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11TH **BERLIN LATE SUMMER MEETING**

OCTOBER 25 – 27, 2018

PROGRAM & ABSTRACTS

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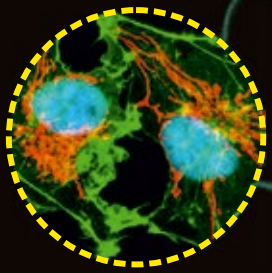

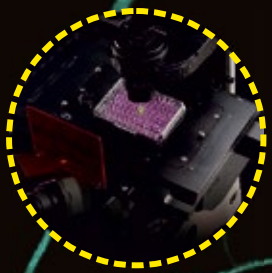

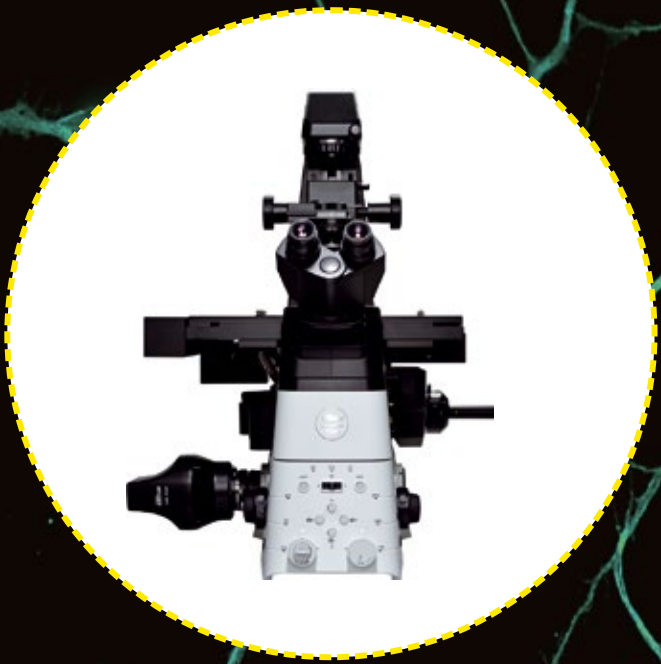

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WELCOME TO THE

11TH BERLIN LATE SUMMER MEETING

OCTOBER 25 – 27, 2018

A conference organized by the Berlin Institute for Medical Systems Biology (BIMSB) at the Max Delbrück Center for Molecular Medicine in the Helmholtz Association, funded by:

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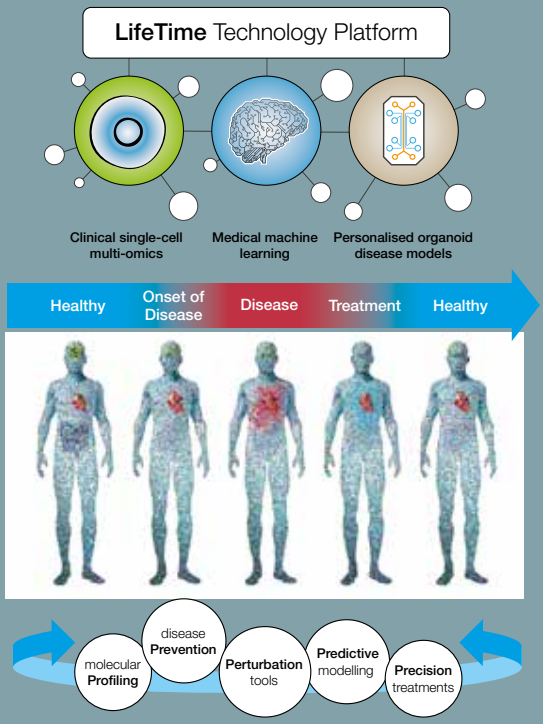
CONTENTS

LifeTime

Revolutionising Healthcare by Tracking and Understanding Human Cells during Disease

FIND US AT POSTER 135



Our Vision

LifeTime aims to track and decipher the activity of our genomes - our "book of life" - in individual cells as they progress through time.
Its unifying goal is to quantify and model cellular trajectories in health, to detect and predict their deviation during disease and to find targeted perturbations to steer disease trajectories back to health. LifeTime wants to inform the physician about the molecular history and future of a patient's tissues, and the consequences of medical treatments, leading to early diagnosis and effective interception of disease.

The Consortium

LifeTime was founded by researchers from >50 leading research institutions with partners across 18 European countries. The consortium is co-chaired by Nikolaus Rajewsky (Max Delbrück Center for Molecular Medicine) and Geneviève Almouzni (Institut Curie).

Join LifeTime > lifetime-fetflagship.eu

CONFERENCE INFORMATION

Conference Venue

Langenbeck-Virchow-Haus
Luisenstraße 58/59
10117 Berlin

Date
Thursday, October 25 to Saturday, October 27, 2018

Organization & Contact
Galina Hoppe, Verena Maier, Irena Perko Radulovic
Lien-Georgina Dettmann, Matthias Runow, Timkehet Teffera

Max Delbrück Center for Molecular Medicine
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Homepage:	https://berlin-summer-meeting.org
Twitter:	#bimsb2018

Registration

Regular fee:	350,00 €
Student fee:	150,00 €

The registration fee includes attendance at all scientific sessions, October 25 – 27, conference documents, name badge, final program and abstract book, conference bag, coffee breaks, lunch, social evening and free internet access.

Poster
Posters will be displayed during the meeting in 2 sessions.

Poster numbers 1- 67 will be in Poster Session I.
Poster numbers 68-134 will be in Poster Session II.

Authors are asked to be present at their poster during the poster session. You will find the number of your poster in this abstract volume. Posters, in poster session I, should be mounted on Thursday, 12:00-15:00 h and removed on Thursday at 18:00 h at the latest.

Posters, in poster session II, should be mounted on Friday, 13:00-16:00 and removed on Friday at 19:00 h at the latest.

Internet

Internet access via Wireless LAN is free of charge in the Langenbeck-Virchow-Haus.

The current login and password is provided at the registration desk.

Social Event

Friday, October 26, 2018 | 19:30 – 00:00

Venue

Kalkscheune
Johannisstraße 2
10117 Berlin

BIG PARTY with Hot Live Music, Food, Drinks & Dancing (featuring live band MR ŽARKO followed by DJ Auflegewitsch)

Live Band

MR ŽARKO from Berlin is a Force of Nature, taking Balkan Brass to the next level.

The seven piece band, comprising musicians from Turkey, Bulgaria, Serbia, Tunisia and Germany plays only original compositions that cross the boundaries of musical genres.MR ŽARKO has played in Festivals, such as Rudolstadt (GER), Sziget (Budapest HU), Lowlands (Biddinghuizen, NL) and Nilüfer Festivals (Bursa, TR).

Copyright: Andrey Kezzyn

DJ Auflegewitsch

Datschadance - Eastern European Dance Turmoil

WELCOME ADDRESS

Dear Friends, Colleagues and Guests!

We are delighted to welcome you to the 11th Berlin Summer Meeting – our "Grand BIMSB Opening Symposium". Each of the past 11 Berlin Summer Meetings was devoted to a particular biological topic at the interface between computation and experiment. This year's symposium is different! For the past 10 years, we have been waiting for a dedicated BIMSB research building - we have been incubating at the Campus Berlin-Buch, growing from one scientist to 16 groups and ~250 scientists from many countries and continents. To celebrate the opening of the new building, we have invited 16 scientists who have contributed in truly important ways to our overall scientific education, ideas, and plans. Our Scientific Mission is to integrate the many levels of gene regulation to better understand phenotypes in health and in disease. This mission requires bridging boundaries in many ways - scientists from different areas need to collaborate intensely, new interdisciplinary methods have to be developed, and computational and experimental approaches must truly come together. Our Motto is therefore "Breaking Boundaries"- you will see this alive on many levels - perhaps most and literally visibly in the beautiful light art "SPLASH" by Berlin-based artist Barbara Trautmann, installed on the roof garden and illuminated at night.

