods, such as Hi-C. To overcome these limitations, we adopted Genome Architecture Mapping (GAM), a ligation-independent method that maps chromatin structure by sequencing the genomic content of a collection of nuclear slices. Here, we applied GAM to a hybrid mouse embryonic stem cell (ESC) line with high SNP density. Most genomic windows could be phased to the parental genomes, enabling the construction of haplotype-specific 3D structures with high sensitivity. We detected extensive parental-specific folding at all genomic length scales, in compartments, topologically associating domains (TADs) and long-range contacts at specific genomic loci. Genotype differences in 3D genome folding often coincided with allele-specific differences in gene expression, in association with diverse mechanisms of gene regulation, namely polycomb repression, loops, and proximity to lamina-associating domains (LADs).

METABOLIC ANALYSIS OF RESISTANCE TO CIS-PLATIN TREATMENT

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A project of the MASTARS consortium

Background: Head and neck squamous cell carcinoma (HNSCC) ranks as the sixth most common cancer worldwide. Cisplatin (CDDP) constitutes a pivotal element of HNSCC chemotherapy. It forges intra-strand crosslinks with purine bases, triggering DNA damage-induced apoptosis. Nevertheless, half of the patients exhibit no response to initial treatment, and the emergence of CDDP resistance is frequent. Therefore, our objective is to comprehend the molecular mechanisms of CDDP resistance at a metabolic level and identify predictive biomarkers to facilitate patient selection for specific therapies.

Methods: We employed CDDP-resistant and CDDP-sensitive subclones from an HNSCC cell culture model (FaDuRes and FaDuSens). Post CDDP treatment, we examined nucleotide levels, incorporation of 13C glucose-derived labels into metabolites of central carbon metabolism, and proteome data across four distinct subclones.

Results: Proteomic analysis reveals profound alterations in FaDuSens following CDDP treatment, whereas the proteomes of FaDuRes show diminished adaptability and appear less influenced by CDDP. FaDuRes exhibit heightened expression of glycolytic enzymes and regulators, accompanied by increased 13C-labeled intermediates of glycolysis and the TCA cycle. Nucleotide analysis discloses an overall decrease in nucleotide pools in FaDuSens upon CDDP treatment relative to the control. Conversely, thymidine-derived nucleotides increase following CDDP treatment in sensitive subclones and, to a lesser extent, in resistant subclones. Interestingly, extracellular nucleoside species mirror intracellular changes. Correspondingly, thymidylate synthetase and dihydrofolate reductase elevate with CDDP treatment in all subclones, while ribonucleotide reductase is elevated solely in FaDuRes. This corroborates earlier findings that thymidine nucleotides rise upon CDDP treatment, and only resistant subclones can sustain other deoxy-nucleotide levels. Building upon these results, we

tested metabolic inhibitors of nucleotide synthesis, glycolysis, and one-carbon metabolism for potential synergistic drug interactions. A subset of the chosen inhibitors effectively counteracted cisplatin resistance in the FaDu cell model.

Outlook: We intend to evaluate the effectiveness of co-treatment involving cisplatin and metabolic inhibitors in tumor models derived from patients. Furthermore, we will establish metabolic markers indicative of resistance to cisplatin treatment, which can be employed for clinical testing.

RETINOIC ACID-DEPENDENT SPATIOTEMPORAL 3D CHROMATIN ARCHITECTURE DURING ZEBRAFISH EMBRYONIC DEVELOPMENT USING NANOPORE ACCESSIBILITY C-WALKS

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Three-dimensional (3D) chromatin structure is essential for gene regulation and function. Many methods capturing 3D chromatin folding rely on PCR amplification and short read sequencing, resulting in amplification artifacts, low mappability in repetitive regions, and loss of endogenous DNA modification information. Furthermore, most of these methods do not measure more than one mode of genomic architecture. We have developed Nanopore Accessibility C-walks (NPAC-walks), a quantitative, tri-modal, long-read assay, providing information about multi-way chromosome confirmation, DNA methylation, and chromatin accessibility profiles in single molecules without PCR amplification. We benchmark this assay in human SH-SY5Y cells where it yields exceptional interaction data with high cis-trans ratios, read length, and concatemer order, while displaying nucleosome periodicity evident in DNA methylation and chromatin accessibility patterns around cis regulatory elements. We have applied our new assays to posterior and anterior zebrafish embryo dissections at several developmental stages, with and without blocking production of the key developmental morphogen, retinoic acid (RA), and will integrate the data with single-cell resolved multi-modal measurements to achieve unprecedented temporal and spatial resolution of in vivo, celltype specific, RA-dependent 3D genome organization and chromatin architecture.

RESOLVING GENE REGULATORY NETWORKS AFFECTED BY THE LOSS OF NPAS4L USING MULTI-OMICS AND SINGLE-MOLECULE SPATIAL MEASUREMENTS OF ZEBRAFISH EMBRYONIC TAILS.

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The npas4l gene has been established as a crucial transcriptional regulator during zebrafish vascular and hematopoietic development. Dissecting the gene regulatory networks (GRNs) involving Npas4l will shed light onto transcriptional regulatory mechanisms underlying tissue specification and differentiation. Our previous study using sci-ATAC-seq revealed unexpected changes in muscle and pronephros cell populations in cloche mutants, but the underlying GRNs were unclear. To achieve higher resolution, we now dissect the tails of 24 hpf zebrafish embryos in combination with cutting edge multi-modal single-cell assays to perform a genome-wide identification of the GRNs that change in response to the loss of Npas4l. While our results confirmed the loss of hematopoietic cells and the majority of endothelial cells in the mutants, high variability in cell-type content between replicates precluded statistical quantification of changes in cellular makeup between conditions. To overcome these limitations, we have turned to the 10x Genomics Xenium platform for single-molecule spatial imaging with the intention of constructing a custom probe panel based on differential expression analysis between and within single-cell clusters. We have therefor implemented an agarose preembedding strategy using custom 3D-printed molds to allow FFPE sectioning of many embryos simultaneously with near-perfect plane aligment and spatial single-molecule quantification of many sections simultaneously on a single Xenium slide. Through the combination of these cutting-edge assays we expect to achieve unprecedented spatial resolution of cellular and molecular consequences following loss of an essential, tissue-determining transcription factor during vertebrate embryonic development.

