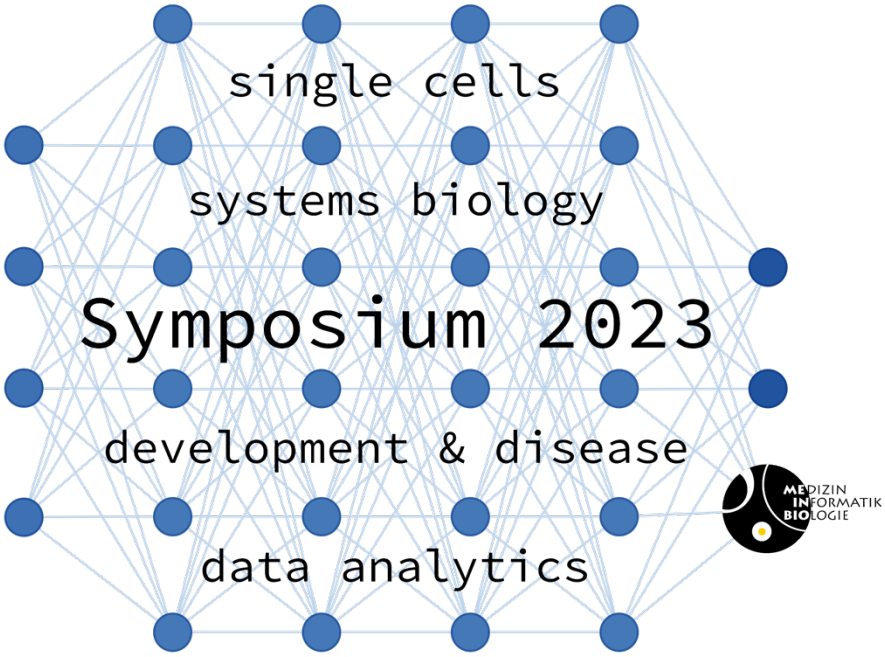

Symposium

Single cell, systems biology and data analytics approaches to understand cellular mechanisms in development and disease

Freiburg, Oct 04-06 2023

Programme

universität freiburg

Oct 04

afternoon

4:00 **registration**

4:30 **welcome note**: C. Fullio

4:40 **talk session I** – *chair I. Akol*

- 4:40 keynote: M.C. Gambetta, Lausanne:
  "Chromosome-scale organization of the regulatory genome in the fly central nervous system"
- 5:20 E. Lichtenberg, Freiburg:
  "The fate of mRNAs in Cyanobacteria: Subcellular localization of RNAs in Synechocystis"
- 5:40 L. Hemm, Freiburg:
  „Identification of RNA-based interactions and regulation in the cyanobacterial model Synechocystis sp. PCC6803"
- 6:00 F. Cardamone, Freiburg:
  "Cell type-specific chromatin states at regulatory regions instruct ZGA and cell fate specification"
- 6:20 Y. Teng, Freiburg:
  "Control of TGFβ1-induced pEMT and collective invasion of intestinal organoids by a Sox11 Pdgf signaling axis"

6:40 „Poster + beer": **poster session I** - odd poster numbers

Oct 05

morning

9:00 **talk session II** – *chair S. Jäger*

9:00 invited talk: M. Bartosovic, Stockholm:
"Multimodal single-cell profiling of three epigenetic modalities in the mouse brain by nanobody-based CUT&Tag"

9:30 C. Fullio, Freiburg:
"DOT1L-Mediated Basal Progenitor to Interneuron Signaling and Its Impact on Cerebral Cortex Development"

9:50 T. Hohl, Freiburg:
"The role of Dot1l in neuronal development – a single-cell story"

10:10 C. Alfonso-Gonzalez, Freiburg:
"Sites of transcription initiation drive mRNA isoform selection"

10:30 coffee break

10:50 **talk session III** – *chair A. Saenz Espinoza*

10:50 F. Zenk, Lausanne:
"Single-cell epigenomic reconstruction of developmental trajectories in human neural organoid systems"

11:10 I. Akol, Freiburg:
"Unraveling the Molecular Mechanisms of FOXG1: Insights into Transcriptional Control in Mice"

11:30 D. Armbruster, Freiburg:
"An anatomical and molecular characterization of subpallial dopaminergic neurons in zebrafish larvae"

11:50 F. Gather, Freiburg:
"The lincRNA Pantr1 is a critical mediator of dendritic outgrowth in a murine and human model of FOXG1 syndrome"

12:10 lunch break

afternoon

1:00 **poster session II -** even poster numbers

3:00 **talk session IV** – *chair: P. Bousounis*

3:00 invited talk: M. Schulz, Frankfurt:
"Computational methods for linking regulatory elements to target genes"

3:30 M. Hackenberg, Freiburg:
"Dimension reduction and temporal patterns in scRNA-seq data"

3:50 A. Ozier-Lafontaine, Nantes:
"Kernel-based testing for global comparison of single-cell RNA Sequencing datasets"

4:10 invited talk: S. Krishnaswamy, Yale (virtual):
"Multiscale diffusion geometry for (deep) learning of graphs, manifolds, and flows"

4:40 F. Barkmann. Zürich:
"CanSig: discovery of shared transcriptional states across cancer patients from single-cell RNA sequencing data"

Oct 06

Morning

9:00 **talk session V** – *chair: C. Fullio*

9:00 invited talk: F. Theis, Munich (virtual)
"Modeling single-cell dynamics and fate decisions across modalities, time and space"

9:30 C. Zechner, Dresden:
"Bayesian inference of chromatin looping dynamics from live-cell measurements"

10:00 P. Videm, Freiburg:
"Galaxy and single-cell developments"

10:20 M. Lange, Zürich:
"Unified fate mapping in multiview single-cell data"

10:40 coffee break

11:00 **talk session VI** – *chair: Y. Yu*

11:00 invited talk: J. Cosgrove, Paris:
"Fuelling Innate Immunity"

11:30 J.U. Verga, Galway:
"Identification of potential therapeutic targets to revert natural killer cell exhaustion in Multiple Myeloma through single-cell RNA-Seq analysis"

11:50 M.C. Romero Mulero, Freiburg:
"Mouse multipotent progenitor 5 cells are located at the interphase between hematopoietic stem and progenitor cells"

12:10 P. Bousounis, Freiburg:
"Deciphering the role of Mda5 in HSC aging"

12:30 E. Szegezdi, Galway
"HSC Interactome: An RShiny App for Exploring Cell-Cell Interactions Between Hematopoietic Stem Cells and Other Bone Marrow Cell Types"

12:50 N. Cabezas-Wallscheid, Freiburg
"Regulation of dormant hematopoietic stem cells"

1:10 **Concluding remarks**

Talk abstracts

Talk session I

Chromosome-scale organization of the regulatory genome in the fly central nervous system

Maria Cristina Gambetta
Center for Integrative Genomics, University of Lausanne

Topologically associating domains (TADs) have been determined as basic units of genome organization. We present evidence of a previously unreported level of genome folding, where distant TAD pairs, megabases apart, interact to form meta-domains. Within meta-domains, gene promoters and structural intergenic elements present in distant TADs are specifically paired. The associated genes are involved in axonal guidance and adhesion. These long-range associations occur in a large fraction of Drosophila neurons but support transcription in only a subset of neurons. Meta-domains are formed by diverse transcription factors that are able to pair over long and flexible distances. We present evidence that two such factors, GAF and CTCF, play direct roles in this process. The relative simplicity of higher-order meta-domain interactions in flies, compared with those previously described in mammals, allowed the demonstration that genomes can fold into highly specialized cell-type-specific scaffolds that enable megabase-scale regulatory associations.

The fate of mRNAs in Cyanobacteria: Subcellular localization of RNAs in Synechocystis

Elisabeth Lichtenberg[1], Dominik Rabsch[2], Annegret Wilde[1]
[1]Institute of Biology III, University of Freiburg, Freiburg, Germany,
[2]Bioinformatics Group, Department of Computer Science, University of Freiburg

RNAs are known to localize heterogeneously throughout eukaryotic cells. Here, different mechanisms involved in RNA-trafficking were discovered over the past few years. Special cis-acting sequential motifs determine the final RNA localization and further provide binding sites for RNA-binding proteins, necessary for RNA-transport. Although those processes were thought to solely occur in eukaryotes, recent evidence suggest that translation- independent, directed RNA-trafficking could be important for local regulation of gene expression in prokaryotes as well[1]. We aim to get a better understanding of RNA organization using two independent experimental approaches. The RNA-sequencing technique “RNA localisation-sequencing (Rloc-seq)” combining cell fractionation and RNA-sequencing and the visualization technique “fluorescence in situ hybridization (FISH)” combined with high-resolution microscopy[2,3]. Using Rloc-seq we further intent to identify different sequential or structural motifs responsible for directed RNA-transport. Current results show, that different transcripts encoding proteins involved in the photosynthesis accumulate at the thylakoid membrane in a translation- independent manner. However, not only the process of RNA-transportation plays an important role determining the fate of RNAs. Other players like RNA polymerase, RNA degradasomes and ribosomes could be involved in spatio-temporal RNA organization. Therefore, we try to unravel the fate of mRNAs in *Synechocystis* sp. PCC 6803 by investigating not only the subcellular RNA organization, but also RNaseE mediated RNA degradation occurring in a 5’-phosphorylation dependent manner[4].

references:

1. Irastortza-Olaziregi, M. & Amster-Choder, O. RNA localization in prokaryotes: Where, when, how, and why. Wiley Interdiscip. Rev. RNA 12, 1–27 (2021).
2. Mahbub, M. et al. mRNA localization, reaction centre biogenesis and thylakoid membrane targeting in cyanobacteria. Nat. Plants 6, 1179–1191 (2020).

3. Kannaiah, S., Livny, J. & Amster-Choder, O. Spatiotemporal Organization of the E. coli Transcriptome: Translation Independence and Engagement in Regulation. Mol. Cell 76, 574-589.e7 (2019).
4. Hoffmann, U. A. et al. Transcriptome-wide in vivo mapping of cleavage sites for the compact cyanobacterial ribonuclease E reveals insights into its function and substrate recognition. Nucleic Acids Res. 49, 13075–13091 (2021).