Our scientific mission has defined the basic functional features of our new building, and we thank the Team of Volker Staab Architects and our Department of Construction for having the motivation and patience to make this dream a reality. Group leader offices are in close physical proximity, communication hubs are inserted throughout the building, the labspace is shared between labs, computer and bench are only separated by a few steps. Moreover, the location on the Life Science Campus of the Humboldt University, a stone throw away from the Medical University/Hospital Charité, is crucial for our future scientific and educational plans. We believe that with the expansion of the MDC with BIMSB to Mitte, many new opportunities arise not only for us, but also for the MDC at large.

We are looking forward to a rewarding, memorable, interactive, and last but not least fun Berlin Summer Meeting 2018. Don't miss the evening party on October 26 - we present superb, fast playing Eastern European musicians who will make you dance.

Please see the “Thank you” page with all names listed - too many for here.

Enjoy the Meeting,

The BIMSB Group Leaders

Altuna Akalin, Marina Chekulaeva, Jan Philipp Junker, Stefan Kempa, Markus Landthaler, Darío Lupiáñez, Irmtraud Meyer, Uwe Ohler, Ana Pombo, Stefan Preibisch, Nikolaus Rajewsky, Sascha Sauer, Roland Schwarz, Baris Tursun, Andrew Woehler and Robert Zinzen

THANK YOU!

A heartfelt “thank you” goes to the many people involved in the organization of this year’s Berlin Summer Meeting and the realization of the new BIMSB building.

Berlin Summer Meeting 2018

Irena Perko-Radulovic, Verena Maier, Jutta Steinkötter, Galina Hoppe, Lien Dettmann, Timkehet Teffera Mekonnen, Matthias Runow, Fouad El-Haj, BIMSB scientists & employees

Conception, Planning and Construction of the New BIMSB Building

MDC Construction Department

Volker Staab Architects

Nikolaus Rajewsky

Jutta Steinkötter

Eurolabors (Lab Design)

Winter Ingenieurs (HVAC Design)

Schröder and Partner (Electricity Design)

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PROGRAM

THURSDAY, OCTOBER 25TH, 2018

08:30 – 09:00	Registration & Breakfast
09:00 – 09:10	Welcoming Address **Martin Lohse** (Scientific Director, MDC) **Peter Frensch** (Vice President for Research, HU Berlin) **Karl Max Einhäupl** (Chief Executive Officer, Charité – Universitätsmedizin Berlin)
09:10 – 09:25	Introduction by **Nikolaus Rajewsky** (Scientific Head, BIMSB)
09:25 – 12:00	**SESSION 1: GENE REGULATION DURING DEVELOPMENT** **CHAIR: ROBERT ZINZEN**
09:25 – 10:10	**Christiane Nüsslein-Volhard**, Max Planck Institute for Developmental Biology, Tübingen, Germany How fish colour their skin: A paradigm for development and evolution of adult patterns
10:10 – 10:30	**COFFEE BREAK**
10:30 – 11:15	**Jürgen Knoblich**, Institute of Molecular Biotechnology, Vienna, Austria Cerebral organoids: modelling human brain development and tumorigenesis in stem cell derived 3D culture
11:15 – 12:00	**Claude Desplan**, NYU, USA The development of the Drosophila motion vision circuit
12:00 – 15:00	**LUNCH BREAK & Poster session I** Guided tours of the new BIMSB building will be offered
15:00 – 19:00	**SESSION 2: PLURIPOTENCY AND DIFFERENTIATION** **CHAIR: ANA POMBO**
15:00 – 15:45	**Edith Heard**, Institute Curie, Paris, France Exploring the dynamic relationship between gene expression and chromosome organisation during X-chromosome inactivation
15:45 – 16:00	**Manuel Irimia**, Centre for Genomic Regulation, Barcelona, Spain Amphioxus functional genomics reveals the evolution of vertebrate regulatory traits

16:00 – 16:45	**Ruth Lehmann**, NYU School of Medicine, New York, USA Protecting Immortality: Germ granule organization by phase transition
16:45 – 17:15	**COFFEE BREAK**
17:15 – 18:00	**Amanda Fisher**, MRC London Institute of Medical Sciences, United Kingdom Epigenetics and inheritance
18:00 – 18:15	**Leonie Ringrose**, Humboldt University Berlin, Germany Comprehensive theoretical analysis of the Polycomb – Trithorax system predicts that poised chromatin is robustly bistable and minimally bivalent
18:15 – 19:00	**Bing Ren**, UCSD School of Medicine, San Diego, USA Remodeling of Chromatin Architecture during Human Cardiomyocyte Differentiation

FRIDAY, OCTOBER 26TH, 2018

08:30 – 09:00	Registration & Breakfast
09:00 – 12:45	**SESSION 3: MOLECULAR MECHANISMS OF GENE REGULATION IN HEALTH AND DISEASE 1** **CHAIR: MARINA CHEKULAEVA**
09:00 – 09:45	**Patrick Cramer**, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany A molecular mechanism for RNA polymerase II activation
09:45 – 10:45	**BIMSB PI Presentation** Hosts: Laura Breimann and Marvin Jens
10:45 – 11:15	**COFFEE BREAK**
11:15 – 12:00	**Lior Pachter**, California Institute of Technology, Pasadena, USA Computational and experimental foundations for single-cell genomics
12:00 – 12:45	**Charles Swanton**, The Francis Crick Institute, London, United Kingdom Cancer Evolution and Immune Escape: TRACERx

12:45 – 13:00	Group Photo
13:00 – 16:00	**LUNCH BREAK & Poster session II** Guided tours of the new BIMSB building will be offered
16:00 – 19:15	**SESSION 4: MOLECULAR MECHANISMS OF GENE REGULATION IN HEALTH AND DISEASE 2** **CHAIR: ROLAND SCHWARZ**
16:00 – 16:45	**Mike Levine**, Princeton University, Princeton, USA A high-resolution view of gene activity during development
16:45 – 17:00	**Jane Reznick**, Max Delbrück Center for Molecular Medicine, Berlin, Germany A switch to fructose metabolism under extreme hypoxia in the naked mole-rat
17:00 – 17:30	**COFFEE BREAK**
17:30 – 18:15	**Hans Clevers**, Hubrecht Institute, Utrecht, The Netherlands Lgr5 Stem Cell-based organoids in human disease
18:15 – 18:30	**Giuseppe Testa**, University of Milan and European Institute of Oncology, Milan, Italy A novel computational approach to single-cell transcriptomics uncovers the dynamics of tumor stem cells in ovarian cancer organoids
18:30 – 19:15	**Peter Lichter** – German Cancer Research Center, Heidelberg, Germany Newly emerging mechanisms of genomic instability and their impact on tumor evolution
19:15 – 19:30	Walk to Kalkscheune
19:30	BIG PARTY with Hot Live Music, Food, Drinks & Dancing

SATURDAY, OCTOBER 27TH, 2018

08:30 – 09:00	Registration & Breakfast
09:00 – 12:30	**SESSION 5: SINGLE-CELL APPROACHES TO MOLECULAR MECHANISMS AND FUNCTION** **CHAIR: BARIS TURSUN**
09:00 – 09:45	**Ido Amit**, Weizmann Institute of Science, Rehovot, Israel Single-cell genomics: a stepping stone for future immunology discoveries