CELL-TYPE SPECIFIC PREDICTION OF RNA STABILITY FROM RNA-BINDING PROTEINS BINDING

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RNA-binding proteins (RBPs) influence many processes including stability, and translational activity of RNA. Combinatorial action of the RBPs bound to the same transcript are believed to determine the post-transcriptional properties of the mRNA. We compiled a set of matched stability and ribosome profiling datasets for four human cell lines, using existing data and performing metabolic labeling to determine RNA degradation rate in several human cell lines.

We then used machine learning methods to predict RNA degradation rate and translation level from RBP binding information, which comprised existing in vivo RBP binding datasets and computationally predicted RBP binding sites. We show that: 1. We can predict RNA degradation rate and translation level from RBP binding alone. 2. In vivo binding sites have higher importance for prediction compared to computationally predicted binding sites. 3. Model trained on one cell type can be applied to a different cell type. We further explore the feature importance of different RBPs for stability prediction in the context of differential stability of 3’UTR isoforms.

PROGRAMMED EXTRUSION OF BMP2+ SURFACE COLONOCYTES PROMOTES STROMAL REMODELING AND TISSUE REGENERATION

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The colon epithelium frequently incurs damage through toxic influences. Repair is rapid, mediated by cellular plasticity and acquisition of the highly proliferative regenerative state. Mechanisms that promote the regenerative state are not well understood. Here, we reveal that upon injury and subsequent inflammatory response IFN-γ drives widespread epithelial remodeling that includes uninjured compartments. IFN-γ promotes rapid apoptotic extrusion of BMP-2-expressing surface colonocytes. By contrast, crypt-base stem and progenitor cells are reprogrammed by IFN-γ into “preregenerative colonocytes”, a cell cycle-arrested state that shares features of colonocytes and transitamplifying cells. Unlike homeostatic colonocytes, these cells neither respond to nor produce BMP-2.

Meanwhile, the loss of BMP-producing surface cells causes a remodeling of the mesenchymal niche, inducing high expression of HGF, which releases the cell cycle arrest in the pre-regenerative colonocytes to promote regeneration. This mechanism of lineage replacement enables tissue-wide adaptation to injury and efficient repair.

MULTI-OMIC TRACING OF TUMORIGENESIS USING A MURINE MODEL OF TRIPLE NEGATIVE BREAST CANCER (TNBC)

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Among breast tumor subtypes, triple-negative breast cancer (TNBC) displays a particularly aggressive phenotype and a high rate of metastasis that result in a poor prognosis for the patients. Important abnormalities in TNBC include gain of function (GOF) mutations in TP53 and PIK3CA but also spurious activation of the WNT pathway that supports the maintenance of mammary stem cells. In this regard, we developed a murine model for TNBC by mammary-specific expression of the most common breast cancer GOF mutations in Trp53 and Pik3ca combined with over-activation of the WNT pathway.

The animals consistently develop multiple, rapidly growing tumors with different histologies. To trace the progressive remodelling of the tumor and its associated micro-environment we performed spatial and single-cell transcriptomic approaches at different malignant stages.

We identify distinct fibroblast and immune stromal niches that are physically associated to specific tumor state. Interestingly, different subclasses of CAFs (cancer associated fibroblasts) show exclusive localization in vicinity of advanced tumor ducts. Moreover, we could characterize the transition of healthy luminal cells towards a basal-like cancer state. In parallel, we derived an ex vivo organoid model from healthy and tumor breasts to disentangle cell-autonomous tumorigenic mechanisms from the influence of the micro-environment.

This study sheds light on the dynamic microenvironment and molecular dynamics of TNBC tumors. Altogether we anticipate these results to open new therapeutic avenues centered on the targeting of the micro-environment of TNBC.

ESTABLISHMENT OF COLON ASSEMBLOIDS TO STUDY EPITHELIAL-STROMAL INTERACTION

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The cellular organization of gastrointestinal crypts is orchestrated by different cells of the stromal niche but available in vitro models fail to fully recapitulate the interplay between epithelium and stroma. Here, we establish a colon assembloid system comprising the epithelium and diverse stromal cell subtypes. These assembloids recapitulate the development of mature crypts resembling in vivo cellular diversity and organization, including maintenance of a stem/progenitor cell compartment in the base and their maturation into secretory/absorptive cell types. This process is supported by self-organizing stromal cells around the crypts that resemble in vivo organization, with cell types that support stem cell turnover adjacent to the stem cell compartment. Assembloids that lack BMP receptors either in epithelial or stromal cells fail to undergo proper crypt formation. Our data highlight the crucial role of bidirectional signaling between epithelium and stroma, with BMP as a central determinant of compartmentalization along the crypt axis.

VOLTRON: A SPATIAL OMIC ANALYSIS TOOLBOX FOR MULTIOMIC INTEGRATION USING IMAGE REGISTRATION

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The introduction of “Spatial Transcriptomics” (ST) in 2016 and the large-scale utilization of fluorescence in situ hybridisation (FISH) techniques have motivated many to analyse omics data more in a spatially resolved manner. This has been followed by a rapid increase in the number of commercially available spatial omics instruments, such as Visium (10x), GeoMx (NanoString), PhenoCycler (Akoya Biosciences) etc., that introduced fine-tuned workflows capturing spatial omics profiles in diverse levels of resolutions (regions of interest, single cells, molecules etc.). Hence, there is a need for downstream computational analysis tools capable of investigating spatial datasets with multiple modalities and resolutions. Such platforms should provide workflows for spatially aware multiomic integration across tissue sections as well as data modalities. To this end, we have developed VoltRon (bioinformatics.mdc-berlin.de/VoltRon), a novel R package for spatial omics analysis with a unique data structure that accommodates spatial data readouts across many levels of resolutions and modalities including regions of interests (ROIs), spots, and even single cells. VoltRon accounts for spatial organization of tissue blocks (samples), layers (sections) and assays given a collection of spatial readouts and provides spatial data integration between these assays. An easy-touse computer vision toolbox, OpenCV, is fully embedded in VoltRon that allows users to seamlessly register spatial coordinates across layers for data/label transfer without the need for configuring externals tools such as Python or ImageJ/Fiji. VoltRon tutorials include (i) integration workflows across serial tissue sections, (ii) integration between spatial transcriptomics and scRNA datasets as well as (iii) multiple end-to-end analysis workflows for all spatial data modalities using the same API.