Identification of RNA-based interactions and regulation in the cyanobacterial model Synechocystis sp. PCC6803

Luisa Hemm, Wolfgang R. Hess
Institute of Biology III, University of Freiburg

Cyanobacteria are exposed to various changing environmental conditions, such as nutrient availability, temperature shifts and varying light intensities. In response to these changes, cyanobacteria have evolved different regulation mechanisms.
Various types of regulatory RNA molecules contribute to the regulation of gene expression in bacteria. One part of this RNA-based regulation is carried out by small noncoding RNAs (sRNAs) which frequently modulate the translation of their target mRNAs via complementary base pairing (1). Furthermore, it was shown that the localization of the mRNA in the cell also plays an important role for the protein expression and function of these proteins (2). Both of these mechanisms require assistance by RNA-binding proteins (RBPs). RBPs regulate a variety of processes in the bacterial cell, ranging from transcription termination and translation initiation to RNA decay (3). We contributed to work which showed that Rbp2 and Rbp3, belonging to a family of conserved cyanobacterial RBPs, are important for the correct localization of photosynthetic mRNAs, such as psbA and psaA, at the thylakoid membrane (4). This suggests that RNA binding proteins play an important role in the correct function of cellular mechanisms. Therefore, the in-depth characterization of these photosynthesis-relevant RBPs and the identification of new in the cyanobacterial model Synechocystis sp. PCC 6803 is being addressed in this study.
References:
1 Georg J, Hess WR. Microbiol Spectr. 2018 Jul 13;6(4):6.4.12.
2 Irastortza-Olaziregi M, Amster-Choder O. WIREs RNA. 2020;12(2):e1615.
3 Holmqvist E, Vogel J. Nat Rev Microbiol. 2018 (10):601–15.
4 Mahbub M, et al. Nat Plants. 2020 (9):1179–91.

Cell type-specific chromatin states at regulatory regions instruct ZGA and cell fate specification

Francesco Cardamone, Bastian Eichenberger, Annamaria Piva, Fides Zenk, Eva Löser, Yinxiu Zhan, Nicola Iovino
Max-Planck-Institute of Immunobiology and Epigenetics, Freiburg

The establishment of the different germ layers during early development is crucial to guarantee the correct propagation of cellular identities and body formation to adulthood. This is achieved, in part, through maternally inherited transcription factors, proteins and histone modifications. However, little is known about the conubium of events that link those different processes and drive the selective activation or repression of specific genes in a cell-type specific fashion. To address this, we simultaneously dissected the epigenetic and transcriptomic state of each single nucleus of wild-type, E(z) and CBP depleted Drosophila embryos at ZGA to elucidate the crosstalk between chromatin states, regulatory elements usage and cell fate specification. We propose that the early epigenetic landscape at regulatory regions is instructive for proper genome activation via two mechanisms: a

novel pre-zygotic H3K27me3-repressive action to prevent aberrant expression of germ layer-specific genes in other tissues, and a H3K27ac-active zygotic role to direct the cells from a pluripotent state towards proper germ layer specification.

Control of TGFβ1-induced pEMT and collective invasion of intestinal organoids by a Sox11 Pdgf signaling axis

Yu-Hsiang Teng[1,2,6], Bismark Appiah[2,5], Severin Dicks[2,5], Monika Schrempp[1], Kameron Hahn[1,6], Melanie Boerries[3,4,5,6], and Andreas Hecht[1,2,6]

[1] Institute of Molecular Medicine and Cell Research, Faculty of Medicine, University of Freiburg
[2] Faculty of Biology, University of Freiburg
[3] German Cancer Consortium (DKTK), Freiburg
[4] German Cancer Research Center (DKFZ), Heidelberg
[5] Institute of Medical Bioinformatics and Systems Medicine, Medical Center - University of Freiburg
[6] MeInBio DFG research training group, GRK2344

Epithelial-mesenchymal transition (EMT) is a cellular program implicated in facilitating cancer cell invasion and metastasis, which are the cause of most cancer-related deaths. Notably, many cancer cells undergoing EMT simultaneously exhibit epithelial and mesenchymal characteristics, which is referred to as partial EMT (pEMT). However, the mechanisms leading to pEMT phenotypes during cancer progression are not fully understood. To study this phenomenon, we employ genetically modified intestinal organoids with colorectal cancer driver mutations (Apc-/-, KrasG12D/+, Trp53R172H/+, TKA organoids) as a model system. TGFβ1-treated TKA organoids undergo pEMT and show a collective invasion behavior which is characterized by the maintenance of cell-cell adhesion and the formation of so-called leader cells located at the migratory front. Aiming to decipher the regulatory mechanisms controlling pEMT and collective invasion, we performed scRNA-seq of TKA organoids treated with TGF-β1 for different time periods. By Uniform Manifold Approximation and Projection (UMAP) visualization nine transcriptionally distinct cell clusters were identified. Calculation of EMT and leader cell scores based on the MSigDB EMT gene expression signature and a custom leader cell gene expression profile suggested cluster 2 cells to represent a leader cell population associated with the most advanced pEMT state. Analyses of transcription factor (TF) gene expression in bulk and scRNA-seq data sets identified Sox11 as the most highly upregulated TF in TGFβ1-treated TKA organoids with specific enrichment of Sox11 transcripts in cluster 2. Immunofluorescence analysis of epitope-tagged Sox11-HA expressed from the endogenous locus showed nuclear Sox11 to be highly expressed at the organoid migratory front upon TGFβ1 treatment, confirming that Sox11 is enriched in leader cells. CRISPR/Cas9-mediated knockout (KO) of Sox11 attenuated TKA organoid pEMT and decreased mesenchymal marker expression. Additional bioinformatic analyses of scRNA-seq data from TKA organoids and clinical samples from the TCGA database revealed that expression of Sox11 was positively correlated with that of platelet-derived growth factor subunit B (Pdgfb). Moreover, KO of Sox11 decreased Pdgfb expression in TGFβ1-treated TKA organoids. Further, treatment with crenolanib, a Pdgf receptor inhibitor, impaired TGFβ1-induced collective invasion of TKA organoids and increased epithelial marker gene expression. These results suggest that a Sox11 and Pdgf signaling axis may regulate pEMT and collective invasion downstream of TGFβ signaling in an organoid model of colorectal carcinogenesis.

Talk session II

Multimodal single-cell profiling of three epigenetic modalities in the mouse brain by nanobody-based CUT&Tag

Marek Bartosovic
Department of Biochemistry and Biophysics, Stockholm University

Cell-type specific regulation of gene expression is controlled through combination of epigenetic regulatory mechanisms. Recent advances in single-cell chromatin mapping technologies have allowed for profiling of histone marks at the single-cell resolution. Now, we developed a novel improved technology to multimodally profile the chromatin at single-cell resolution - single-cell nano-CUT&Tag (nano-CUT&Tag). Nano-CUT&Tag makes it for the first time possible to simultaneously analyse open chromatin and two histone modifications from thousands of single cells. In addition to being multimodal, nano-CUT&Tag has lower input requirements, yields more fragments per cells and improves clustering resolution over previous generation of scCUT&Tag.
We have applied nano-CUT&Tag to the juvenile mouse central nervous system and uncovered unprecedented epigenetic heterogeneity. The obtained multimodal profiles can be used to deconvolute the individual cell identities in the brain and generate high quality multimodal epigenetic profiles. Moreover, we used nano-CUT&Tag to uncover the dynamics of the chromatin during a differentiation process in vivo across a pseudotime trajectory. We then leveraged the relationship between chromatin opening and enhancer activation to define chromatin velocity and predict the lineage progression during differentiation. Altogether, nano-scCUT&Tag provides unprecedented insights into chromatin regulatory landscape in the mouse CNS.

DOT1L-Mediated Basal Progenitor to Interneuron Signaling and Its Impact on Cerebral Cortex Development

Camila Fullio, Tanja Vogel
Institute of Anatomy and Cell Biology, University of Freiburg

Proper cortical development relies on generating and maintaining an equilibrium between excitatory neurons and inhibitory interneurons. Excitatory neurons originate from apical and basal progenitors (APs, BPs) in the dorsal telencephalon, which emit chemoattractant signaling molecules crucial for the attraction and migration of interneurons from the ventral telencephalon into the dorsally located cortex.
In our prior research, we established that DOT1L activity serves as an epigenetic barrier in the excitatory, glutamatergic cell lineage of the dorsal telencephalon. Here, DOT1L controls the fate of apical progenitors by balancing cell division and differentiation in part by regulating the expression of genes impacting metabolic pathways. DOT1L has similar effects in the inhibitory, GABAergic cell lineage. In this study, we investigate cell-type resolved roles of DOT1L in cortical development and its impact on the coordinated integration of interneurons in the glutamatergic network. For this purpose, we analyzed single-cell RNA sequencing (scRNA-seq) data from DOT1L conditional knockout (cKO) mice that either lost DOT1L in APs or in BPs. In both model systems loss of DOT1L occurs in glutamatergic progenitors, and we analyzed molecular alterations at multiple developmental time points and in regard to the coordinated development of both the glutamatergic and GABAergic cell lineages.
We noted a significant increase in the population of cortical interneurons in comparison to control mice when DOT1L is lost in APs of the dorsal telencephalon. Inferred cell-to-cell communication

revealed an intensified and prolonged expression of both known and novel signaling molecules, specifically connecting BPs and interneurons. Notably, loss of DOT1L in BPs had opposite effects, i.e. decreased numbers of interneurons and decreased signaling. The same pathways were affected but in the opposite manner. These findings demonstrate that DOT1L exerts not only a cell-autonomous influence on glutamatergic cell fate determination but also plays a crucial non-cell-autonomous role in regulating the integration of interneurons into the dorsal telencephalic networks by tuning specific signaling pathways. This underscores the multifaceted significance of DOT1L in the intricate process of cortical development of excitatory and inhibitory neurons.

The role of Dot1l in neuronal development – a single-cell story

Tobias Hohl[1,2], Camila Fullio[2,3], Laura Arrigoni[1], Tanja Vogel[3], Thomas Manke[1]
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[2] Faculty of Biology, Freiburg University
[3] Institute of Anatomy and Cell Biology, Department of Molecular Embryology, Faculty of Medicine, Freiburg University

During embryonal neuronal development, the epigenetic state of chromatin plays a crucial role in the spatiotemporal regulation of gene expression. Previously we have shown that the in vitro differentiation of murine embryonic stem cells to neuronal progenitor cells globally increases levels of H3K79me3, a histone modification deposited by Dot1l. Inhibiting DOT1L led to decreased accessibility of Sox2-binding regions as well as negatively affected Sox2 binding to known related enhancers. Therefore, the activity of Dot1l is crucial for the differentiation of neuronal progenitors. To follow up on this work, we generated a comprehensive single-cell multi-omics dataset with temporal resolution using a conditional Dot1l-KO mouse model to investigate on cell type specific effects of Dot1l during the development of the cerebral cortex and its intricate layered structure. A first knockout validation study revealed a possible alternative astrocyte differentiation pathway that needs further verification through inclusion of spatial transcriptomics data. Continuative multimodal and integrative data analysis will let us infer developmental trajectories and cell-type specific transcription factor networks, as well as unravel the role that DOT1L plays in both the differentiation of the numerous cell types involved and the activities of transcription factors that are crucial for the development of the different cell populations in the cerebral cortex.