09:45 – 10:00	**Nikos Karaiskos**, Max Delbrück Center for Molecular Medicine, Berlin, Germany De novo spatial reconstruction of single-cell gene expression
10:00 – 10:45	**Eileen Furlong**, The European Molecular Biology Laboratory, Heidelberg, Germany Understanding enhancer usage during embryonic development at a single cell level
10:45 – 11:15	**COFFEE BREAK**
11:15 – 11:30	**Florian Erhard**, Würzburg University, Germany Record-seq reveals core features of transcription dynamics in single cells
11:30 – 12:15	**Phil Sharp**, Massachusetts Institute of Technology, Cambridge, USA RNA regulates transcription through phase transitions?
12:15 – 12:30	**Walter Birchmeier**, Max Delbrück Center for Molecular Medicine, Berlin, Germany
12:30 – 13:00	Meeting Wrap-Up & Suprise Performance
13:00	Farewell Lunch

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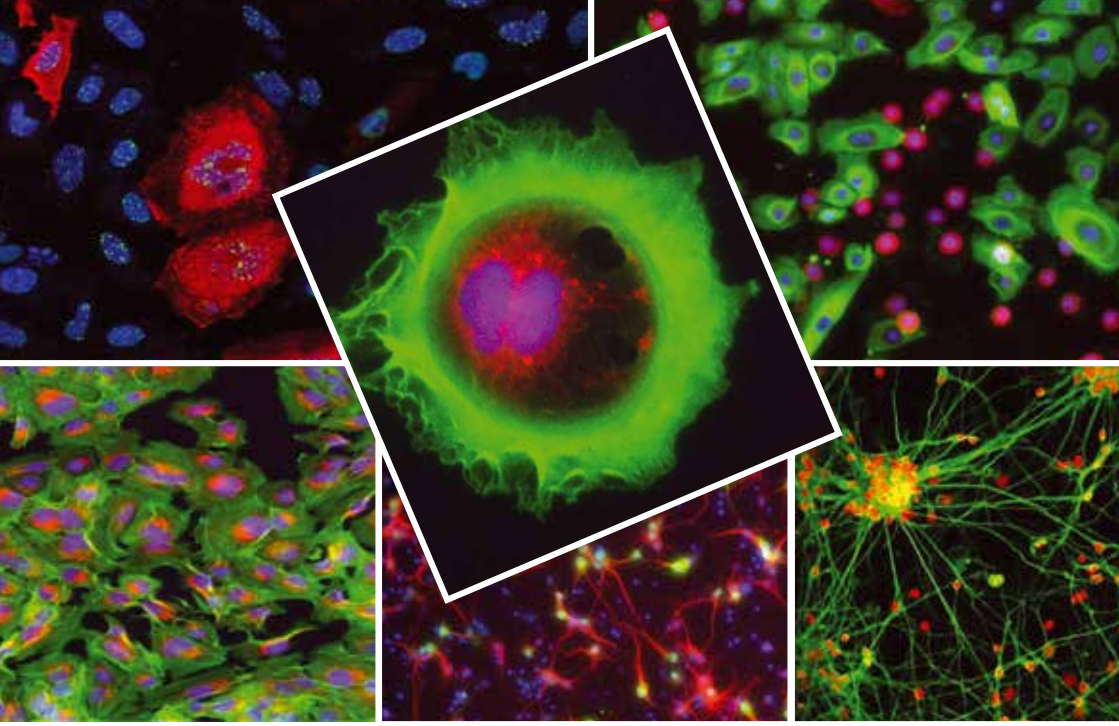

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SPEAKER ABSTRACTS

(IN ORDER OF THE TALKS IN THE PROGRAM SCHEDULE)

CHRISTIANE NÜSSLEIN-VOLHARD

Max Planck Institute for Developmental Biology, Tübingen, Germany

How fish colour their skin: A paradigm for development and evolution of adult patterns

Colour patterns are prominent features of most animals; they are highly variable and evolve rapidly leading to large diversities between species even within a single genus. As targets for natural as well as sexual selection, they are of high evolutionary significance. The zebrafish (Danio rerio) displays a conspicuous pattern of alternating blue and golden stripes on the body and on the anal- and tailfins. Pigment cells in zebrafish – melanophores, iridophores and xanthophores – originate from neural crest-derived stem cells associated with the dorsal root ganglia of the peripheral nervous system. Clonal analysis indicates that these progenitors remain multipotent and plastic beyond embryogenesis well into metamorphosis, when the adult colour pattern develops. Pigment cells share a lineage with neuronal cells of the peripheral nervous system; progenitors spread along the spinal nerves. The proliferation of pigment cells is regulated by competitive interactions among cells of the same type. An even spacing involves collective migration and contact inhibition of locomotion of the three cell types distributed in superimposed monolayers in the skin. This mode of colouring the skin is probably common to fish, whereas different patterns emerge by species specific cell interactions among the different pigment cell types. These interactions are mediated by channels involved in direct cell contact between the pigment cells, as well as unknown cues provided by the tissue environment.

The colour patterns in closely related Danio species are amazingly different; their variation offers a great opportunity to investigate the genetic and developmental basis of colour pattern evolution in vertebrates. Exciting technical developments of the recent years, especially next-generation sequencing technologies and the novel possibilities of genome editing with the CRISPR/Cas9 system, allow to easily expand from model organisms into other species and directly test the function of genes by targeted knock outs and allele replacements. Thus, models and hypotheses about pigment pattern formation derived from zebrafish can now be tested in other Danio species. These studies will lay the foundation to understand not only the genetic basis of colour pattern variation between Danio species, but also the evolution of colour patterns in other vertebrates.

References:

Irion, U., Singh, A. P. and Nüsslein-Volhard, C. (2016): The developmental genetics of vertebrate colour pattern formation: Lessons from Zebrafish. Current Topics Developmental Biology, http://dx.doi.org/10.1016/bs.ctdb.

Nüsslein-Volhard, C. and Singh, A. P. 2017, Bioessays 39: 3, March 2017

NOTES

JÜRGEN KNOBLICH

Institute of Molecular Biotechnology of the Austrian Academy of Science, Vienna, Austria

Cerebral organoids: modelling human brain development and tumorigenesis in stem cell derived 3D culture

The human brain is unique in size and complexity, but also the source of some of the most devastating human diseases. While many of these disorders have been successfully studied in model organisms, recent experiments have emphasized unique features that cannot easily be modeled in animals. We have therefore developed a 3D organoid culture system derived from human pluripotent stem cells that recapitulates many aspects of human brain development. These cerebral organoids are capable of generating several brain regions including a well-organized cerebral cortex. Furthermore, human cerebral organoids display stem cell properties and progenitor zone organization that show characteristics specific to humans. We have used patient-specific iPS cells to model microcephaly, a human neurodevelopmental disorder that has been difficult to recapitulate in mice. This approach reveals premature neuronal differentiation with loss of the microcephaly protein CDK5RAP2, a defect that could explain the disease phenotype. More recently, we have been able to generate organoid-based models for human brain cancer and demonstrated their feasibility for drug testing. Our data demonstrate an in vitro approach that recapitulates development of even this most complex organ, which can be used to gain insights into disease mechanisms.