METABOLIC ENZYMES AS REGULATORS OF CHROMATIN AND GENE EXPRESSION

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Accurate control of metabolite concentration is essential for gene regulation. For example, the abundance of specific metabolites in the nucleus can control the activity of chromatin modifying enzymes by providing the required acetyl and methyl groups for histone modifications. It was long thought that metabolites passively diffuse across the cytoplasm, yet metabolic enzymes have also been found in the nucleus. Some of these enzymes interact with specific transcription factors and chromatin modifying enzymes and it has been suggested that they could be involved in the specific regulation of gene expression by locally affecting the metabolite pool. Here we explore metabolic enzyme localization and find that some enzymes show cell-to-cell variability in nuclear localization in a seemingly homogeneous population of cells. In cancer, single-cell variation in gene expression and the proteome is thought to contribute to cell survival and drug resistance. In this study, we aim to identify if the variability in nuclear localization of metabolic enzymes is linked to non-genetic cellular heterogeneity in gene expression and the proteome across cells. We further aim, by using genomics and proteomics tools, to uncover how metabolic enzymes affect gene regulation through their nuclear localization and thereby possibly enhance the proliferative capacity and survival of cancer cells.

USING TEMPORAL FLOW MODELS FOR DISSECTING EPIGENETIC FUNCTION AND INTERCELLULAR SIGNALING DURING EARLY EMBRYONIC DEVELOPMENT

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Mouse embryonic development is a canonical model system for studying mammalian cell fate acquisition. Recently, we introduced a temporal flow model for mouse gastrulation, inferring precise timing of differentiation flows and lineage specification dynamics over the embryonic transcriptional manifold. Here we present a general computational framework for inferring cellular differentiation trajectories from temporal single-cell atlases. We next describe how to use inferred trajectories for assessing the function of epigenetic gene regulation and inter-cellular signals in-vivo and ex-vivo.

First, we compare the lineage composition, developmental time and gene expression fidelity of embryos consisting entirely or partially of cells in which the TET DNA de-methylation machinery is eliminated. This analysis shows how mild intrinsic effects of TET in the epiblast and nascent mesoderm escalate to dramatic tissue level effects unless normal signaling from wild-type cells is available as well. As a second example we study the function of Bmp4 in both embryonic and extraembryonic tissues during gastrulation. We find that early Bmp4 signal from the extraembryonic ectoderm is important for both maintenance of extraembryonic tissues as well as endoderm/mesoderm bifurcation and specification of extraembryonic mesoderm progenies in the embryo proper. In contrast, later embryonic-derived Bmp4 balances the differentiation flux of extraembryonic mesoderm to allantois and primordial germ cells. Together, temporal atlases are emerging as essential tools for testing the impact of genes' perturbation on intra-cellular gene regulation and the subsequent intercellular, tissue-level effects.

UNRAVELLING THE CLONAL DYNAMICS OF SOMATIC MUTATIONS TO LEARN MECHANISMS OF TREATMENT RESISTANCE

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A wide range of somatic mutations have been implicated in the pathogenesis of human cancer, including single nucleotide variants (SNVs), small insertions and deletions (indels) and large-scale structural variations (SVs). While the mutational landscape is known to change during cancer progression, the complex interplay between the subclonal evolution of mutations and the selection pressures (e.g. the inflammatory environment and treatment exposure) acting on a tumour remains to be fully explored.

To decipher the role of somatic mutations in the initiation, evolution and treatment resistance of human cancer, we require methods that accurately identify all mutation classes at single-cell resolution. We are therefore developing computational workflows to characterise a sample's complete spectrum of somatic variation, which we will then use to study the temporal order of mutation acquisition and subsequent changes in prevalence as the clonal composition of a tumour evolves. We leverage data from Strand-seq: a haplotype-resolved, single cell DNA sequencing technique that has greater power to resolve somatic SVs than conventional methods, and are exploring the contribution of somatic SNVs found in both the nuclear and mitochondrial genomes.

Our preliminary results indicate that cancer subclones can be better resolved by integrating nuclear and mitochondrial DNA (mtDNA) SNVs into our SV-based phylogenies. For instance, a T-cell acute lymphoblastic lymphoma sample has somatic mtDNA mutations that are private to an aggressive chromothriptic subclone that emerged at relapse, which we will use to trace clonal lineages.

We will use these mutational profiles to unravel the phylogenetic relatedness of both subclones and individual cells, which will allow us to model the contribution of different mutation types to clonal architecture. By integrating new variant classes into our analyses, we expect to gain new insights into how the different disease-relevant selective pressures experienced by a tumour shape the somatic mutational landscape.

BIOELECTRONICS MEET NEURO-MUSCULAR ORGANOIDS: ENHANCING THE COMPLEXITY AND MATURATION OF ORGANOIDS

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In the Gouti Lab, we have successfully generated human neuromuscular organoids (NMOs), that capture key aspects of the neuromuscular system. Inspired by early embryonic developmental principles, we use human derived axial stem cells, namely neuromesodermal progenitors, to generate in parallel both spinal cord neural and mesodermal lineages, which interact and selforganise into complex NMOs, exhibiting contractile and electrophysiological activity driven by functionally and morphologically compliant neuromuscular junctions and central pattern generators. Similar to other organoid models and despite their promising features, NMOs do not reach adult stages of maturation, which is required to better understand developmental phenomena and to model neurodegenerative diseases that develop late in life, such as Amyotrophic Lateral Sclerosis.