Sites of transcription initiation drive mRNA isoform selection

Carlos Alfonso-Gonzalez
Max-Planck-Institute of Immunobiology and Epigenetics, Freiburg

The generation of distinct messenger RNA isoforms through alternative RNA processing modulates the expression and function of genes, often in a cell-type-specific manner. Here, we assess the regulatory relationships between transcription initiation, alternative splicing, and 3′ end site selection. Applying long-read sequencing to accurately represent even the longest transcripts from end to end, we quantify mRNA isoforms in Drosophila tissues, including the transcriptionally complex nervous system. We find that in Drosophila heads, as well as in human cerebral organoids, 3′ end site choice is globally influenced by the site of transcription initiation (TSS). “Dominant promoters,” characterized by specific epigenetic signatures including p300/CBP binding, impose a transcriptional constraint to define splice and polyadenylation variants. In vivo deletion or overexpression of dominant promoters as well as p300/CBP loss disrupted the 3′ end expression landscape. Our study demonstrates the crucial impact of TSS choice on the regulation of transcript diversity and tissue identity.

Talk session III

Single-cell epigenomic reconstruction of developmental trajectories in human neural organoid systems

Fides Zenk
Brain Mind Institute EPFL, Lausanne

Pluripotent progenitors, through a tightly controlled series of cell fate restrictions events, give rise to the diversity of human cell types. These fate restrictions are governed by epigenetic mechanisms that control the activity of target genes and regulatory elements. However, exploring these pathways in human early brain development has been challenging. Here, we use single-cell profiling of histone modifications (H3K27ac, H3K27me3, and H3K4me3) in human central nervous system organoid cells across a developmental time course in order to reconstruct the epigenomic trajectories driving cell identity. We analyse transitions from pluripotency to neuronal and glial terminal states as well as differentiation from progenitors to retinal and brain area specification through the neuroepithelium. We discover that decisions at each level can be predicted by switching between repressive and activating epigenetic modifications. We further establish a temporal census of regulatory elements and transcription factors and characterise them within the gene regulatory network of human cerebral cell fate acquisition. We confirm this regulatory mode in a primary tissue using transcriptome and chromatin accessibility measures in the same cell from a human developing brain. We demonstrate that by inhibiting the polycomb group protein Embryonic Ectoderm Development (EED) at the neuroectoderm stage, fate restriction is disrupted, and abnormal cell fate acquisition occurs, which ultimately affects the cell type composition in brain organoids. Our single-cell genome-wide atlas of histone modification changes during the development of human brain organoids serves as a guide for further research into the cell fate choices made in human brain development in healthy physiology and in neurodevelopmental disorders.

Unraveling the Molecular Mechanisms of FOXG1: Insights into Transcriptional Control in Mice

Ipek Akol, Tanja Vogel
Institute of Anatomy and Cell Biology, University of Freiburg

Despite the significance of FOXG1 in neurodevelopmental disorders, there have been limited studies investigating the molecular processes underlying its function. In our published research, we harnessed a comprehensive multi-omics dataset to delve into the molecular changes linked with diminished FOXG1 levels and their implications for neuronal dysfunctions.
Specifically, we examined the influence of FOXG1 on neuronal maturation at the chromatin level in the mouse hippocampus. Our findings illuminated substantial alterations in the epigenetic landscape. These alterations encompass shifts in chromatin accessibility and modifications to histones at enhancer sites, subsequently exerting an influence on the transcriptome within mature mouse hippocampal neurons in vitro.
Furthermore, our investigations brought to light that FOXG1 engages with the proneuronal transcription factor NEUROD1 and HDACs to orchestrate gene transcription. These intricate interactions underscore the multifaceted and multimodal nature of FOXG1's functions in relation to neuronal maturation and overall function.

By providing an integrated multi-omics perspective, our study unveils a previously unrecognised multimodality of FOXG1 functions, all converging on the process of neuronal maturation. These findings contribute to a better understanding of FOXG1 syndrome and its underlying molecular mechanisms.

An anatomical and molecular characterization of subpallial dopaminergic neurons in zebrafish larvae.

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In mammals, midbrain dopaminergic (DA) neurons of the mesolimbic and mesostriatal pathways project into the telencephalon and play critical roles in emotion and motor control. Degeneration of these neurons in Parkinson's disease comprises proper function of the striatum and limbic system. While ongoing research about the transplantation of DA progenitors into the striatum holds promise as a biomedical application, a deeper understanding of the differentiation and maturation of DA neurons in the telencephalic environment is necessary. While zebrafish lack midbrain DA neurons, they form DA neurons in the subpallial telencephalon itself, which offers a unique model for investigating telencephalic DA neuron differentiation and their integration into modulatory DA circuits. However, the exact anatomical location, molecular heterogeneity, and function of zebrafish subpallial DA neurons are still not understood. Recent studies suggest that several DA populations line the striatum and might be situated within regions homologous to the mammalian amygdala (Porter and Mueller, 2020). We used fluorescent in situ hybridization co-expression studies to demonstrate that in the larval zebrafish brain subpallial DA neurons form part of the extended medial amygdala. Moreover, our co-expression studies revealed molecularly distinct subpopulations within these presumptive amygdalal DA neurons. To further characterize their molecular heterogeneity, we FACS-sorted catecholaminergic (CA) neurons from a transgenic reporter line driving GFP expression in CA neurons, and performed 10x Genomic based single-cell RNA sequencing. Our dataset contains the transcriptomes of over 2000 cells that represent the majority of CA populations in the 5 dpf zebrafish brain. Based on published data, we could assign most cell clusters to defined CA populations and progenitors. Further, our ongoing analysis revealed previously uncharacterized subtypes of subpallial DA cells, in line with the findings of our co-expression studies. We anticipate that this analysis will provide valuable insights into the molecular characteristics of subpallial DA subgroups, enhancing our understanding of mechanisms underlying telencephalic DA differentiation and circuit development in zebrafish.

References:

Porter, B. A., & Mueller, T. (2020). The Zebrafish Amygdaloid Complex – Functional Ground Plan, Molecular Delineation, and Everted Topology. Frontiers in Neuroscience, 14(July). https://doi.org/10.3389/fnins.2020.00608

The lincRNA Pantr1 is a critical mediator of dendritic outgrowth in a murine and human model of FOXG1 syndrome

Fabian Gather[1],*, Tudor Rauleac[1,2],*, Ipek Akol[1,2], Ganeshkumar Arumugam[1,3], Teresa Müller[4], Dimitrios Kleidonas[2,3,5], Marion Scheibe[6], Andre Fischer[7,8,9], Andreas Vlachos[5,10], Rolf Backofen[4], Tanja Vogel[1,10,11]

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* equal contribution

Rett- and Rett-spectrum disorders, such as FOXG1 syndrome, arise from disturbances in gene expression programs involving transcriptional and posttranscriptional mechanisms. Although non-coding RNAs are involved in brain development and aberrant expression has been associated with a wide range of neurological diseases, the role of long non-coding RNAs (lincRNAs) in FOXG1 syndrome development remains unexplored yet.
Therefore, we investigated the expression of lincRNAs in the hippocampus and cerebral cortex of adult and developing mice, as well as in human brain organoids. Our findings revealed differential expression of Pantr1 (Pou3f3 adjacent non-coding transcript 1) upon heterozygous loss of FOXG1. Pantr1 localized to the nucleus, cytoplasm, and dendrites, and it exhibited interactions with various proteins. One such protein identified through pulldown experiments and mass spectrometry is PURB. Upon knockdown of FOXG1, Pantr1, or PURB using shRNA in cultured hippocampal neurons and neural progenitor cells, we observed differently expressed shared target genes and altered binding of FOXG1 by RNAseq, ChIPseq, and RT-qPCR. Additionally, Sholl analysis demonstrated changes in neuronal morphology and dendritic outgrowth, while immunoprecipitation assays confirmed interactions between Pantr1, FOXG1, and PURB.
In conclusion, our study suggests that the expression and function of FOXG1, PURB, and Pantr1 are critical for dendritic outgrowth and neuronal differentiation by establishing an interactive network that regulates gene transcription associated with dendritic complexity during brain development. Given that aberrant Pantr1 expression contributes to defects in dendritic outgrowth, it holds considerable potential as a therapeutic target for neurodevelopmental disorders such as FOXG1 syndrome.

Talk session IV

Computational methods for linking regulatory elements to target genes

Marcel Schulz
Institute of Cardiovascular Regeneration, Uniklinikum and Goethe University Frankfurt

Identifying regulatory regions in the genome is of great interest for understanding the epigenomic landscape in cells and study regulation of gene expression. One fundamental challenge in this context is to find the target genes, whose expression is affected by the regulatory regions. In this talk I will summarize recent advances for computational methods that address this challenge in data scenarios with as little as one epigenomic dataset or in projects that deal with consortia size datasets. I will present an application in the context of finding novel disease genes using regulatory mutations and discuss efforts of the International Human Epigenomics Consortium (IHEC) to define a catalogue of all human regulatory elements.

Dimension reduction and temporal patterns in scRNA-seq data

Maren Hackenberg, Harald Binder
Institute of Medical Biometry and Statistics, Faculty of Medicine and Medical Center, University of Freiburg

Generating single-cell RNA-sequencing (scRNA-seq) data at several time points, e.g., during a developmental process, promises insights into mechanisms controlling cellular differentiation at the level of individual cells. As there is no one-to-one correspondence between cells at different timepoints, a first step in a typical analysis workflow is to reduce dimensionality to visually inspect temporal patterns. Here, one implicitly assumes that the resulting low-dimensional manifold captures the central gene expression dynamics of interest. Yet, commonly used techniques are not specifically designed to do so and their representations do not necessarily coincide with the one that best reflects the actual underlying dynamics.\nWe thus investigate how visual representations of different temporal patterns in time-series scRNA-seq data depend on the choice of dimension reduction, considering principal component analysis (PCA), t-distributed stochastic neighbourhood embedding (t-SNE), uniform manifold approximation and projection (UMAP) and single-cell variational inference (scVI), a popular deep learning-based approach. We specifically focus on comparing visual representations of each dimension reduction technique as such visual inspection of temporal patterns is often a crucial first step that guides the choice of further downstream analyses.\nTo characterize the approaches in a controlled setting, we introduce an artificial time series in a snapshot scRNA-seq dataset from one experimental time point by simulating an underlying low-dimensional developmental process and generating corresponding high-dimensional gene expression data. Specifically, we apply a specific dimension reduction approach (say, tSNE) to the snapshot data and transform the low-dimensional representation according to biologically meaningful temporal patterns, e.g., dividing cell clusters during a differentiation process. We train a deep learning model to generate synthetic high-dimensional gene expression profiles corresponding to the simulated pattern at each time point, and apply the different dimension reduction approaches on the high-dimensional time-series data to compare how well they reflect the underlying temporal pattern. Subsequently, we vary the dimension reduction method used to introduce the temporal pattern, the temporal pattern itself, and the underlying dataset based on which the development is simulated, to generalize our findings.
We thus characterize the different perspectives of each technique on different temporal patterns. The results illustrate how the choice of the dimension reduction approach can dramatically alter temporal structure. We further demonstrate this effect on a real-world time-series scRNA-seq dataset. To alleviate such problems, we provide directions for designing dimension reduction

techniques that explicitly respect temporal structure and allow for visualizing development patterns of small groups of cells.