NOTES

CLAUDE DESPLAN

Center for Developmental Genetics, Department of Biology, New York University, New York, USA

The development of the Drosophila motion vision circuit

The Drosophila optic lobes receive retinotopic inputs from photoreceptors specialized in motion vision (lamina), or color and polarized light vision (medulla). At least 100 types of neurons in the optic lobes process these inputs for extracting visual information. They are organized in 800 columns corresponding to the 800 unit eyes in the retina (ommatidia). How is this variety of neurons generated and how is retinotopy established?

Neural stem cells that produce neurons in the medulla, the main part of the optic lobes, sequentially express six transcription factors in a temporal manner. Different neurons emerge in each temporal window, therefore generating a series of 800 neurons of each type: These 'Uni-columnar neurons' are generated throughout the neuroepithelium and have a 1:1 stoichiometry with the photoreceptors that innervate the medulla. The less numerous 'multi-columnar' neurons that have larger receptor fields and are present at a lower stoichiometry with photoreceptors emerge from the same neural stem cells but differ in distinct regions of the medulla neuroepithelium. In spite of their restricted origins, these neurons still contribute to the entire retinotopic map through dispersion of their cell bodies. Therefore, the generation of 80 cell types involves the integration of temporal and spatial patterning that preserves retinotopy of neurons present at different stoichiometry.

Once neurons are generated, they must incorporate into a neuronal network that relies on precise connectivity between neurons. Understanding the genetic control of the complexity of brain wiring during development is one of the greatest challenges in neurobiology. In the optic lobe, the direction-selective T4 and T5 neurons represent a conspicuous example of such complex retinotopic organization. Within each column, there are four subtypes of T4 and of T5 neurons that receive their inputs in the medulla (T4, which respond to the motion of a bright edges) or lobula (T5, which respond to the motion of a dark edges). Each T4 and T5 subtype responds to one direction of local motion along each of the four cardinal directions (vertical up-down and down-up, and horizontal front-to-back and back-to-front). We investigated how the identity of the four subtypes of T4 and of T5 neurons is specified and how their retinotopic organization is established. I will show that T4 and T5 neurons are produced by a specific type of neurogenesis: Vertical and horizontal motion sensitive T4/T5 neurons

originate from two distinct neuroprogenitors. These divide in an asymmetric Notch-dependent manner to produce two distinct intermediate progenitors that generate neurons sensitive to opposite directions (e.g. front-to-back or back-to-front). The intermediate progenitors then divide one last time, also in a Notch-dependent manner, producing sibling T4 (NotchOFF) and T5 (NotchON) neurons of the same subtype. I will propose a model in which retinotopy results from patterns established by neuronal birth order. This illustrates how a complex neuronal organization can be implemented by simple developmental rules.

NOTES

EDITH HEARD

Institut Curie – Centre de Recherche, Unité de Génétique et Biologie du Devéloppement, Paris, France

Exploring the dynamic relationship between gene expression and chromosome organisation during X-chromosome inactivation

X-chromosome inactivation during early female development is an essential epigenetic process that is required to achieve appropriate dosage for X-linked gene products. We are interested in understanding how the differential treatment of the two X chromosomes in the same nucleus is set up during development and how this differential expression is then maintained, or reversed in certain circumstances such as the inner cell mass of the mouse embryo or in the germ line. The establishment of X inactivation involves the non-coding Xist RNA that triggers chromosome-wide chromatin re-organisation and gene silencing. Recent insights have been made into the nature of these chromosome-wide changes, including the global loss of topologically associated domains (TADs)1,2,3. However little is known about the underlying mechanisms and the precise relationship between 3D chromosome structure and altered gene expression states on the X chromosome. Results of our recent studies, using a combination of single-cell chromosome-conformation capture technologies and high-resolution microscopy in differentiating embryonic stem cells and *in vivo* mouse embryos, will be presented where we have investigated (i) the degree to which 3D chromatin organization into topologically associated domains (TADs) is involved in monoallelic Xist regulation during development and (ii) the relationship between 3D structure and regional escape from X inactivation on the inactive X chromosome.

References:

1. Nora, E.P. et al (2012). Spatial partitioning of the regulatory landscape of the X-inactivation centre. Nature 485, 381–385.
2. Giorgetti, L. et al (2014) Predictive polymer modeling reveals coupled fluctuations in chromosome conformation and transcription. Cell, 157: 950–963
3. Giorgetti L., Lajoie, B., Carter, A.C., Attia, M. et al (2016) Structural organization of the inactive X chromosome. Nature 535: 575-579.

NOTES

MANUEL IRIMIA[5]

F. Marletaz[1], P. N. Firbas[2], I. Maeso[2], J. Tena[2], O. Bogdanovic[3], M. Perry[4], C. Wyatt[5], B. lanceolatum Genome Consortium, R. Lister[6], B. Lenhard[4], P. Holland[1], H. Escriva[7], J. L. Gomez-Skarmeta[2]

[1]University of Oxford, Oxford, United Kingdom,
[2]Centro Andaluz de Biología del Desarrollo, Sevilla, Spain,
[3]Garvan Institute of Medical Research, Sydney, Australia,
[4]Imperial College London, London, United Kingdom,
[5]Centre for Genomic Regulation, Systems Biology Unit, Barcelona, Spain,
[6]The University of Western Australia, Perth, Australia,
[7]Sorbonne Universités, Banyuls/Mer, France

Amphioxus functional genomics reveals the evolution of vertebrate regulatory traits

All chordates share a fundamental body plan that was greatly elaborated in vertebrates. Vertebrates also evolved highly distinctive genomes, sculpted by two whole genome duplications (WGD). To investigate the evolution of genome regulation in chordates, we characterized promoters, methylation, chromatin accessibility, histone modifications and transcriptomes in multiple tissues and throughout development of the cephalochordate amphioxus. These data revealed multiple striking results. Amphioxus seems to represent an intermediate stage in the evolution of differentially methylated enhancers: although its genome is largely unmethylated (as non-vertebrates), it presents adult-specific enhancers whose activation is associated with demethylation (as vertebrates). Second, we found high conservation of gene expression and its underlying cis-regulatory logic between amphioxus and vertebrates, maximally at a developmental phylotypic period. We also unraveled the principal route of regulatory evolution following WGD. We found that over 80% of gene families with multiple paralogs in vertebrates have members that restricted their ancestral expression. However, we found that specialization of specific paralogs rather than subfunctionalization (as defined by the DDC model) is the major fate for multi-gene families. Counter-intuitively, vertebrate genes that underwent expression restriction increased the complexity of their regulatory landscapes. Taken together, our results indicate that the two rounds of WGD not only caused an expansion and diversification of gene repertoires in vertebrates, but also allowed functional and expression specialization of the extra copies by increasing the complexity of their gene regulatory landscapes. In summary, these data pave the way for a better understanding of the regulatory principles underlying key vertebrate innovations.

NOTES

RUTH LEHMANN

Skirball Institute, NYU School of Medicine, New York, USA

Protecting Immortality: Germ granule organization by phase transition

Germ cells are the only cell in the body that are able to generate an entire new organisms. The distinction between germ cell lineage and somatic cells is important to preserve generational continuity. In Drosophila germ cells form in a specialized cytoplasmic region, the germ plasm. During the last 30 years genetic screens and molecular analysis have provided us with a parts list of proteins and RNAs that are important for the assembly and function of germ granules, the specialized ribonucleoprotein (RNP) granules of the germ plasm. Nevertheless, we still have only a limited understanding of how germ granules are structured and how this structure may influence the timed translation of RNAs localized to the germ plasm. We have begun to analyze the mRNA and protein components of the germ plasm combining super-resolution microscopy and biophysical characterization and found that cytoplasmic and nuclear granules form by phase transition initiated by the assembly of particles containing the short isoform of the Oskar protein. Within the protein scaffold of the cytoplasmic granules, RNAs organize at discrete positions by RNA-RNA self-organization into homotypic clusters. We are exploring the mechanisms underlying protein-protein and RNA-RNA demixing and sorting within this specialized cytoplasmic environment.