Here, our aim is to establish a framework to accelerate and enhance the differentiation and maturation status of human NMOs to resemble the in vivo adult tissues. During embryonic development, movements are spontaneous. After birth, the neural circuits gradually mature, while supraspinal input is necessary to drive locomotor activity. To achieve such levels of neural circuit complexity and tissue maturation, we will employ a multidisciplinary approach, focusing on interfacing our NMOs with organic bioelectronic platforms as a means to integrate bioelectrical cues, which are known to play an essential role in development and maturation of tissues, but have, atleast until recently, been largely ignored by the stem cell and organoid community. Specifically, we will interface our NMOs with conformable bioelectronic devices to i)record, in real time, in situ and with high spatiotemporal resolution, NMO electrophysiological activity, and ii) deliver brain-like input via electrical stimulation to push NMO maturation status towards post-natal/adult stages. Upon validating our findings with cutting-edge technologies, including advanced microscopy, spatial transcriptomics and optogenetics, we will ultimately deliver a sophisticated framework for generating next-generation biohybrid organoids, as advanced tools for studying human physiology and disease.

THE TUMOUR MICROENVIRONMENT ALTERS THE DIFFERENTIATION OF GENOMICALLY NORMAL EPITHELIAL CELLS IN COLORECTAL CANCER

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Single-cell transcriptomics provides intricate insights into the cellular heterogeneity of tumours, necessitating clear delineation of admixed tumour and normal cell populations. While different genomics and transcriptomics methods have been used to identify malignant tumour cells, their applicabilities across diverse tumour subtypes and the characteristics of non-malignant normal cells require further exploration. Here, we used complementary tools based on copy number alterations (CNAs), single nucleotide somatic variants (SNVs), and gene expression subtypes of cells from colorectal cancer (CRC) tumour and matched normal samples. Our consensus calls for tumour cells with genomic alterations and genomically normal cells underscore the essential role of genomics data, e.g., for enhanced resolution in microsatellite instable (MSI) CRCs exhibiting minimal CNAs.

Moreover, we analysed epithelial cell type composition and differentiation states within genomically normal cells and compared to normal samples. Remarkably, genomically normal cells exhibit enriched intermediate cell states and a depletion of terminally differentiated cell. Our findings suggest that signals originating from the tumour microenvironment (TME) influence the developmental trajectory of neighbouring non-malignant cells. Investigation into intrinsic pathway activities and intercellular signalling will improve our understanding of cellular interactions in the TME.

DISSECTING IDP CONDENSATE ARCHITECTURE ACROSS SCALES

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Biomolecular condensates are widely considered as an important mode of subcellular organization in diverse biological contexts. Intrinsically disordered proteins (IDPs), in particular, feature extensively in many condensates and are thought to contribute to their formation via transient intermolecular contacts [1, 2]. At the same time, a multiscale picture of the spatial organization and dynamics of IDP condensates, connecting the conformational properties and interaction patterns encoded in the sequences of individual polypeptides with the features of the condensates they build, remains unclear. Here we present a newly derived fractal model that provides a quantitative link between the atomistic features of an IDP with the spatial organization of the biomolecular condensate it forms. The proposed model resonates with the emerging concepts of coupling between phase separation and percolation in condensate formation [3].

For an 80-aa IDP fragment of yeast transcriptional regulator Lge1 (Lge1(1-80)), we connect single and multi-copy, microsecond molecular dynamics simulations with the experimentally observed, micrometer-scale behavior of the protein's condensate [4]. Specifically, our formalism describes condensate architecture across length-scales as a function of protein valency and compactness. Importantly, simulation-derived fractal dimensions of condensates of Lge1(1-80) and its mutants are shown to match the in vitro observations. Finally, the formalism provides an atomistic model of a micrometer-size condensate starting from individual simulated protein conformers. The presented modeling framework enables a multiscale description of biomolecular condensates and embeds their study in a wider context of colloid self-organization, as further applied to study self-assembly of functionally important IDP fragments of FUS and Pol II CTD.

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SYSTEMS APPROACHES TO IDENTIFYING MOLECULAR PATHWAYS CONTROLLING ORGANO-TYPICAL VASCULARIZATION

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During evolution, higher vertebrates developed intricately branched vascular networks that perfuse organs according to a form follows function principle. Vascular endothelial growth factor (VEGF) and its receptors are key-regulators of developmental angiogenesis and branching remodeling in most organs including the nervous system. How this relatively limited set of molecules can account for the great diversity in organ vessel patterning is unknown but considered clinically relevant for designing organo-typical vascular therapies to treat neurodegenerative and cardiovascular diseases. Pathway analyses of single cell sequencing data sets obtained from WT, and zebrafish mutants with a VEGF gain of function scenario identified several pathways contributing to organo-typical vascularization.

In the context of the peripheral nervous system, we identified neural Apelin and VEGF to be required for precisely titrating VEGF signaling strength and angiogenesis of juxta-positioned venous endothelial cells at the neuro-vascular interface. Single cell sequencing of FACS sorted endothelial cells identified a specific subset of venous endothelial cells expressing the Apelin-receptor (aplnr) and VEGF receptor-2/Kdrl rendering them angiogenic when exposed to both VEGF and apelin. This subpopulation resides in the cardinal vein, and migrates against the direction of blood flow toward the neuro-vascular interface. Here, upon exposure to neural Apelin and VEGF, these venous cells generate spinal cord vessels. Mechanistically, Aplnr and Kdrl genetically interact to amplify VEGF signaling output and promote angiogenic cell behavior. We conclude that organo-typical vasculatures require a specialized endothelial cell population displaying genetic interaction between growth factor receptors promoting endothelial angio-potential specifically in response to a distinct combination of multiple tissue derived cues. This opens novel therapeutic avenues for interfering with vascular remodeling defects in multiple disease conditions.