Kernel-based testing for global comparison of single-cell RNA Sequencing datasets

Anthony Ozier-Lafontaine
Laboratoire de Mathématiques Jean Leray - Ecole Centrale de Nantes

Single-cell RNA sequencing (scRNAseq) is a high-throughput technology quantifying gene expression at the single-cell level, for hundreds to thousands of observations (i.e. cells) and tens of thousands of variables (i.e. genes). New methodological challenges arose to fully exploit the potentialities of these complex data. A major statistical challenge in scRNAseq data analysis is to distinguish biological information from technical noise in order to compare conditions or tissues. The principal approach to do this is Differential Expression Analysis (DEA), which is basically gene-wise univariate two-sample tests. However, DEA misses the multivariate aspects of scRNAseq data, which carries information about gene dependencies and gene regulatory networks, and does not inform about the global similarity of the compared datasets. Thus there is a need to develop specific multivariate two-sample tests to test for any global difference between two scRNAseq datasets.
I will present a kernel based two-sample test called truncated Kernel Fisher Discriminant Analysis (tKFDA) test that can be used for DEA as well as for multivariate two-sample tests.
The tKFDA test have been introduced in the seminal work of Harchaoui et al. and we propose its first ready-to-use implementation dedicated to scRNAseq data. The tKFDA test can be interpreted as a regularized version of the famous Maximum Mean Discrepancy (MMD) test. This regularization is based on a Kernel Principal Component Analysis (KPCA) like dimension reduction, allowing to distinguish insightful information from technical noise. Moreover, the regularization is designed such that the associated empirical statistic has an asymptotic chi-square distribution, which allows for asymptotic testing.
Besides reaching state of the art performances in DEA, our approach has a geometrical interpretation of finding the optimal nonlinear transformation of the data that discriminates between the two compared conditions. Visualization tools dedicated to highlighting the main differences can be derived from this interpretation and be the basis of a cell-wise investigation to identify the cells or populations of cells that are the more different between the two conditions.

Multiscale diffusion geometry for (deep) learning of graphs, manifolds, and flows

Smita Krishnaswamy
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In this talk we show how to learn the underlying geometry of data using multiscale data diffusion, and then combine this with deep learning for prediction and inference in several different settings. First we look at capturing graphs using multiscale diffusion based geometric scattering within neural frameworks. We show how to make such networks end-to-end differentiable in order to learn rich representations spaces from which to classify and generate graphs. We then show how to extend this type of analysis to manifolds, where point-clouds of data can be similarly featurized using cascades of wavelets on data graphs to create a manifold scattering transform. Next we show how to derive Wasserstein distances between pointclouds of such data using multiscale diffusion distances. Finally we move from static to dynamic optimal transport using neural ODEs in order to learn dynamic trajectories from static snapshot data-a key problem in inference from single cell data. Throughout the talk, we present examples of such techniques being applied to massively high throughput and high dimensional datasets from biology and medicine.

CanSig: discovery of shared transcriptional states across cancer patients from single-cell RNA sequencing data

Florian Barkmann
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Multiple cancer types have been shown to exhibit heterogeneity in the transcriptional states of malignant cells across patients and within the same tumor. The intra-tumor transcriptional heterogeneity has been linked to resistance to therapy and cancer relapse, representing a significant obstacle to successful personalized cancer treatment. However, today there is no easy-to-use computational method to identify heterogeneous transcriptional cell states that are shared across patients from single-cell RNA sequencing (scRNA-seq) data.\n\nTo discover shared transcriptional states of cancer cells, we propose a novel computational tool called CanSig. CanSig automatically preprocesses, integrates, and analyzes cancer scRNA-seq data from multiple patients to provide novel signatures of shared transcriptional states and associates these states with known biological pathways. CanSig uses a VAE-based approach to correct for patient-specific effects. To improve the integration of samples stemming from different patients, CanSig leverages inferred CNVs and a Gaussian MIxture prior.\n\nIn our benchmarks, we show that using inferred CNVs and a Gaussian MIxture as a prior distribution improves dataset integration. Further, CanSig re-discovers known transcriptional signatures on previously published cancer scRNA-seq datasets. We illustrate CanSig's investigative potential by uncovering signatures of novel transcriptional states in four additional cancer datasets. Some of the novel signatures are linked to cell migration and proliferation and to specific genomic aberrations and are enriched in more advanced tumors. \n\nIn conclusion, CanSig detects transcriptional states that are common across different tumors. It facilitates the analysis and interpretation of scRNA-seq cancer data and efficiently identifies transcriptional signatures linked to known biological pathways. The CanSig method is available as a documented Python package at https://github.com/BoevaLab/CanSig.

Talk session V

Modeling single-cell dynamics and fate decisions across modalities, time and space

Fabian Theis
Institute of Computational Biology, Helmholtz Munich and Department of Mathematics and Department of Life Sciences, TU München

Single-cell technologies are revolutionizing our understanding of cellular dynamics across biological processes. However, analyzing and interpreting these data poses computational and conceptual challenges, in particular with recent developments regarding spatio-temporal profiling and lineage tracing. Here, I will discuss approaches for studying single-cell dynamics and fate decisions across molecular modalities, time, and space.
After a brief review of pseudotemporal ordering and RNA velocity, I will show how optimal transport can be used consistently across biological applications, including temporal, spatial, and spatio-temporal single cell problems, such as aligning multi-modal single-cell data across space and time. Finally I will discuss CellRank and a recent extension beyond RNA velocity to learn dynamics based on any pseudotime, developmental potential, real-time information, and metabolic labeling data.

Bayesian inference of chromatin looping dynamics from live-cell measurements
Christoph Zechner
Max-Planck-Institute of Molecular Cell Biology and Genetics and Center for Systems Biology Dresden

Recent live-cell microscopy techniques allow the simultaneous tracking of distal genomic elements, providing unprecedented ways to study chromatin dynamics and gene regulation. However, drawing robust conclusions from such data is statistically challenging due to substantial technical noise, intrinsic fluctuations and limited time-resolution. I will present recent progress we have made in addressing some of these challenges; specifically, we developed a new statistical method to quantify CTCF/cohesin-mediated chromatin looping dynamics from two-point live-cell imaging experiments. The method combines a simple polymer model with a Bayesian filtering approach to infer loop lifetimes and frequencies. Its application to experimental data revealed that chromatin loops are surprisingly rare (~5% looped fraction) and short-lived (~20mins loop lifetime). I will discuss potential implications of these findings and outline future challenges.

Galaxy and single-cell developments
Pavankumar Videm
Bioinformatics Group and Galaxy Europe Team, University of Freiburg

Galaxy is a workflow-management system that simplifies data analysis in many fields of life sciences including single-cell omics. To deal with the challenges in integration of an ever-growing list of single-cell data analysis tools into Galaxy, maintenance of existing tools, creation of comprehensive training materials, we aim to unite tool developers through to users to experts to trainers. As a Galaxy single-cell working group, we integrated more than 90 different single-cell related tools from 15 different tool suites. We also created workflows and developed training materials to guide the users with best practices in single-cell data analysis. As of now, Galaxy Training Network offers more than 20 single-cell specific training resources on single-cell matrix creation, quality control, clustering, trajectory inference and bulk RNA deconvolution. With an aim to expand beyond scRNA-seq, we are currently developing tools and workflows on Galaxy for scATAC-seq data analysis and further to analyse single-cell multiomics and spatial omics data. In this talk, I will give an overview of Galaxy single-cell developments with future aims, challenges and training infrastructure.

Unified fate mapping in multiview single-cell data
Marius Lange
Department of Biosystems Science and Engineering, ETH Zürich

Single-cell RNA sequencing allows us to model cellular state dynamics and fate decisions by using expression similarity or RNA velocity to reconstruct state-change trajectories. However, trajectory inference does not incorporate valuable time point information or utilize additional modalities, while methods that address these different data-views cannot be combined and do not scale. Here, we present CellRank 2, a versatile and scalable framework to study cellular fate using multiview single-cell data of up to millions of cells in a unified fashion. We demonstrate that CellRank 2 consistently recovers terminal states and fate probabilities across data modalities in human hematopoiesis and mouse endodermal development. Our framework also allows combining transitions within and across experimental time points, a feature we use to recover genes promoting medullary thymic epithelial cell formation during pharyngeal endoderm development. Moreover, we enable estimating

cell-specific transcription and degradation rates from metabolic labeling data, which we apply to an intestinal organoid system to delineate differentiation trajectories and pinpoint regulatory strategies.

Talk session VI

Fuelling Innate Immunity
Jason Cosgrove
Institut Curie Paris

Innate immune cell production is one of the dominant biosynthetic processes in the human body, in which hematopoietic stem and progenitor cells (HSPCs) give rise to tens of billions of cells each day in homeostasis, and even greater numbers of cells to combat infection. Despite these significant biosynthetic demands, the nutrients and metabolites that fuel this process are poorly characterized.
In this talk, I will present novel single-cell and systems biology approaches we have developed to estimate the bioenergetic demands of hematopoeisis and to perform metabolic state-fate mapping of single cells in vivo. Using this strategy together with challenge models and targeted genetics, we identifya subset of metabolically distinct myeloid-biased HSPCs that are a major source of innate immune cells in steady state and inflammatory conditions. In addition we show that immune cell production dynamics can be modulated by increasing enzyme expression in HSPCs. Collectively our results highlight a key role for metabolism in regulating the type and amount of immune cells that HSPCs produce in vivo.