NOTES

AMANDA FISHER

Dounia Djeghloul, Chiara Prodani, Karen Brown, Andrew Dimond,
Holger Kramer, Matthew Van de Pette, Matthias Merkenschlager

MRC Clinical Sciences Centre,
Imperial College London, Hammersmith Hospital, London, UK

Epigenetics and inheritance

We are interested in how epigenetic identity is established and how this information is transmitted through cell division and across generations. To tackle these important questions we have developed some new approaches that span a range of different experimental scales. In the first approach we assess the repertoire of proteins that remain bound to individual chromosomes during mitosis. This reveals a cadre of proteins that may be important for re-establishing cellular identity (so-called bookmarking factors), or mediating transition (pioneer factors), in daughter cells. By performing experiments with cells that lack specific chromatin components, we can ask whether these factors require a specific chromatin context to bind and operate. The second approach uses a series of luciferase-based mouse reporters that were designed to detect the allelic expression of different imprinted and X-linked genes. These animals enable us to non-invasively image epigenetic changes that occur during normal development and in response to specific exposures. Our experiments show that maternal over- and under-nutrition can fundamentally alter the epigenetic profile of the developing embryo and suggest that once induced, these alterations can be retained throughout life.

NOTES

LEONIE RINGROSE[2]

K. Sneppen[1]

[1]University of Copenhagen, Nils Bohr Institute, Copenhagen, Denmark,
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Comprehensive theoretical analysis of the Polycomb – Trithorax system predicts that poised chromatin is robustly bistable and minimally bivalent

Polycomb (PcG) and Trithorax (TrxG) proteins give stable epigenetic memory of silent and active expression states for several hundred target genes, but also allow poised states for many of those same genes in pluripotent cells. It has been proposed that poised chromatin at PcG/TrxG target genes is bivalent, simultaneously carrying active and silent histone modifications, that are later resolved to purely active or silent forms. PcG/TrxG biochemistry is exquisitely complex, and a coherent theoretical framework has been lacking. Thus it has been difficult to formalise observations of individual modifications and enzymatic reactions into a coherent whole. To systematically address the relationship between poised, active and silent chromatin, we integrated 64 publications on PcG/TrxG biochemistry into a mathematical model, comprising 12 nucleosome states, 40 transitions and 8 enzymatic reactions. We explored dynamic system properties using stochastic simulations. The model predicts that poised chromatin is robustly bistable and minimally bivalent. Bivalent chromatin, containing opposing active and silent modifications, is present as a rare and unstable background population in all system states. Specific forms of bivalent chromatin co-occur with active and silent states. In contrast, bistability, in which the system switches frequently between stable active and silent states, occurs under a wide range of conditions. Bistable chromatin typically represents an essential transition between monostable active and silent system states. Importantly, poised bistable chromatin differs from monostable modes only in its higher frequency of switching. We show that several published observations strongly support the model predictions. By proposing that bistability and not bivalency is associated with poised chromatin, this work has profound implications for understanding the molecular nature of pluripotency.

NOTES

BING REN

Department of Cellular and Molecular Medicine, UCSD School of Medicine, Ludwig Cancer Research, La Jolla, USA

Remodeling of Chromatin Architecture during Human Cardiomyocyte Differentiation

Restructuring of chromatin architecture is an essential process for establishing cell type-specific gene regulatory programs in eukaryotic cells including cardiomyocytes. Supporting its importance, recent studies have reported that a substantial number of mutations discovered in congenital heart disease (CHD) patients reside in genes encoding chromatin remodeling factors; yet, how chromatin structure reorganizes to assemble gene regulatory networks crucial for controlling human cardiomyocyte development remains to be elucidated. Here, we reveal novel molecular insights into how human PSC chromatin architecture is iteratively remodeled to build gene regulatory networks directing cardiac lineage specification through comprehensively analyzing high-resolution genomic maps that detail the dynamic changes of chromatin architecture, chromatin accessibility and modifications, and gene expression during human pluripotent stem cell (PSC) cardiomyocyte differentiation. Specifically, we uncover a new class of human PSC-specific topologically associating domain (TAD) that is created by the active transcription of HERV-H primate-specific endogenous retrotransposons. Silencing of these HERV-Hs during the initial stages of human PSC differentiation or by genome-editing results in the elimination of corresponding TAD boundaries and reduced transcription of genes upstream of lost TADs. Supporting their role in maintaining pluripotency, we discovered that genome-edited deletion of specific HERV-Hs leads to accelerated human PSC cardiomyocyte differentiation. Using chromatin interaction maps from these analyses, we also assigned potential target genes to distal regulatory elements involved in cardiac differentiation. Genome-editing of enhancers harboring cardiac-disease risk loci associated with congenital and adult heart diseases further confirmed that these loci regulate predicted target genes. Our results highlight a novel role for HERV-Hs in establishing human-specific PSC chromatin architecture, delineate the dynamic gene regulatory networks during cardiomyocyte development and inform how non-coding genetic variants contribute to congenital and adult heart diseases.

NOTES

PATRICK CRAMER

Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

A molecular mechanism for RNA polymerase II activation

Our laboratory combines structural biology with functional genomics and computational biology to study the mechanisms of gene transcription and its regulation in eukaryotic cells. Recent work includes structural studies of chromatin remodeling by Chd1 (Farnung Nature 2017) and Pol II initiation (Plaschka Nature 2015, 2016; Nozawa Nature 2017; Schilbach Nature 2017). We also developed transient transcriptome sequencing (TT-seq), which canmonitor changes in RNA synthesis and enhancer landscapes at high temporal resolution (Schwalb Science 2016; Demel Mol. Syst. Biol. 2017). I will concentrate on our recent unpublished work and present the structures of paused and activated Pol II elongation complexes (Vos, Farnung et al., unpublished) together with *in vivo* evidence for polymerase pausing during elongation controlling initiation (Gressel, Schwalb et al. Leonhardt, Eick, and Cramer, eLife 2017). The resulting molecular mechanism for Pol II activation supports a large body of work from many groups that was collected over the years on gene regulation.

NOTES

PHIL SHARP

Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, USA

RNA regulates transcription through phase transitions?

The discovery of messenger RNA (mRNA) over fifty years ago presented an intermediate between the repository of genetic information—DNA—and the highly diverse structures and functions of proteins. The constellation of mRNAs in a single cell reveals biological variation at its most fundamental level and the extent of this variation seems to be surprisingly high. From the time of the discovery of mRNA, regulation of gene expression has been assigned almost exclusively to proteins. This changed significantly with RNA becoming a major regulatory factor following the discovery of microRNAs and RNA interference. In vertebrate organisms, the former can be considered the cytoplasmic analogs of protein transcription factors in the nucleus. More recently, non-coding RNAs such as enhancer RNAs and promoter-associated RNAs have been shown to regulate transcription. Many of these probably function through phase transitions facilitating the formation of liquid-liquid type condensates that activate initiation of transcription at promoters. Similar processes are probably involved in selection of exons in RNA splicing. This phase transition process is highly cooperative, dependent mostly upon changes in the valency of interactions and the concentration of components. In these condensates, RNA takes on a new role as a structural regulatory component in the cell. Recent evidence suggests that the activity of super-enhancers in controlling the transcription of genes critical for the development and cancer is mediated through formation of condensates.