GENERATING A HEMATOPOIETIC NICHE IN HUMAN FETAL LIVER-LIKE ORGANOIDS TO UNRAVEL THE MULTICELLULAR RESPONSIVENESS

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Background: Organ niches are specialized environments supporting cell growth, like the hematopoietic niche in the embryonic liver and bone marrow, that controls hematopoiesis via cell interactions and cytokine gradients. Human hematopoietic niche models could improve the development of therapies for leukemias, immune defects and hemoglobinopathoes, but complex cell interactions make their bioengineering challenging.
Aim: Create human fetal liver-like organoids (FLOs) with a functional hematopoietic nice from human induced pluripotent stem cells (iPSCs).
Methods: Our process involves developing 3D cell clusters that become hepatoblasts and stroma, forming a niche expressing crucial signals. We also co-differentiated hematopoietic progenitors (HPs) within these FLOs. We observed their self-sustained behavior with or without external cytokines, confirming the niche's functionality. To enhance hematopoiesis in highly hematopoietic FLOs (hFLOs), we devised a small molecule-cytokine mix. Our study used various methods, such as single-cell RNASequencing, human fetal liver atlas integration, FACS-based experiments, and immunofluorescence, to map the differentiation from FLOs to hFLOs and their interactions.
Results: HPs expressed CD34, RUNX1, HLF, and SPINK2 and self-sufficiently differentiated into erythromyeloid colonies even without our molecule-cytokine mix. FLO niche cells were vital, expressing EPO, CSF and other cytokines. Mesenchyme harboured Nestin+ pericytes, expressing CXCL12 and KITLG. Trajectory analysis from FLOs to hFLOs revealed emergence of 21 hematopoietic populations, known from the Fetal Liver Atlas. B-cell assay identified fetal B1-lymphocytes. hFLOs expanded HPs, macrophages, monocytes, and granulocytes that effectively responded to bacterial and lipotoxic stimuli, displaying phagocytosis and granulocyte expansion
Conclusion: We demonstrate the successful generation of a niche model to investigate human hematopoiesis within a complex–yet completely autologous–microenvironment. The spontaneous differentiation of hematopoietic progenitors in FLOs and the heterogenous hematopoietic populations within hFLOs supports the self- sufficiency and expandability of our model.

MODELLING VIRAL ENCEPHALITIS CAUSED BY HERPES SIMPLEX VIRUS 1 INFECTION IN CEREBRAL ORGANOIDS

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Understanding how the human brain functions in health and disease is one of the greatest challenges of modern science, yet hindered by limited availability of human samples and ethical restrictions. The three-dimensional human brain organoid model has emerged as a cutting-edge, genetically-tractable experimental system to study human brain development and function in health and disease. Here, I will present one exemplary project illustrating how brain organoids can be employed to model human brain infectious disease – viral encephalitis. Herpes simplex encephalitis (HSE) is a lifethreatening disease of the central nervous system caused by herpes simplex viruses (HSV). However, with the standard anti-viral treatment, most patients still experience various neurological sequelae. We employed human brain organoids to model acute HSV-1 infection in a complex neuronal tissue and preformed single- cell RNA sequencing, electrophysiology and imaging to characterize the molecular changes associated with HSV-1 infection. We observed strong perturbations of tissue integrity, neuronal function and cellular transcriptomes. Antiviral acyclovir treatment alone, which reflects clinical treatment, prevented viral replication, but did not rescue HSV-1-driven defects observed in organoids. Using organoid model, we further tested alternative treatments for the acute infection to improve current therapeutic strategies.

IDENTIFYING SHARED TRANSCRIPTIONAL SIGNATURES IN RESPONSE TO T2D-RELATED ENVIRONMENTAL STRESSORS IN MOUSE PANCREATIC ISLET BETA CELLS

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The endocrine pancreas plays a vital role in regulating glucose homeostasis through the secretion of metabolic hormones including insulin. In response to increased metabolic demand, β-cells can enhance insulin production to counter insulin resistance in target tissues. However, chronic exposure to cellular and molecular stressors leads to progressive β-cell dysfunction, resulting in glucose intolerance and type 2 diabetes (T2D). Diverse pre-clinical mouse models, each with strengths and weaknesses, have collectively proposed several mechanisms of β-cell failure. The advent of single-cell RNA sequencing has further enabled assessment of heterogeneity in this context, adding to the degree of study-to-study variation by identifying changes to different β-cell subsets during T2D. By jointly analyzing multiple datasets from several mouse models, we have compiled an atlas of pancreatic β-cells exposed to various environments mimicking the systemic defects of T2D. Integration of diverse datasets enabled simultaneous analysis by circumventing batch effects, thereby providing insights beyond individual studies. We integrated over 100,000 cells from seven datasets from models varying in body composition, glycemic status, and insulin demand. Through this atlas, we were able to identify an enriched "compensating" state associated with increased insulin demand per β-cell as well as a "stressed-immature" state enriched during hyperglycemia. Analysis of key drivers of variation across β-cells identified two phenotypic features related to workload and maturation state. While the "compensating" state exhibited a signature of increased β-cell workload and preservation of β-cell maturity, the "stressed-immature" state was associated with increased workload and loss of maturity. Altogether, these observations suggest that successful β-cell adaptation to increased insulin demand corresponds to preservation of β-cell maturity during enhanced workload. Identifying pathways linked to β-cell workload and maturity loss lays the basis for mechanistic studies, making this resource invaluable for understanding β-cell failure in T2D.

PROTEOGENOMIC LANDSCAPE OF MULTIPLE MYELOMA

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Multiple myeloma is a plasma cell malignancy of the bone marrow. Despite therapeutic advances, it remains incurable, and better risk stratification, as well as new therapies, is therefore highly needed.