Identification of potential therapeutic targets to revert natural killer cell exhaustion in Multiple Myeloma through single-cell RNA-Seq analysis
Jacopo Umberto Verga
University of Galway, Ireland

Introduction
Multiple myeloma (MM) is a blood cancer caused by the accumulation of plasma cells in the bone marrow. Disease progression is associated with immune suppression. Natural Killer (NK) cells, part of the innate immune system, play a pivotal role in anti-tumor surveillance. We aimed to understand whether and how MM may impact the NK cells and how NK cell dysfunction in MM can be reverted.
Methods
scRNA-Seq datasets were integrated using Seurat and scVI. SingleR was used to identify the different cell types. To classify NK cells as active, tissue-resident (rNK), or exhausted (eNK), we developed an algorithm (ScoreMarkers) that scores the cell state based on a gene expression signature. The classification has been validated using GSEA and gene sets from MSigDB. Then NK cells have been characterized through differential gene expression (DGE) with Seurat (method =”LR”, FDR<0.05), gene ontology (GO) enrichment analysis with ClusterProfiler, and GOSemSim. Active ligand-receptor pairs have been identified with LIANA and active Transcription Factors (TF) in NK cells with pyScenic. An immune checkpoint (IC) signaling cascade network has been built with NicheNet using ligands from LIANA and target genes from pyScenic as inputs. Genes in the identified IC cascades were ranked by calculating a score based on PageRank, Betweenness centrality, Percolation, and Ricci Curvature.
Results
Because the NK cell population is heterogeneous, we used single-cell level analyses. We have integrated 6 scRNA-Seq studies containing samples encompassing all MM disease progression

stages. The dataset contains 14,103 and 7,596 NK cells in MM and healthy samples, which were divided into two categories: active, tissue-resident NK cells or exhausted NK cells based on gene expression signatures (Foroutan et al. 2021). MM-specific characteristics of eNK cells were analyzed by determining DGE, enriched biological processes, and the active IC receptors. The proportion of eNK cells increased in all the disease stages (p.value<0.01). eNK cells in MM up-regulated several IC receptors, genes associated with the development of malignancies, and altered tumor immune microenvironment. Only a minority of DGE, enriched Biological Processes (BP) and TF were shared by eNK cells in MM and healthy samples. This suggests disease-specific pathways driving NK cell exhaustion in MM. Cell-cell interactions suggest the tumor microenvironment actively supports immune exhaustion by activating several IC Receptors. With the network analysis, we ranked the IC signaling cascade genes. The expression patterns of the highest-ranking genes exhibited a significant correlation with exhaustion scores determined by ScoreMarkers. These top-ranking genes encompass a diverse range of functional categories, including nuclear receptors, TFs, and phosphatases and many of them play pivotal roles in regulating NK activity, while others have yet to be implicated in this context. We are currently in the process of planning in vitro experiments to validate the identified genes and potentially uncover new functional roles, providing substantial evidence for our findings.

Conclusions

Our investigation has identified distinctive myeloma-specific pathways driving the exhaustion of NK cells, already at the earliest phases of the disease, indicating a pivotal role in disease progression. By accurately delineating the signaling cascades orchestrating immune exhaustion, we have pinpointed potential therapeutic targets. These targets are anticipated for experimental evaluation, to design NK cells resistant to the debilitating influences of the tumor microenvironment.

Mouse multipotent progenitor 5 cells are located at the interphase between hematopoietic stem and progenitor cells

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Hematopoietic stem cells (HSCs) are located at the top of the hematopoietic hierarchy and, together with multipotent progenitors (MPPs), are contained within the Lin−Sca-1+c-Kit+ (LSK) compartment. We and others have previously characterized HSCs and MPP1-4 using diverse surface-marker panels. In this study we address the molecular (RNA-seq at population and single-cell level) and functional (in vivo and in vitro) characterization of the previously unknown MPP5 population (LSK CD34+ CD135- CD48- CD150-; Sommerkamp*, Romero-Mulero* et al., Blood 2021; *first-shared authorship). Upon transplantation, MPP5 cells presented in vivo a distinctive engraftment pattern supporting initial emergency myelopoiesis but long-term contribution to the lymphoid lineage. In addition, in vitro assays showed that MPP5 cells are immediately downstream of the stem cell compartment and represent a dynamic component of the MPP network, as they are capable of generating MPP1-4 populations, but not HSCs. To identify specific transcriptomic landscapes of hematopoietic stem and progenitor cells (HSPCs), we performed RNA-sequencing (RNA-seq) analysis of HSCs and MPP1-5. Expression patterns of MPP5 were similar to HSC and MPP1 populations and were primed towards inflammatory processes. This was complemented by extensive single-cell RNA-seq (scRNA-seq) analysis of the LSK compartment to establish the differentiation trajectories from HSCs to MPP1-5. In agreement with functional assays, MPP5 population is located immediately downstream of HSCs but upstream of the more committed MPP2-4, displaying trajectories to all the different MPP populations. This study provides a comprehensive analysis of the LSK compartment, focusing on the functional and molecular characteristics of the newly defined MPP5 subset.

Deciphering the role of Mda5 in HSC aging

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Aging of the hematopoietic system is characterized by increased sterile inflammation, which leads to susceptibility to malignancies. Here, we investigate the role of the innate immune sensor melanoma differentiation-associated protein 5 (MDA5) in hematopoietic stem cell (HSC) aging. Single-cell RNA-seq analysis performed on the LSK compartment of young and aged, wildtype (WT) and Mda5-/- animals revealed an expansion of the multipotent MPP2/3 populations in the aged WT cells, consistent with a myeloid bias. Functionally, we observed reduced frequency of middle aged and aged Mda5-/- HSCs in the BM compared to the WT counterpart. Furthermore, accumulation of myeloid biased HSCs in aged WT animals was abrogated in Mda5-/- mice. Cell cycle analysis revealed that higher percentage of middle aged and aged Mda5-/- HSCs remain in G0 phase, and single cell division assay showed that they are less prone to divide. To evaluate the repopulation capacity, we performed non-competitive transplants, where aged Mda5-/- HSCs showed a better repopulation capacity than WT HSCs. In order to gain insights on the molecular mechanism, we perform transcriptional analysis on Mda5-/- and WT HSCs. In agreement with the functional data, Gene Set Enrichment Analysis of genes expressed in aged Mda5-/- HSCs indicated an enrichment for “HSCs” and “Dormant_HSCs”, whereas the “Active_HSCs” and “MPPs” were enriched in the WT counterpart. Metascape Analysis indicated an enrichment for terms associated with “HSF1-dependent transactivation” and “protein folding”. Additionally, digital footprinting analysis also revealed reduced motif occupancy for inflammatory transcription factors in aged Mda5-/- HSCs. Finally, treatment with Hsf1 inhibitor rescues the functional differences observed between aged Mda5-/- and WT HSCs. Overall, our results support the hypothesis that lack of Mda5 can delay the effects of aging in the HSC compartment by fine tuning inflammation.

HSC Interactome: An RShiny App for Exploring Cell-Cell Interactions Between Hematopoietic Stem Cells and Other Bone Marrow Cell Types

E. Szegezdi
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The bone marrow (BM) is a complex microenvironment, coordinating the production of billions of blood cells every day. Despite its essential role and its relevance to hematopoietic diseases, this environment remains poorly characterised. As part of a recent publication [1], we generated one of

the largest atlases of single-cell gene expression in healthy and diseased human bone marrow to date, consisting of 339,381 BM cells from normal and acute myeloid leukaemia (AML) samples. Here we present HSC Interactome [2], an RShiny app which allows users to explore the predicted ligand-receptor interactions between haematopoetic stem and progenitor cells with all other BM-resident cell types by looking at the expression of genes that code for ligands and their interacting receptors using the LIANA [3] R package. Users can generate various plots (heatmap, connection, chord diagram, violin) to investigate ligand-receptor interactions of interest [4], or look at interactions of a specific cell type or gene of interest. HSC Interactome is available both on shinyapps.io and as a docker container for users to run locally [2].

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Regulation of dormant hematopoietic stem cells

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Hematopoietic stem cells (HSCs) rely on complex regulatory networks to preserve their function. Due to the scarcity of HSCs, technical challenges have limited our insights into the interplay between HSC metabolism and their transcriptional and epigenetic regulation. We recently established and integrated low-input multi-layer OMICs data revealing distinct metabolic and epigenetic hubs that are enriched in HSCs and their downstream multipotent progenitors (Schönberger et al., Cell Stem Cell 2022; Schönberger et al., STAR Protocols- Cell Press; Schönberger et al., Analytical Chemistry 2023). Mechanistically, we uncovered by extensive in vitro and in vivo functional analysis a non-classical retinoic acid signalling axis that regulates HSC identity. Now we found that by modulating HSC activity in the context of myocardial infarction via vitamin A metabolites is beneficial for long-term recovery of the heart. Our data uncovers a novel strategy to dampen the detrimental and excessive immune response that is observed upon myocardial infarction.

Poster abstracts

Odd poster numbers will be presented in poster session I,
even numbers in poster session II

P 01
Investigation of Arabidopsis root stem cell niche reorganization by single-nucleus transcriptomics
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Pluripotent stem cells in plant meristems give rise to complete new organs throughout the plant's life, which can last more than thousands of years, as in the case of long-lived trees. Furthermore, they can adapt to environmental and developmental signals to regulate their activity. Yet, very little is known about how the organization of plant stem cell niches changes in response to these signals. The previous MeInBio member of our lab, Annika Wein, found that the organization of the columella stem cell niche undergoes stark changes during the first two weeks of the Arabidopsis thaliana seedling development. My project aims to gain insight into the regulatory structure and dynamics of the niche by single nuclei transcriptomics. In the first step, I adapted nucleus extraction and fluorescence-activated nuclei sorting protocols to the columella stem cell niche and performed a pilot single-nuclei-sequencing experiment at different ages of the Arabidopsis thaliana seedlings.

P02
zitools: an R package to analyze zero-inflated count data
Carlotta Meyring
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One fundamental objective of microbiome analyses is to identify differentially abundant taxa among different experimental groups or conditions. However, microbiome data are often overdispersed and zero inflated making data analysis extremely challenging. Although there are several models considering zero inflation, none of them provides functionality for subsequent analyses. Therefore, we propose zitools, an R package allowing for zero inflated count data analysis by either using down-weighting of excess zeros or by replacing an appropriate proportion of excess zeros with NA. Through overloading frequently used statistical functions (such as mean, median, standard deviation), plotting functions (such as boxplots or heatmap) or differential abundance tests, it allows a wide range of downstream analyses for zero-inflated data in a less biased manner.