NOTES

CHARLES SWANTON

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Cancer Evolution and Immune Escape: TRACERx

Increasing evidence supports complex subclonal relationships in solid tumours, manifested as intratumour heterogeneity. Parallel evolution of subclones, with distinct somatic events occurring in the same gene, signal transduction pathway or protein complex, suggests constraints to tumour evolution that might be therapeutically exploitable. Emerging data from TRACERx, a longitudinal lung cancer evolution study will be presented. Drivers of tumour heterogeneity change during the disease course and contribute to the temporally distinct origins of lung cancer driver events. APOBEC driven mutagenesis appears to be enriched in subclones in multiple tumour types. Oncogene, tumour suppressor gene and drug induced DNA replication stress are found to drive APOBEC mutagenesis. Evidence that intratumour heterogeneity and chromosomal instability is finely tuned will be presented, to create sufficient diversity for adaptation mitigating the risks of excessive genome instability resulting in cell autonomous lethality. On-going chromosomal instability, manifested as Mirrored Subclonal Allelic Imbalance (MSAI) is found to be a major driver of intratumour heterogeneity in non-small cell lung cancer, contributing to parallel evolution and selection. The finding of subclonal driver events, evidence of ongoing selection within subclones, combined with genome instability driving cell-to-cell variation is likely to limit the efficacy of targeted monotherapies, suggesting the need for new approaches to drug development and clinical trial design and integration of cancer immunotherapeutic approaches. The clonal neo-antigenic architecture may act as a tumour vulnerability, targeting multiple clonal neo-antigens present in each tumour to mitigate resistance and treatment failure. The role of cancer genome instability driving immune evasion and HLA/MHC loss and immune escape will be presented.

NOTES

MIKE LEVINE

Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, USA

A high-resolution view of gene activity during development

Transcriptional enhancers regulate the on/off activities of target genes in response to a variety of intrinsic and external signals. The human genome is thought to contain hundreds of thousands of enhancers, an average of 10-20 enhancers per protein coding gene. We are using two model organisms, the fruit fly Drosophila melanogaster and the sea squirt Ciona intestinalis, to understand how enhancers control precise patterns of gene expression during embryonic development.

Live imaging methods provide new opportunities for studying enhancer-promoter communication in the early Drosophila embryo. I will present evidence that enhancer-promoter proximity, not direct contact, is sufficient to activate gene expression. To measure these distances we established a transvection assay, whereby an enhancer located on one chromosome can activate a target gene on the other homologue. These studies suggest that the enhancers work over a distance of about ~250 nm.

Single cell RNA sequencing methods were used to obtain comprehensive gene expression profiles of every cell at every stage during Ciona embryogenesis. These studies identified regulatory determinants responsible for the specification of neuronal cell types, such as coronet cells (rudimentary hypothalamus).

The 4D expression atlas also provides new insights into the evolutionary origins of novel vertebrate cell types, such as neural crest, cranial placodes and the telencephalon.

NOTES

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A switch to fructose metabolism under extreme hypoxia in the naked mole-rat

The naked mole-rat has recently emerged as a rodent model of interest to biomedicine due to its extraordinarily long and healthy lifespan (>36 years). Naked mole-rats tolerate hours of extreme hypoxia and survive 18 minutes of total oxygen deprivation (anoxia) without apparent injury. During anoxia, the naked mole-rat switches to anaerobic metabolism fueled by fructose and sucrose. Global expression of fructose specific transporter GLUT5 and ketohexokinase (KHK) were identified in the naked mole-rat suggesting a rewired transcriptome to enable fructose metabolism across the entire organism. We have combined transcriptomics and *in vivo* stable isotope resolved metabolomics analyses and showed that under hypoxia the naked mole-rat activates the polyol pathway to synthesise fructose in the heart whilst enhancing fructolysis in heart and brain. Furthermore, mouse brain also showed a preference for fructose metabolism under hypoxic conditions. Therefore, fructose dependent metabolism could be protective under hypoxic conditions.

NOTES

HANS CLEVERS

Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences & University Medical Centre Utrecht and Princess Maxima Center for Pediatric Oncology, Utrecht, the Netherlands

Lgr5 Stem Cell-based organoids in human disease

The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. We originally defined Lgr5 as a Wnt target gene, transcribed in colon cancer cells. Two knock-in alleles revealed exclusive expression of Lgr5 in cycling, columnar cells at the crypt base. Using lineage tracing experiments in adult mice, we found that these Lgr5+ve crypt base columnar cells (CBC) generated all epithelial lineages throughout life, implying that they represent the stem cell of the small intestine and colon. Lgr5 was subsequently found to represent an exquisitely specific and almost 'generic' marker for stem cells, including in hair follicles, kidney, liver, mammary gland, inner ear tongue and stomach epithelium.

Single sorted Lgr5+ve stem cells can initiate ever-expanding crypt-villus organoids, or so called 'mini-guts' in 3D culture. The technology is based on the observation that Lgr5 is the receptor for a potent stem cell growth factor, R-spondin. Similar 3D cultures systems have been developed for the Lgr5+ve stem cells of human stomach, liver, pancreas, prostate and kidney. Using CRISPR/Cas9 technology, genes can be efficiently modified in organoids of various origins.

NOTES

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A novel computational approach to single-cell transcriptomics uncovers the dynamics of tumor stem cells in ovarian cancer organoids

High Grade Serous Ovarian Cancer (HGSOC) is the most prevalent and the most lethal gynecological malignancy, with only negligible improvement in survival over the last decades. This unresolved emergency derives from our poor understanding of HGSOC biology and with the uncertainty about its cell of origin and the lack of molecular signatures that permit the assignment of patients' tumors to either one of the two candidate tissues: the ovarian surface epithelium (OSE), fimbrial epithelium (FI). Here we advance the field through the integration of two approaches: the first-ever derivation and propagation of 3D organoids from OSE, FI, HGSOCs and its metastatic counterpart; and the identification by single-cell transcriptomics (scRNAseq) of HGSOC stem cell subpopulations that propagate tumor growth. Through a newly developed culture method, we were able to isolate, expand to 3D organoids and functionally define a new subpopulation of metastatic HGSOC propagating cells. A single-cell resolution comparison of 3D organoids with freshly isolated metastatic and 2D cultured samples reveals a major enrichment in 3D organoids for a tumor-propagating epithelial compartment, revealing the retention of patient-specific druggable pathways that are instead depleted from 2D models. Furthermore, to enhance the power of our characterization, we developed a new method that clusters together cells based on expression of specific, previously validated, signatures associated to distinct tumor compartments. This generates in silico aggregations of cells with similar characteristics that we term supercells. This approach increases vastly both the amount of genes per cell and the sequencing depth without, crucially, any loss in rare subpopulations. The result is thus a heightened sensitivity in the precise identification, for each individual patient, of the specific subpopulations functionally validated in 3D assays, establishing transforming resources for precision oncology in ovarian cancer.