We have analyzed samples from 138 patients with plasma cell malignancies within trials of the German Study Group Multiple Myeloma (DSMM) with available clinical data. TMT-based proteomics of multiple myeloma samples identified a total of 8,336 proteins and 25,131 phosphopeptides in at least half of the samples.

Based on the proteome data, we were able to identify the differentially expressed proteins for the main genetic subgroups. The correlation between protein and phosphopeptide levels with patient outcomes revealed a novel protein high-risk signature comprising eight proteins that exhibited a strong predictive value for both progression-free and overall survival in intensively treated patients, independent of classical cytogenetic-based risk stratification.

Our investigation of proteome profiles in multiple myeloma cells, compared to healthy plasma cells, and integration with functional genetic CRISPR screens in myeloma cell lines, led to the identification of specific proteins exclusive to malignant cells. Furthermore, through the integration of our data with the cancer cell surface atlas and leveraging single-cell analyses, we have identified surface proteins that are uniquely expressed in malignant cells. These surface proteins hold significant potential for the development of new targeted immunotherapies, such as CAR-T cell or T-cell engager approaches.

TENETA: PYTORCH FRAMEWORK FOR BIOLOGY-INFORMED DEEP LEARNING

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Machine learning models recognize complex patterns within high-dimensional datasets, outperforming handcrafted feature engineering and traditional statistical approaches. Despite its potential, it faces obstacles, such as demand for large labeled datasets, exposure to overfitting and challenges in interpreting the learned representations.

One way to overcome these challenges in the biomedical research domain is to infuse prior knowledge into the models. Using biological networks as structural hierarchical priors for the neural networks is a natural way of such integration, for instance, using gene regulatory networks for analyzing gene expression data or mapping genomic profiles to phenotypes using gene ontologies as priors.

Although such networks are readily used in biological research, it is hard to ascertain the validity of such an approach, and impossible to compare different models. Addressing this crucial gap, we have built Teneta, a PyTorch framework based. Teneta takes the biological knowledge expressed in the shape of a directed acyclic graph and converts it into a sparse feedforward model. The produced model can be integrated directly into the existing PyTorch-based training pipeline. A source graph used for model creation can be used as a backbone from which multiple variants of the same target model can be produced. For instance, available options are randomized sparse networks, having as many connections as the target model but randomly distributed or fully connected variants, having the same number of nodes as the target model but instead being oversaturated with connections not initially present in the source graph. Having full access to the model's internal parameters via a straightforward interface, Teneta enables fine-tuning of particular blocks or groups of parameters. In addition to the core functionality, Teneta includes minimal training pipelines for classification and regression tasks implemented in PyTorch-Ignite. Teneta model zoo includes models based on the Reactome pathway and Knowledge-primed neural networks.

LOGICAL DESIGN OF SYNTHETIC CIS-REGULATORY DNA FOR GENETIC TRACING OF CELL IDENTITIES AND STATE CHANGES

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Descriptive data are rapidly expanding in biomedical research. Instead, functional validation methods with sufficient complexity remain underdeveloped. Transcriptional reporters allow experimental characterization and manipulation of developmental and disease cell states, but their design lacks flexibility. Here, we report logical design of synthetic cis-regulatory DNA(LSD), a computational framework leveraging phenotypic biomarkers and trans-regulatory networks as input to design reporters marking the activity of selected cellular states and pathways. LSD uses bulk or single-cell biomarkers and a reference genome or custom cis-regulatory DNA datasets with user-defined boundary regions. By benchmarking validated reporters, we integrated LSD with a computational classifier to rank phenotypic specificity of putative cis-regulatory DNA. Experimentally, LSD-designed reporters targeting a wide range of cell states are functional without minimal promoters. In silico, an LSD-unsupervised mesenchymal glioblastoma reporter outperformed previously validated ones. In genome-scale CRISPRa screens, it discovered known and novel bona fide cell-state-drivers. Thus, LSD captures core principles of cis-regulation and is broadly applicable to studying complex cell states and mechanisms of transcriptional regulation.

DIFFERENCES IN THE QUANTIFICATION OF COMPLEX INTERACTIONS BETWEEN GAM AND HI-C MES CELL DATA

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Genome organization in 3D puts regulatory regions into physical contact with distant target genes and enables long-range gene regulation. Whereas Hi-C assesses genomic contacts by measuring the frequency of proximity ligation, Genome Architecture Mapping (GAM) extracts spatial information about 3D genome topology by sequencing the genomic content of hundreds to a few thousand ultrathin, randomly oriented nuclear slices. We have now developed a multiplexed version of GAM and compared GAM contacts from mouse embryonic stem cells to published Hi-C data from the same cell line.

We found that only one-third of the strongest contacts detected by GAM or Hi-C were shared. The strong contacts observed only in Hi-C often connected genomic regions with heterochromatic marks such as H3K9me3 and H3K20me3, whereas strong contacts observed only in GAM often connected euchromatic regions occupied by CTCF, transcription factors, active RNA polymerase II, and/or enhancers. In addition, many contacts that contain CTCF at both anchor points and combine CTCF–CTCF with enhancers or active marks are more strongly detected by GAM than Hi-C.

When counting the number of GAM-specific and Hi-C-specific along the linear genome, we observed GAM "hot spots", extended regions of increased GAM contacts missed by Hi-C. These regions were often associated with increased

gene density, transcriptional activity, presence of enhancer elements and super-enhancers. Since these features are often involved in higher-order chromatin interactions, we mined triplet contact data and found that complex contacts often form strong GAM contacts while Hi-C ligation events preferentially detect pairwise contacts and underestimate high-complexity contacts.

Our work highlights the importance of ligation-independent methods and emphasizes the relevance of higher-order contacts in understanding gene regulation and genetic disease.

ROLES OF UPSTREAM OPEN READING FRAMES IN EARLY ZEBRAFISH DEVELOPMENT

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Translation is a tightly regulated step in gene expression. After transcription, mRNA levels can partially explain protein variability in cells, proving that protein abundance is regulated at the level of translation. Translation initiation can be regulated through short sequence elements which are present in the 5' leader sequence of the transcripts, named upstream open reading frames (uORF). uORFs have a start codon from which translation can start rather than the initiation codon in the main open reading frame of the downstream coding gene. Thus, uORFs can modulate the translation of the main open reading frame.