P03
Dissecting Vemurafenib resistance in melanoma: The role of the PI3K pathway
Aránzazu Sáenz-Espinoza, Melanie Börries, Andreas Hecht, Ella Levit-Zerdoun, Pablo Antón-García, Eyleen Corrales and Silke Kowar,
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In melanoma, the BRAF V600E mutation constitutively activates the MAPK signaling pathway, promoting cell proliferation, invasion, and apoptosis evasion. This mutation can be targeted by Vemurafenib (PLX4032); however, therapy resistance is frequent. A possible mechanism of drug

resistance is PI3K pathway activation, and the loss of this pathway's negative regulator phosphatase and tensin homolog (PTEN) is a common feature in melanoma (Moses *et al.* 2019). Our group has recently reported that in patient-derived malignant melanoma cell lines that harbor the BRAF V600E mutation, loss of PTEN with consequent PI3K/AKT activation correlates with phenotypic changes in cellular morphology, proliferation, migration, and invasion, and importantly, in their sensitivity to MAPK cascade inhibitors (Corrales *et al.* 2022). Therefore, we aim to further elucidate the contribution of PTEN loss to Vemurafenib resistance in the context of the BRAF V600E mutation. For this purpose, we used CRISPR-Cas9 methodology to generate PTEN-deficient clonal cell lines and PTEN-proficient controls derived from Vemurafenib-sensitive cell line MaMel63a. We assessed phenotypic differences between the PTEN-deficient clones and the PTEN-proficient controls. We found that PTEN deficiency leads to PI3K pathway activation, albeit at varying degrees among clones. Interestingly, PI3K pathway degree of activation alone is not indicative of Vemurafenib resistance. Furthermore, we detected significant differences among both PTEN-proficient and deficient clones in Vemurafenib resistance in regard to low MITF and high AXL expression, two proteins, which serve as markers of differentiation and drug resistance, respectively. Preliminary results indicate that the PTEN-deficient and highly Vemurafenib-resistant cell lines with high AXL expression, are re-sensitized to Vemurafenib treatment when co-treated with the AXL inhibitor Bemcentinib, while neither Bemcentinib nor Vemurafenib alone affect the cell viability. Therefore, our results indicate that loss of PTEN and subsequent hyperactivation of the PI3K pathway is not sufficient to confer resistance to Vemurafenib treatment and other factors might be involved, such as differentiation levels, alternative pathways, and intrinsic heterogeneity.

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P04

Involvement of WOX2 in stem cell initiation in the Arabidopsis shoot apical meristem

Frauke Garbsch, Thomas Laux

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Stem cell niches (SCN) are a reserve of undifferentiated cells that provide a steady\nsupply of precursor cells to develop differentiated tissues. The stem cells in the SAM are maintained by a feedback-loop involving the WUSCHEL (WUS) transcription factor (TF) and the CLAVATA (CLV) genes. WUS is restricted to the organizing center (OC) and promotes SC identity and the expression of the small signalling peptide CLV3 in the SC region. In return, CLV3 represses WUS by activating its receptor kinases CLV1 & 2. However, WUS has been shown to be dispensable for SC initiation during embryogenesis as this function is fulfilled by related TFs from the WUSCHEL-RELATED HOMEOBOX (WOX) family. WOX2 is required to induce a SC population in the embryo and in its absence the function can be compensated by WOX1,3 and 5, designated the WOX2 module. WOX2 expression can already be detected in the zygote, much earlier than the appearance of a CLV3 signal marking the established SCs. How and when WOX2 initiates SC establishment and which additional factors contribute to the process is poorly understood. The aim is to analyse the function of WOX2 in SC initiation in Arabidopsis thaliana. Direct targets of WOX2 will be identified and WOX2-regulated pathways will further be integrated into a network of described SC determinants using genetic and molecular approaches.

P05

Trancriptional Mechanisms Controlling Transition States during Neurogenesis

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Distinct neural stem cell (NSC), progenitor (NPC), and early neuron populations progress through temporally structured transcriptome states to generate and maintain the brain. Notch-signalling independent (Her6/9) and dependent (Her4/12/15/2) HES/Her transcription factors contribute to their regulation. Analysis of HES/Her genetic networks in the zebrafish thalamus revealed that Notch-independent her genes, especially her6, prominently contribute to maintenance of specific neural stem cells, while Notch-dependent her genes are also prominently expressed in neural progenitor cells (Sigloch et al., 2023). To reveal transcriptional profiles of NSCs/NPCs, we used VarID (Grün, 2020) to analyse specific subsets from published scRNAseq data of zebrafish brain cells (Raj et al., 2020), and identified stem and progenitor cell clusters differentially expressing HES/Her genes. Her6 has been found to auto-regulate its expression (Sigloch et al., 2023) and oscillate (Soto et al., 2020). Single-cell RNA sequencing of FACS-isolated Her6-mNeonGreen expressing cells and subsequent cluster analysis by VarID revealed distinct stem cell and progenitor states of Her6+ cells. The Her6+ transcriptomes were compared to published scRNAseq data, which were further analyzed to identify genes expressed correlated or anti-correlated to her6 mRNA levels. These genes may have oscillatory expression in-phase or anti-phase to her6 mRNA. The findings were compared to time series RNAseq data of heat-shock Her4 or Her6 overexpression experiments to validate genes with her6 correlated or anti-correlated expression as potential Her6 targets. Our studies will lead to a better understanding of the relative contributions of Notch- dependent and Notch-independent HES/her downstream transcriptional networks to NCS/NPC regulation.

P06

A modelling approach for time resolved single cell data

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In systems biology, ordinary differential equations (ODEs) are frequently applied for investigating dynamic processes such as signalling pathways or gene regulation networks. ODEs are typically defined by translating relevant biochemical interactions into rate equations. These models are not applicable to high-throughput single cell data because they have of the large number of dynamic variables and parameters.
We recently introduced a complementary modeling approach based on curve fitting of a tailored retarded transient response function (RTF) [1]. This approach exhibits amazing capabilities in approximating ODE solutions in case of transient dynamics as it is typically observed for cellular signalling. Besides the broad and easy applicability, a benefit of the RTF is the clear-cut interpretation of its parameters as response time, as amplitudes, and time constants of a transient and a sustained part of the response. Dose-dependencies of these parameters are described via Hill functions, allowing for the calculation of half-maximal activating (EC50) or inhibitory (IC50) effects on these dynamic parameters.
The presented approach offers a data-driven alternative modelling strategy for situations where classical ODE modeling is cumbersome or even infeasible such as for time-resolved single cell data.
[1] https://doi.org/10.3389/fphy.2020.00070

P07

Molecular characterization of pityriasis rubra pilaris

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Non-communicable inflammatory skin diseases (ncISDs) are a major global burden and affect up to 20% of the human population. Psoriasis and atopic eczema are the most common non-communicable inflammatory skin diseases, however pityriasis rubra pilaris (PRP) remains an ultra-rare skin disorder with a yet unknown pathogenesis. In addition, PRP patients are often misdiagnosed due to overlapping symptoms, i.e. thickening of epidermis and itchy ness, which often leads to choosing ineffective treatments. In order to address this, we want to shed light on PRP by investigating the hypothesis whether PRP is a more keratinocyte driven inflammatory skin disease unlike psoriasis and atopic eczema which are more T-cell driven ncISDs. Therefore, we took punch biopsies from lesional and non-lesional skin of PRP, psoriasis and eczema patients. These were deep molecular characterised with spatial, single cell, and bulk transcriptomics technologies, thus enabling us analysing the gene expression profiles on different resolutions.\nFirst, we extracted molecular biomarkers by analysing whole tissue transcriptomics based on age and sex matched PRP samples in comparison to psoriasis and atopic eczema. Next, we investigated the expression of the biomarkers on a spatial and single cell resolution for gaining insights in the localization in the tissue and their expression level in different cell types, respectively. As next steps, we want to investigate whether our single cell population shows a highly differentiated keratinocyte population and lower amounts of T helper cells. In addition, we are interested in the difference of PRP to psoriasis in specific tissue layers using our spatial transcriptomics data set.\nBulk transcriptomics revealed that PRP differentially regulates genes including IL17C, SERPINB1 and PYDC1 against all samples and CCL20 and IL23A against lesional samples of psoriasis and eczema. Especially the upregulation of IL17C suggested that PRP is a more keratinocyte driven disease. This is also supported by the upregulation of SERPINB12 which plays a critical role sustaining the skin barrier function while PYDC1 induces keratinocyte differentiation. Pathway enrichment analysis reveals the downregulation of pathways related to adaptive immune response and upregulation of keratinization and its differentiation in PRP compared to psoriasis and eczema samples.\nFor clinical translation, we extracted stable signatures empowering an expression based multi-classifier distinguishing between PRP, psoriasis and eczema.

P08

Telencephalic Neural Stem Cell Proliferation Dynamics and Dopaminergic Neurogenesis in the Larval Zebrafish

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The zebrafish exhibits high and lifelong neurogenic potential throughout the brain. Heterogeneity within neural stem cells (NSC) stems from differences in their cellular state (quiescent versus proliferating), molecular signatures established during development in their precursors, and results in different lineages of their progeny. While in the embryo progenitor cells have a regionalized

character, regionalization among NSCs at adult and larval stages beyond the major dorsoventral and prosomeric domains is still under debate. In this project, we want to investigate telencephalic populations of NSCs that are the source of progenitors differentiating into dopaminergic neurons of the subpallium. Subpallial dopaminergic neurogenesis appears continuous throughout larval and into adult stages. We examine if the same population of ventricular wall NSCs gives rise to early and late forming subpallial dopaminergic neurons, which may indicate a distinct molecular regional identity of the NSCs generating a dopaminergic lineage. We optimized clonal analysis by CreERT-Lox system lineage labelling using limiting dilutions of tamoxifen to achieve sparse labelling selectively in NSCs through driving CreERT expression under Sox2 control. Preliminary data indicate that this technique identified NSC and progenitor domains for subpallial dopaminergic neurons. To analyze the transcriptomes and find defining factors of the dopaminergic lineage we will apply an intersectional FACS strategy using fluorescently labelled transgenic lines for the telencephalic marker foxg1a, the NSC marker sox2 and the dopaminergic marker th to perform scRNAseq. These data should help to identify potential microheterogeneity of NSCs, focusing on regional identity, and, due to the extended stability of the fluorescent protein expressed in Sox2 NSCs, should also identify early and late progenitors of the dopaminergic lineage.

P09

RDPMspecIdentifier - Identification of RNA Dependent Proteins.

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The importance of RNA binding Proteins (RBPs) in the regulation of cellular processes has been demonstrated multiple times. These proteins bind to their RNA targets, e.g. leading to the up- or down-regulation of mRNA levels. While there are more than 1000 such proteins known in humans, little is known about the RNA-interactome of less closely related organisms like Synechocystis sp.
Density gradient sedimentation of Proteins, followed by RNase treatment and quantitative mass spectrometry is an approach that targets this knowledge gap. This experiment focuses on identifying RNA-dependent Proteins (RDPs) which include RBPs, by examining mass shifts resulting from RNase treatment. The output of such an experiment is converted to a distribution of the relative protein amount across the gradient. The only published tool fits multiple Gaussian distributions to the data and tries to identify shifts of the fitted peaks. Unfortunately, this might miss candidates where only a low amount of the protein is bound to RNA, even if this is supported via multiple biological replicates.
Here we present RDPMSpecIdentifier, a tool that makes use of the multivariate nature of the experimental data to rank proteins based on their likelihood to be RNA-dependent. We will further demonstrate its ability to recognize a specific RBP in Synechocystis even when it is only minimally bound to RNA. Our tool provides a user-friendly web server-based interface for the ranking and other features like clustering proteins according to the patterns of peak shifts.