NOTES

PETER LICHTER

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Newly emerging mechanisms of genomic instability and their impact on tumor evolution

Processes causing genomic instability are known as potential drivers of tumor etiology and progression. Large-scale deep sequencing of tumor cell genomes revealed comprehensive landscapes of genomic alterations uncovering previously unrecognized features of genomic instability. An emerging pattern is the frequent hi-jacking of regulatory DNA elements, such as enhancers and promoters, resulting in the deregulation of the activity of genes that drive oncogenesis. These studies also uncovered a previously unrecognized phenomenon of genomic instability termed chromothripsis, where single chromosomes or parts thereof are shattered into tens to hundreds of fragments and the subsequent impaired repair process results in highly aberrant chromosomes. Typically, the loss of multiple fragments during this process yields in DNA copy number profiles oscillating between two or three copy numbers states. Notably, chromothripsis is associated with bad prognosis in a number of tumor entities. We and others accumulated evidence for the cellular processes that contribute to the formation of chromothriptic chromosomes, such as telomere attrition, poly-ploidization, micronuclei formation, DNA repair deficiency and replication stress. We developed novel in vitro and in vivo test systems allowing us to assess the role of these processes in more detail. The recent status of our attempts to recapitulate the chromothripsis event will be presented and the impact on tumor evolution will be discussed.

NOTES

IDO AMIT

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Single-Cell Genomics: A Stepping Stone for Future Immunology Discoveries

The immune system is a complex, dynamic and plastic network composed of various interacting cell types that are constantly sensing and responding to environmental cues. From very early on, the immunology field has invested great efforts and ingenuity to characterize the various immune cell types and elucidate their functions. However, accumulating evidence indicates that current technologies and classification schemes are limited in their ability to account for the functional heterogeneity of immune processes. Single cell genomics hold the potential to revolutionize the way we characterize complex immune cell assemblies and study their spatial organization, dynamics, clonal distribution, pathways, and crosstalk. This emerging field can greatly affect basic and translational research of the immune system. I will discuss how recent single cell genomic studies are changing our perspective of various immune related pathologies from cancer to neurodegeneration. Finally, I will consider recent and forthcoming technological and analytical advances in single cell genomics and their potential impact on the future of immunology research and immunotherapy.

NOTES

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De novo spatial reconstruction of single-cell gene expression

The ongoing quest to decipher the identity of single cells, the building blocks of tissues or whole organisms, has proven deeply fruitful. However, there are important questions about tissue-level functions and cell-cell communication seemingly inaccessible without further information about the spatial configuration of these cellular building blocks. Recent computational work has shown that inferring such spatial configuration from single-cell RNA sequencing data can be achieved when provided with an extensive reference atlas of spatial expression patterns. However, removing the reliance on such additional information remains an important task, since it is often not available. To this end, we propose to exploit structural information that captures the relationships between expression profiles of single cells in expression space, as well as the relationships between locations the single cells could be embedded in across the tissue. We show that despite existing sharp boundaries in expression patterns, the relationship between pairwise cellular distances for different tissues and organisms in these two spaces is many times increasingly monotonic. We leverage this as the basis for novoSpaRc (de novo Spatial Reconstruction), a computational framework based on optimal transport, for the reconstruction of spatial expression patterns across canonical tissues given only single-cell information, such as generated by single-cell RNA sequencing data. novoSpaRc is successful in reconstructing full transcriptomic spatial expression.

NOTES

EILEEN FURLONG

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Understanding enhancer usage during embryonic development at a single cell level

Complex patterns of temporal and spatial gene expression are regulated by enhancers; *cis*-regulatory elements that recruit multiple transcription factors, leading to a very defined output of expression. Enhancers can be located in close proximity to, or at great distances from, their target gene. Our previous work[1] indicates that embryonic enhancers are often in close proximity to their target promoters hours before the gene is expressed during embryogenesis, suggesting that pre-formed chromatin topologies may prime the system for rapid activation (when the enhancers presumably come in even closer proximity), at least within the rapidly developing Drosophila embryo.

To better understand the relationship between chromatin organization and transcriptional regulation, we are integrating single cell genomic approaches with single cell imaging and genetic deletions to determine inherent properties of enhancer usage during embryogenesis, and when and how their three dimensional topologies are first established during development. Using 3D nuclear FISH we have quantified the distances of enhancer-promoter interactions in individual nuclei at specific development stages, within perturbations both in *trans* and *cis*. In parallel, we are using single cell ATAC-seq[2] to measure enhancer usage and to try to link enhancers to their target genes at the same stages of embryogenesis. We have also uncovered new functions for enhancers themselves, indicating that these elements can have multiple roles[3,4] in the regulation of developmental programs.

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NOTES

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Record-seq reveals core features of transcription dynamics in single cells

Current single-cell RNA sequencing (scRNA-seq) approaches analyze total RNA profiles at a single time point but convey little information about the underlying temporal dynamics. They all share the major limitations that (i) responses to perturbations cannot be measured directly, (ii) kinetics of transcription (e.g. bursts) cannot be investigated, (iii) short-term changes due to a perturbation or stimulus within a timescale of a few hours are masked by pre-existing RNA and (iv) changes in RNA synthesis and decay cannot be differentiated. Here, we present Record-seq, which integrates metabolic RNA labeling, biochemical nucleoside conversion and scRNA-seq to directly record transcriptional activity in addition to transcript levels. Key to the analysis of Record-seq data was a new computational approach (Globally Refined Analysis of Newly transcribed RNA and Decay rates using SLAM-seq (GRAND-SLAM)). Based on a rigorous statistical model and Bayesian inference it enabled us to precisely quantify newly transcribed RNA for thousands of genes in hundreds of individual cells. We applied Record-seq to decipher intercellular heterogeneity in the initial cellular response to lytic cytomegalovirus (CMV) infection. Record-seq visualized gene-specific fluctuations in transcriptional activity, on-off dynamics and bursts at single-cell level. We show that heterogeneity is associated with promoter-intrinsic features (TATA-boxes, CpGs) indicating that DNA methylation renders promoters non-permissive in between transcriptional bursts. The non-permissiveness was resolved in infected cells for NF-κB and IFN induced genes. Thus, without increasing the maximal levels of newly synthesized RNA in individual cells, target genes are upregulated on population level, revealing a fundamental principle of their transcriptional regulation. This establishes Record-seq and GRAND-SLAM as powerful and broadly applicable tools to study transcriptional activity in single cells.

NOTES

LIOR PACHTER

California Institute of Technology, Pasadena, USA

Computational and experimental foundations for single-cell genomics

NOTES

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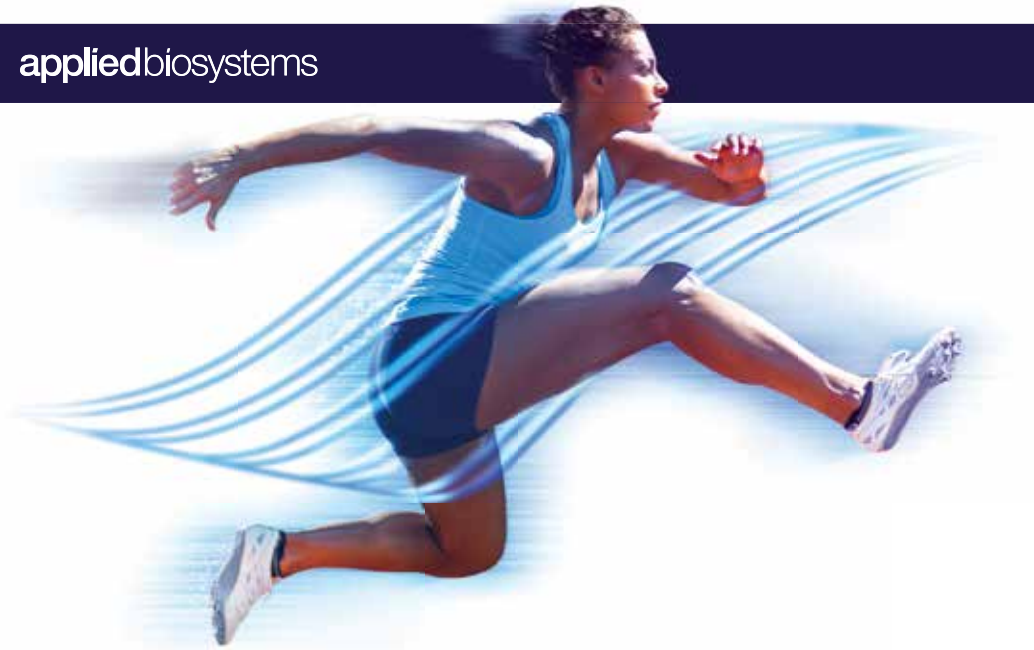