During early development, tightly controlled genome regulation is necessary to determine the fate of non-specified cells. In addition to the regulation at the transcription level, translational control also affects the specification of multiple cell types.

CRISPR-based perturbation assays have become an essential tool for researchers to understand and study the function of genes and regulatory elements. To investigate the effects of uORFs in cell typespecification through gene regulation, in this project, we would like to establish an in vivo CRISPR screen in zebrafish embryos to functionally study the selected uORFs in the context of early development. As a first step, in order to choose candidate uORFs for the screen, we analyzed published zebrafish ribosome profiling data consisting of different embryonic developmental time points. Respectively, we identified genes with uORFs that might have essential roles in cell type specification. In particular, genes that are crucial in neural crest cell development appeared as a result of the analysis. Further, we design guide RNAs targeting candidate uORFs, deliver CRISPR components into the embryos, and observe possible phenotypic and molecular changes at the singlecell level. In conclusion, by combining ribosome profiling, in vivo CRISPR screening, and scRNA-seq, we aim to link uORF function and the regulation of cellular diversity in the post-transcriptional landscape.

FLEXYNESIS: A COMPREHENSIVE DEEP LEARNING FRAMEWORK FOR BULK MULTIOMICS INTEGRATION AND ANALYSIS FOR PRECISION ONCOLOGY

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Deep learning offers immense potential for the integration of multiomics datasets with various applications for precision oncology, yet many current methodologies suffer from lack of code transparency, absence of scalable features, and narrow task applicability. To address these limitations, we introduce Flexynesis, a versatile tool designed with pytorch and pytorch-lightning, packaged with Conda and Guix. This tool streamlines data processing, from removing uninformative features to normalization, enforcing structured data splitting, and ensuring rigorous model evaluation. Critical to its adaptability, Flexynesis offers unsupervised feature selection, flexible omics layer fusion, and scikit-optimize powered hyperparameter tuning. Users can choose from three distinct architectures – fully connected networks, variation autoencoders with MMD loss, and multitriplet networks, accommodating multiple target and batch variables. Each model is complemented with a straightforward input interface and standardized training, prediction, feature importance quantification, and sample embedding extraction methods, which makes it easy to incorporate into pipelines. Features such as on-the-fly task determination and compatibility with both regression and classification tasks accommodating multi-task prediction with a mixture of numerical/categorical outcome variables make Flexynesis user-friendly. We also develop an extensive benchmarking pipeline, showcasing the tool's capability across diverse real datasets. In future developments, we aim to incorporate into Flexynesis capabilities such as survival modeling, data augmentation techniques, model fine-tuning, and a wider range of network architectures.

THE IMPACT OF SLEEP DEPRIVATION ON GENOME REGULATION IN DORSAL CA1 REGION OF HIPPOCAMPUS

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Sleep deprivation (SD) has evolved as a major risk in the modern society and is known to be associated with many disorders and altered physiological responses. Prior work has identified extensive changes in gene expression in different brain regions upon SD, including hippocampus. Hippocampus is responsible for learning and memory and is particularly susceptible to SD. SD disrupts circadian rhythm which is, among others layers of control, regulated by genome topology.

Nevertheless, how SD affects 3D genome architecture and what effect it has on gene expression on a single cell level, is not yet understood. In this study, we mapped changes in chromatin topology upon sleep deprivation using genome architecture mapping (GAM), as well as in gene expression and chromatin accessibility using 10x multiome approach in pyramidal glutamatergic neurons, one of the most SD-affected cell types in the hippocampus. The results show extensive changes in genome architecture on multiple scales and an overall repression in gene expression. Amongst most affected genes are learning/memory-associate genes (such as Satb2 and Dcc), receptor genes (Grm5, Gabrg3, Gabr5, Grin2b), neurexin-family genes (Nrxn3, Nrxn1) and epigenetic regulators (Hdac9, Hdac4). Ourresult suggests a strong imbalance between excitation and inhibition (E-I) upon SD in all studied modalities. E-I imbalance is one of the key features of neurodevelopmental disorders and our results suggest that sleep problems might be contributing to their aetiology, in particular in case of autism spectrum disorder.

A GAM-BASED APPROACH TO EXPLORE THE CONTRIBUTION OF PROTEINS OR PTMS TO 3D GENOME FOLDING AND CELLULAR STATE TRANSITIONS

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Cell function and identity are controlled by fine-tuned gene regulatory networks, involving signalling cascades and transcription factors (TFs) that are tightly and dynamically interlinked with the threedimensional (3D) architecture of the genome at multiple length scales. A variety of important mechanisms have emerged, including chromatin contacts, loop extrusion, phase separation and association of genomic regions with specific nuclear microenvironments, that are orchestrated by various regulatory proteins and RNAs. Despite these advances, we still lack a quantitative understanding of how different molecular players cooperate to drive chromatin folding and their direct implications for gene regulation, their dynamics over time and cell type specificity. We have developed Genome Architecture Mapping (GAM), a high-throughput technique that quantifies multiple aspects of 3D genome folding by sequencing genomic DNA from ultrathin cryosections of cells or tissue samples. Importantly, RNAs and proteins are well preserved within the well-fixed thin cryosections used for GAM, enabling their simultaneous quantification with genomic DNA at single-cell resolution. Here, we develop Ab-GAM, a novel version of the GAM technology that simultaneously quantifies relative concentrations of regulatory proteins and/or their posttranslational modifications (PTMs) and maps 3D genome structure. To combine epitope (protein/PTM) measurements with GAM, we use immunodetection by sequencing, where oligoconjugated antibodies recognizing target epitopes are applied to cryosections followed by laser microdissection of single cellular slices, and parallel single-cell detection of genomic DNA and antibody-derived barcoded oligonucleotides. We show that antibody conjugates access and specifically recognize their targets inside GAM-grade slices, and that epitope levels can be quantified by converting antibody binding into DNA reads through sequencing of the oligos. Using Ab-GAM, we currently study

the dynamic changes in genome structure that accompany cell state transitions in populations of mouse embryonic stem cells, where changes in abundance of pluripotency TFs and PTMs of signalling proteins define functionally distinct cell states.