P10

The role of CYLD and SPATA2 for pro-inflammatory gene expression in intestinal cells

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Inflammation is a crucial response of the body to eliminate invading pathogens. Mediated by immune and cytokine receptors, tight regulation of their pathways is of upmost importance in order to avoid

inadequate immune responses, including pathologies such as autoimmune diseases or chronic inflammation. It has been found that non-degradative M1- and K63-linked ubiquitylation of subunits of such receptors can impact their assimilation and stability, thus impacting activation of downstream effector such as the transcription factors NF-κB and AP-1, thereby regulating inflammatory gene expression. The deubiqitinases (DUBs) CYLD and OTULIN facilitate removal of M1- and K63-linked ubiquitin chains from the tumor necrosis factor (TNF) receptor I (TNFR1). We could recently show that SPATA2, an adaptor protein for CYLD, could also function without CYLD to attenuate pro-inflammatory signalling of the TNFR1 signalling complex (TNFR1-sc). Combined loss of SPATA2 and CYLD led to embryonic lethality in mice, while mice with singular loss of either CYLD or SPATA2 were healthy. It is however not clear how the singular or combined loss of CYLD and SPATA2 affects the global NF- κB/MAPK (JNK) dependent inflammatory gene response. As SPATA2 is highly expressed in intestinal epithelial cells (IECs), changes in gene expression in certain cell subpopulations and the subsequent effects on the cellular composition in case of singular and combined losses are of high interest, especially considering pathologies like inflammatory bowel disease. By analysing pro- inflammatory gene expression of intestinal subpopulations, the role of SPATA2 and CYLD for intestinal inflammation, but also, the embryonic lethality of $CYLD^{-/-}SPATA2^{-/-}$ mice may be explained.

P11

Establishment of single nucleus CUT&Tag for cardiac tissue

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Cardiovascular diseases (CVDs) are the leading cause of morbidity and mortality worldwide. CVDs are associated with extensive changes in gene expression, histone modifications and cis-regulatory elements including enhancers. Thus, a comprehensive understanding of the epigenetic programmes is crucial for understanding the molecular basis of CVDs. Heart tissue consists of different cell types with distinct transcriptional and epigenetic profiles. Single-cell profiling can overcome tissue heterogeneity and chromatin accessibility profiling using single nucleus ATAC-seq (snATAC-seq) is a powerful method to annotate cis-regulatory elements in cardiac cell types. However, it cannot discern active from poised cis-regulatory elements or provide information about repressive chromatin domains in different cell types. Thus, the aim of this study was to optimize single nucleus CUT&Tag (snCUT&Tag) for heart tissue to profile histone modifications in different cardiac cell types.
First, we adapted CUT&Tag to HL-1 cells, a cardiac mouse cell line, and characterized four histone marks (H3K4me3, H3K4m1, H3K27ac, and H3K27me3). Next, we optimized nuclei preparation from frozen heart tissue for CUT&Tag and profiled H3K4me3 and H3K27me3. We could show that libraries generated with custom or commercial pA-Tn5 libraries yielded comparable results. Based on these findings, we combined the droplet-based 10x Genomics single cell ATAC-seq platform with the cardiac CUT&Tag method to achieve single cell resolution. Utilizing antibodies against H3K27me3 from two different sources (Active Motif, Abcam), we examined the repressed chromatin state at the single cell level in the mouse heart. 8,579 and 6,406 nuclei passed quality control with a median number of 326 and 286 fragments per nucleus, respectively. We clustered nuclei and were able to differentiate between cardiomyocytes and non-cardiomyocytes. Cardiomyocyte-associated transcription factors (TFs) such as Gata4, Nkx2-5 showed high H3K27me3 signal in non-cardiomyocyte clusters, while typical cardiomyocyte marker genes such as Nppa and Myh7 showed no strong signal of H3K27me3 in non-cardiomyocytes. \nThis study demonstrates the feasibility of snCUT&Tag to profile histone modifications in heart tissue. Multi-omics techniques will be used in future experiments to analyze both histone modifications and RNA from the same nucleus in order to gain deeper insight into epigenomic regulation to CVDs.

P12

Integrative proteogenomics, large-scale terminomics and metabolomics reveal molecular rearrangement in recurrent glioblastoma

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Background
Glioblastoma multiforme (GBM) is the most common, malignant, and aggressive brain tumor. Nearly 100% of cases recur and at that point, the surgery is possible in only 20-30% of cases. This leads to a poor survival rate despite novel therapeutic approaches. Hence, there is the need for a better understanding of the mechanisms of GBM for the potential discovery of markers of the onset of recurrence (rGBM).
Methods
We obtained patient-matched initial (iGBM) and recurrent (rGBM) samples and performed large-scale explorative mass-spectrometry (MS)-based proteogenomics and lipidomics analysis. We used linear models including random patient effects for the differential abundance tests (DAA), and multi-omics approaches to integrate proteomics and lipidomics data. We performed DAA of semi-specific peptides from the MS proteomics data (terminomics) to explore proteolytic dysregulation in rGBM.
Results
The DAA of 4464 proteins identified in 8 patient-matched samples revealed 146 differentially abundant proteins. ASAH1, GPNMB and SYNM were noticeable hits based on statistical stringency and clinical relevance. Consistent with increased ASAH1 expression, lipidomics data showed a downregulation of ceramides. The terminomics analysis revealed GPNMB as an important target of proteolysis during rGBM, together the increased proteolysis of GFAP with a cleavage pattern indicating caspase activity. Our multi-omics analysis showcases a molecular fingerprint of immune infiltration (ASAH1), extracellular matrix organization (GPNMB, SYNM) and apoptotic signaling (ASAH1, ceramide accumulation, caspase products) in the context of rGBM. Finally, we used ELISA tests to explore if serum levels of ASAH1 at the moment of initial surgery can be associated with a shorter or longer time to recurrence. We observed that higher concentrations of ASAH1 at the moment of initial surgery are significantly associated with longer time to recurrence. Although preliminary and limited in sample size, this observation can point out a novel prognostic marker and highlighting a crucial immune rearregement.

P13

Pathogenic instruction of CD8 T cell differentiation in Crohn's disease

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Crohn's disease (CD), an immune-mediated inflammatory bowel disease (IBD) and involves a significant role for T cells in regulating immunopathology. While CD4 T helper cells are known for causing IBD inflammation, the role of CD8+ T cells has been less clear. Recent studies in CD patient cohorts have identified CD8+ T cell subpopulations. Single-cell exhaustion profiling revealed activated CD39+ and PD-1+ T cells at low frequencies, and high-dimensional profiling showed an abundance of Tc17 cells in active CD, a potential target for anti-CD6 therapy. These findings emphasize CD8+ T cell differentiation's importance in IBD development. This research project has four main goals. We aim to explore PD-1+ T cell populations, deciphering their transcriptional and epigenetic characteristics in inflamed versus non-inflamed states. We also seek to understand differentiation trajectories, identifying transcriptional hubs and developmental checkpoints for PD-1+ cell populations. Additionally, we would like to investigate T cell receptor (TCR) clonality in intestinal

and peripheral T cells, exploring shared clonotypes and their potential antigenic implications. Finally, we aim to grasp microenvironmental interactions' impact on intestinal T cell differentiation, using techniques like Imaging Mass Cytometry and single-cell RNA sequencing profiling. This project sheds light on CD8 T cell dynamics in IBD, vital for targeted therapeutic strategies.

P14

Genome-wide Analysis of the Role of TIP60 Phosphorylation in Transcriptional Regulation

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TIP60 is an acetyltransferase that has been demonstrated to be a haplo-insufficient tumor suppressor. It can acetylate lysines of both histones and non-histone targets, act as a transcriptional coregulator of a great number of genes, such as targets of c-Myc and NfκB, and plays a crucial role in the response to DNA damage. TIP60 activity and binding affinity are regulated by phosphorylation of specific serine residues by PI3K/AKT/GSK-3 and CDK-9. In this project we investigate the effect of wildtype and phosphorylation-deficient TIP60 (TIP60S86A, TIP60S90A,\nTIP60S86A/S90A) on global gene expression by RNA-Seq to assess if the specific phosphorylation states of TIP60 correlate with the transcriptional regulation of distinct sets of genes. As genetic editing of the TIP60 locus has proven to be challenging, we have generated HCT116 cells that combine expression of wildtype or phosphorylation-deficient TIP60 from the ‘safe-harbour’ AAVS1 locus with a knockout of the endogenous TIP60 gene.

P15

Comparative Assessment of Copy Number Variant Calling Tools for the Identification of Malignant Cells in an Acute Myeloid Leukaemia Single-Cell RNA Sequencing Dataset

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Background

Acute myeloid leukaemia (AML) is an aggressive malignancy, resulting in the accumulation of poorly differentiated white blood cells in the bone marrow (BM). In AML, normal and malignant blood production (haematopoiesis) takes place simultaneously with both non-malignant and malignant hematopoietic cells being altered by the disease. However, separation of these cell populations would be crucial to identify the unique features of the malignant cells in order to design effective and selective treatment strategies, which can eliminate the leukemic cells while causing minimal damage to the non-malignant cell population.

Single cell transcriptomics (scRNA-seq) have allowed for the characterisation of genomic alterations in AML, such as single nucleotide variants [1] and copy number variants [2] (CNVs), and exploiting this knowledge may aid in identifying malignant cells. We hypothesised that AML cells, including the LSCs, may differ from normal haematopoietic cells in the complement of expressed CNVs which could be used to demarcate the malignant cells from their normal counterparts.

Methods
We previously performed scRNA-seq on 28 longitudinal samples (diagnosis, n=10; remission, n=7; relapse, n=11) from BM aspirates of 10 AML patients [3]. In the current project we evaluated the ability of three different methods (inferCNV [4], CopyKAT [5], Numbat [6]) to identify the leukemic cell populations based on altered CNV profiles. InferCNV and CopyKAT use expression levels of adjacent genes to infer genomic copy numbers, while Numbat integrates additional allelic ratio and haplotype data to identify CNVs present.
Results
The three tools differ in their use of references and method of prediction of malignancy. CopyKAT and Numbat generated malignant cell predictions (normal/tumour) as part of their output, while inferCNV only provides clustering of cell populations with similar CNV profiles. The malignant cell predictions consisted of broadly similar cell type proportions being labelled as malignant with haematopoetic stem and progenitor cells constituting the greatest proportion, aligning with the biology of this disease. However, CopyKAT predicted almost twice as many cells to be malignant than Numbat across the dataset, with significant variability observed between the tools' predictions for individual samples.
Conclusion
While CNVs show promise for identifying malignant AML cells, a few significant obstacles remain, namely determining the most accurate and sensitive method to label CNV profiles as malignant or not. Finally, the discordant results emphasise that additional features such as an LSC/AML-specific gene-expression signature should be incorporated to improve the precision of the malignancy identification method, which will be the focus of future work.