EPIGENETIC MECHANISMS OF INFORMATION STORAGE IN THE ONSET OF DRUG ADDICTION

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Dopaminergic neurons (DNs) are a highly heterogeneous cell type based on their physiology,mcircuitry, disease susceptibility, and transcriptome. DNs located in the Ventral Tegmental Area (VTA) of the midbrain are associated with addiction and are the first neurons in the brain to respond to a single dose of cocaine, leading to long-term potentiation. Gene expression and synaptic activity are reported to recover within the first 24h, but a memory of the first insult is thought to prevail, as a second exposure to cocaine leads to more expansive activation both in the midbrain and elsewhere in the brain. We have recently shown that VTA-DNs engage in highly cell-type specific threedimensional (3D) chromatin structures linked to specialized cell functions. We found that addictionassociated genes engage in specific 3D contacts enriched for activity of transcription factors highly expressed in DNs. To investigate whether one single dose of cocaine induces long-lasting changes to the epigenome and 3D genome structure of DNs, we produced ImmunoGAM maps of VTA-DNs of mice injected with saline or a single dose of cocaine 24h and 14d before tissue collection. We found extensive rewiring of 3D chromatin structures at 24h many of which remain altered at 14 days. To gain mechanistic insights into the chromatin structure alterations seen by 24h and understand the cascade of events that disturbs networks of regulatory sequences and genes, we have generated single-cell multiome (RNA-ATAC) datasets at different times after drug treatment (0.5, 1, 4, 8, 24 hours and 14 days). We extracted 2500 VTA-DNs containing all described VT-DN subtypes. Our current work explores the dynamic changes in gene expression and the regulatory landscape oftranscription factor binding to discover the underlying mechanisms governing the recovery from a single cocaine administration.

TOWARDS A QUANTITATIVE UNDERSTANDING OF CLONAL DYNAMICS AND CELL FATE IN HUMAN HEMATOPOIESIS

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Self-renewing hematopoietic stem cells (HSCs) are at the apex of a cellular differentiation hierarchy ultimately maintaining everyday blood production. However, we still lack a fundamental understanding of (individual) HSC clonal activity and its regulation in humans, in both homeostatic conditions and disease settings. Here, we leverage single-cell multi-omics to detect somatic mitochondrial DNA (mtDNA) mutations as naturally occurring clonal genetic markers to develop a more quantitative understanding of clonal population dynamics during hematopoietic differentiation, the underlying functional heterogeneity of HSCs, and their cell fate decisions. For example, we investigate these dynamics in i) healthy donors, ii) upon allogeneic and autologous hematopoietic stem cell transplantation, iii) in young cancer survivors exhibiting accelerated aging phenotypes and functional decline of the hematopoietic system due to exposure to radio-/chemotherapy, and iv) in elderly individuals with clonal hematopoiesis of indeterminate potential (CHIP), a condition dominated by the expansion of individual HSC clones and associated with increased mortality rate and elevated inflammation.

SINGLE-CELL MITOCHONDRIAL GENETICS – FROM MITOCHONDRIOPATHIES TO AGEING

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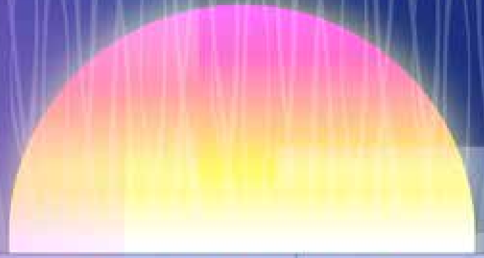
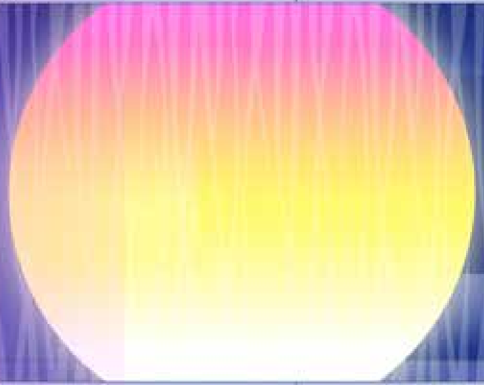
Mitochondria are central to metabolism and carry their own multi-copy number genome. Pathogenic mitochondrial DNA (mtDNA) mutations are the underlying cause for a group of disorders known as 'mitochondriopathies', affecting 1 in 4,300 individuals. However, there is a remarkable paucity of our understanding of the molecular and cellular consequences of altered mitochondrial genetic integrity in primary human cells. Utilizing single-cell multi-omics, we recently discovered the purifying selection of pathogenic mtDNA mutations in human T cell subsets in patients with mitochondriopathies, such as Pearson or MELAS syndrome, suggesting cell type-specific metabolic vulnerabilities arising over the course of attaining or maintaining specific T cell states. Notably, in a healthy donor, we also identified a synonymous mtDNA variant that has been negatively selected against in CD8+ T effector memory cells by impairing mitochondrial protein translation of the mitochondrial MTCO1 gene, highlighting major aspects of mitochondrial genetics that are not yet fully appreciated.

Finally, an increasing mtDNA mutational burden has been associated with ageing phenotypes. Currently, we are subjecting single-cell genomics methods to quantify the mtDNA mutational burden across different age groups and blood

cell types in humans and utilize genetically modified mice that acquire mtDNA mutations at a high rate, to investigate the impact of accumulated mitochondrial genetic variation on cellular and organismal phenotypes and pathologies associated with ageing.

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