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P16
VAEs with Learnable Priors for Learning a Robust Latent Space Representation of Single-Cell RNA Sequencing Data
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In recent years, single-cell RNA sequencing data analysis has allowed us to gain insides into many mechanisms of diseases on a cellular level. Analyzing this data has been complicated by so-called batch effects, non-biological factors influencing the experimental readout. Removing these effects is crucial in order to analyze the biological signals in the data.
One method used successfully to achieve this, is using Variational Autoencoders learning a latent representation of the data. These models have been shown to have limitations using the fixed Standard Normal prior, while learnable priors have been identified as a promising approach to mitigate to these problems.
The main goal of this work was to investigate the potential of learnable priors to improve the latent space learned by Variational Autoencoders with regard to batch integration and biological variance conservation. In order to do this, we implemented the Mixture of Gaussians, Vamp, and Normal Flow priors for the scVI model and benchmarked the learned latent space. In addition to the main goal, we investigated the behavior of the latent space during the training and the impact of scaling of the KL-term and prior parameters on benchmarks. We conducted the experiments mainly on healthy cells, but experimented with our model on datasets with malignant cells as well.
All in all, our work demonstrated that the learnable priors achieve better batch integration while achieving similar performance on biological variance conservation compared to the Standard Normal prior. Additionally, the learnable priors were shown to enable the model to improve batch integration continuously during training. Furthermore, we were able to utilize the number of epochs trained and the scaling of the KL-term to trade off biological variation conservation and batch integration. Overall, we can conclude that learnable priors, in particular, the Mixture of Gaussians prior can significantly improve the learned latent space and should be further investigated to further single-cell RNA sequencing data analysis.

P17
Proteomic analysis of cancer samples
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P18
Understanding the Role of the PI3K/AKT Pathway in Melanoma Microenvironment Remodeling
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Melanoma is one of the most aggressive forms of skin cancer, characterized by high immunogenicity, which enables cancer cells to initiate or influence the adaptive immune response. The tumor suppressor gene Phosphatase and Tensin Homolog (*PTEN*) plays a critical role in regulating the PI3K-AKT pathway within melanoma, and its absence or dysfunction increases cell proliferation while decreasing cell death. Therefore, the project's objective is to gain deeper insights into cell communication within the melanoma tumor microenvironment (TME) in the absence of *PTEN*.

The project plan entails the development of an in vitro 3D model. This model aims to elucidate how the PI3K/AKT pathway impacts the TME by co-culturing CRISPR/Cas9 patient-derived melanoma cell lines with isolated immune cells (T and B cells) and human dermal fibroblasts (HDF). Furthermore, it involves investigating transcriptional variations at the single-cell level among melanoma cells and their TME using single-cell RNA sequencing (scRNA-Seq). Additionally, we will explore spatial transcriptional changes within fresh-frozen sections of 3D matrices, shedding light on how melanoma alters its transcriptional profile as it invades its surrounding environment. The

subsequent step involves the development of an *in silico* model to predict crucial signaling pathways between interacting cells, encompassing both juxtacrine (cell-to-cell contact) and paracrine (cell-to-cell signaling through soluble factors) interactions.

In conclusion, our work aims to enhance our understanding of the PI3K/AKT pathway in melanoma, with the ultimate goal of discovering novel approaches to control the growth of melanoma tumors.

P19

Using scATAC and scRNA-seq for Enhanced Biological Interpretation

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Recent advancements in single-cell omics technologies have revolutionized our understanding of the cellular world. Single-cell RNA sequencing (scRNA-seq) has allowed for the characterization of gene expression profiles at the single-cell level, providing valuable insights into cell type identification and transcriptional dynamics. Similarly, single-cell ATAC-seq (scATAC) has enabled the assessment of chromatin accessibility landscapes in individual cells, shedding light on regulatory elements and their activity. However, each of these techniques alone has limitations in comprehensively elucidating cellular states and functions. In this study, we present an analytical strategy that enhance the biological interpretation of single-cell data.
In the context of hippocampus development in mouse, scATAC and scRNA-seq data where sequenced and analyzed to obtain cell type classification and information on both gene expression profiles on one side and regulatory element accessibility such as enhancers and promoters on the ohter. To obtain a better understanding of cells state, we then associated regulatory element with specific gene expression patterns. Indeed, we used scATAC data to explore epigenetics changes that can explain transcriptional changes that we previously saw in scRNA data. The correlation of transcriptional changes to chromatin accessibility (or TF binding) give an insight into the regulatory mechanisms underlying cell state transitions and differentiation.
Ultimately, comparing the separate information of scATAC and scRNA-seq (via ArchR, Seurat or Scanpy for example) can lead to improved biological interpretation of single-cell data, allowing for a deeper understanding of cellular differentiation trajectories, and regulatory networks in complex biological systems. In the future, we aim to compare the separate analysis (with combination of the interpretation) described here to a full integrative approach (multiome or scATAC enrichment of scRNAseq).

P20

FAIR Data Management: Principles, Challenges and Solutions

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Keywords: FAIR data management, data sharing, data governance, research data, code availability.
The management of research data plays a crucial role in ensuring its discoverability, accessibility, interoperability, and reusability. In this work, we present the key concepts and principles of FAIR data management and discuss the challenges associated with its implementation. FAIR is an acronym for Findable, Accessible, Interoperable, and Reusable and represents a set of guiding principles that enable effective data sharing and utilization across disciplines.

We highlight the importance of making data and code findable by assigning persistent identifiers (like DOI), creating comprehensive metadata. It emphasizes the significance of accessibility through the provision of clear licensing and data preservation practices (often open access). Additionally, the interoperability aspect is discussed, with data standardization and ontologies strategies to facilitate seamless data integration and exchange.
The challenges in implementing FAIR data management are also addressed. These challenges encompass issues related to data privacy, security, as well as, time required for data preparation. We outline various strategies and solutions to overcome them, such as development of data management plans, and the adoption of data management tools (ELN, Fredato …).
In conclusion, we provide a comprehensive overview of FAIR data management principles, explores the associated challenges, and offers solutions for their implementation. By embracing FAIR data management practices, researchers and institutions can unlock the full potential of their data, increase their research diffusion, and enable broader societal impacts.

P21
Interactions between non-coding RNAs, mRNAs and RNA-binding proteins as facilitators of post-transcriptional regulation of gene expression in bacteria
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RNA-binding proteins are a ubiquitous class of proteins found in prokaryotes and eukaryotes, and they regulate RNA lifecycle including transcription, post-transcriptional regulation, translation, and stability. Thus, they possess well-defined RNA-binding domains that allow non-coding RNA recognition and interaction with their target mRNA.
Extensive research in eukaryotes has been conducted on RNA-binding proteins molecular properties, their interactions with non-coding RNAs, and their target mRNAs properties to understand post-transcriptional regulation of gene expression. However, their relevance in bacteria is still a conundrum. Advancements in profiling methods identified novel functions of RNA-binding proteins in bacteria in addition to protein synthesis including, post-transcriptional gene regulation to overcome environmental stress, and genome defense. Novel discoveries revealed non-canonical RNA-binding domains, suggesting unidentified modes of RNA recognition and interaction leading to novel functions of RNA-protein interactions.
The Hess group has investigated the role of regulatory small RNAs in cyanobacteria, a photosynthetic prokaryote, and analysis showed their involvement in oxygenic photosynthesis, nitrogen assimilation, and bacterial cell differentiation. However, little is known about RNA chaperone and other novel unidentified RNA-binding proteins involvement in non-coding RNA regulation.
In this project, we aim to characterize the RNA structure, proteins, and protein domains previously identified by Hess group in cyanobacteria model system "Synechocystis sp. PCC 6803". To investigate unknown RNA-protein interactions and understand their function, previously identified unknown RNA chaperone candidates will be analyzed under different growth conditions using bioinformatics and experimental approaches including RNA-seq, iCLIP analysis of bound RNAs and biochemical characterization.

P22

Tackling current limitations in modeling cell-cell communication

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Single-cell RNA sequencing has paved the way for modeling cellular heterogeneity at single-cell resolution. Recently, there has been increased interest in investigating cell-cell communication on top of identifying cell clusters. Cell-cell communication plays a critical role in several aspects, including cell differentiation, functioning, and organization in the tissue. Yet, there are still major limitations in methods for disentangling cell-cell communication. For example, methods are based on fixed cell clusters, mostly do not incorporate the spatial arrangement of cells, and cannot deal with multiple data modalities, such as single-cell Assay for Transposase-Accessible Chromatin data in addition to RNA-sequencing data. As a first step, we will evaluate the most prominent approaches to see how well they are suited for lifting these limitations. To enable a systematic evaluation, we will work with artificially modified real and synthetic data. Subsequently, we will develop methods that follow the more general principles of faithfully modeling distributions to reflect uncertainty in results and of not relying on rigid assumptions such as cluster memberships. We will do so via end-to-end modeling, where different model components can be optimally fitted simultaneously.

P23

DOT1L deletion impairs the development of cortical parvalbumin-expressing interneurons

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The cortical plate is composed of excitatory and inhibitory interneurons, the latter of which originate in the ganglionic eminences. From their origin in the ventral telencephalon they migrate during embryonic development over large distance to reach their final destination in the cortical plate. DOT1L, a histone methyltransferase, is necessary for proper cortical plate development and layer distribution of glutamatergic neurons, however, its specific role on interneuron development has not yet been explored. Here, we demonstrate that DOT1L affects interneuron development in a cell-autonomous manner. Deletion of *Dot1l* in Nkx2.1-expressing interneuron precursor cells results in an overall reduction and altered distribution of GABAergic interneurons in the cortical plate at postnatal day (P) 0. Furthermore, there is an altered proportion of GABAergic interneurons in the cortex at P21 with a significant decrease in the proportion of Parvalbumin interneurons. Moreover, an increased fraction of cells exiting the cell cycle and a decreased number of mitotic cells at the embryonic day E14.5 was observed upon Dot1l deletion. Altogether, our results indicate that reduced numbers of cortical interneurons upon DOT1L deletion result from premature cell cycle exit, but effects on postmitotic differentiation, maturation, and migration are likely at play as well.