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POSTER ABSTRACTS

(IN ALPHABETICAL ORDER)

P1

Unravelling the role of RNA-binding proteins in neural tissue differentiation during *Drosophila melanogaster* embryogenesis

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Embryonic development requires tight control of gene expression. In Drosophila, neurogenesis initiates through the emergence of neural stem cells (neuroblasts) from distinct lateral columns of the early embryo, eventually giving rise to neurons and glia in a characteristic spatial-temporal manner. We have generated data that dissect the dynamic and tissue-specific transcriptomes of early neurogenic cell population including columnar domains, neuroblasts, neurons and glia, which can be used to further address any specific layer of regulation underlying this developmental process.

Although much emphasis has traditionally been put on regulation at the transcriptional level, the importance of post-transcriptional regulation is becoming increasingly clear, partly due to the recent discovery of hundreds of RNA-binding proteins (RBPs) that regulate virtually every process in the lifespan of mRNAs. Based on a comprehensive list, some RBPs were observed to be specifically expressed in components of the embryonic nervous system, and a screen was conducted to test their requirement. Subsequently, analysis of its cognate transcripts, as well as their mechanism of regulation (i.e transcript stability and/or translational control) will further be dissected. In the end, this approach will help to elucidate the role of RBPs in neurogenesis in particular, and the role of post-transcriptional regulation in general.

P2

Alternative polyadenylation dynamics in neuronal differentiation at single-cell resolution

J. Alles, Nikolaus Rajewsky
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3' Untranslated Regions (3'UTRs) are major regulators of an mRNA's fate, thereby affecting RNA stability, localization and translational output. More than half of all protein-coding genes produce isoforms with alternative 3'UTRs. Alternative polyadenylation is a highly tissue-specific process and equips individual mRNAs with regulatory features, such as miRNA binding sites, in a highly context dependent manner. Many studies so far compared alternative polyadenylation between tissues and across experimental conditions, yet little is known about how changes in alternative polyadenylation are temporally orchestrated for instance throughout cellular differentiation. We thus attempt to use single-cell transcriptomics to reconstruct the differentiation process of human induced pluripotent stem cells (hiPSC) to spinal motor neurons. We further employ a custom sequencing protocol to unambiguously map the 3'UTR end of each transcript, such that we can additionally infer dynamics of alternative polyadenylation throughout differentiation. We further plan to extend this approach to investigate 3'UTR dynamics in hiPS cells derived from patients with motor neuron diseases as Spinal Muscular Atrophy (SMA) or Amyotrophic lateral sclerosis (ALS).

P3

Subcellular compartmentalization of proteins

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Membrane-enclosed organelles are an essential characteristic of eukaryotic cells: this compartmentalization helps the cell to assign specific functions to a defined subcellular region. The localization of a protein in one of this compartment is a crucial feature to determine its physiological functions and interactions. Aberrant protein trafficking can lead to disease. We took advantage of a locally restricted promiscuous biotin ligase (BirA*) to map *in vivo* all proteins in the vicinity of the outer endoplasmic reticulum membrane and outer mitochondrial membrane in HEK293 cells. Mass spectrometry identified 832 proteins enriched in the vicinity of ER membranes, while 539 at the mitochondrial ones. The overlap with existing protein datasets at these sites is good, but leaves many proteins with an unprecedented localization at these compartments, that must be further validated.

P4

Structure function relations in the single cardiomyocyte

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Alternative splicing (AS) is a fundamental process of gene regulation and generation of functional diversity. In the heart, AS controls determinative changes during development, perinatal adaptation and in cardiovascular diseases such as dilated cardiomyopathy (DCM). The splicing factor RNA-binding motif 20 (RBM20) has been linked to hereditary cardiomyopathy and adjusts protein isoform expression and remodeling of the heart by directing the splicing of titin, a giant sarcomeric protein with structural and passive elastic functions. Yet, the regulation of titin isoform expression and splicing factor-mediated cardiac function are not fully understood. To tackle these questions we use whole-cell patch clamp and single-cell RNA-seq of embryonic, adult and iPSC-derived cardiomyocytes. For the latter, we will compare cells in a monolayer versus cells derived from 3D cultures, i.e. engineered heart tissue (EHT). We aim to better understand how cardiomyocytes adapt their mechanical and electrical properties at the single-cell level based on their maturity, position in space, supply of oxygen and nutrients, mechanical input or pharmacological stimulation. Eventually, these insights could serve as a reference to map the heterogeneity of cellular and molecular phenotypes in health and disease.

P5

Computational Approaches for Modeling Model Organisms using High Resolution Microscopy

E. Bahry, S. Preibisch

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Image analysis takes a prominent role in modern biological research, where large quantities of images of model organisms need to be analyzed. The first step in such analyses is often to bring the images into the same coordinate space and to learn a model representation of the specimen in order to be able to compare across different conditions, study variability, stereotypy, etc. In this work we present three such models. First, a high resolution nuclei model of the *C. elegans* dauer, which we use to study the mechanisms that initiate dauer exit and to better understand chromatin architecture and its relation to cellular fate. The model is constructed from a one nanometerresolved electron microscopy dataset of an entire dauer larva to which we map live and fixed light microscopy datasets. Nuclei extraction from the datasets is performed using dynamic programming, random forest classification, and a 3D convolutional neural network. Nuclei matching is implemented using a combinatorial graph matching approach. Second, we present a generic tool for automatic specimen registration using landmarks, and its application to Drosophila wing registration to study enhancer function. The registration tool is constructed as a user friendly pipeline with image segmentation and landmark graph matching applications. Finally, we demonstrate a tool using machine learning to predict the embryonic stage of a *C. elegans* embryos based on the texture of the image without performing actual cell counting. With these three examples, we demonstrate both generic approaches to computational modeling of modeling organisms, as well as bespoke solutions to specific problems. Such tools will be integral to biology research in the future, as automatic microscopes proliferate and data analysis becomes a bottleneck.

P6

Modelling the pathological long-range regulatory effects of structural variation in the neural crest with patient-specific hiPSC

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Congenital abnormalities can be caused by structural variants that disrupt the topology of gene regulatory landscapes. Yet, elucidating the mechanisms by which structural variation can cause a disorder *in vivo* is often limited by differences in gene dosage sensitivity between mice and humans and/or the access to suitable patient material. Here we describe a unique patient with Branchio-Occulo-Facial-Syndrome (BOFS), a rare congenital syndrome that arises due to defects during NC development and is thus considered as a human neurocristopathy. All reported BOFS cases are caused by heterozygous mutations within TFAP2A, a neural crest (NC) master regulator. This patient, though, presents a de novo heterozygous inversion in which one of the breakpoints is located downstream of TFAP2A, within a large Topologically Associating Domain (TAD) containing enhancers essential for TFAP2A expression in NC cells (NCC).

Since humans, but not mice, are haploinsufficent for TFAP2A, here we use NC cells derived from patient-specific human induced pluripotent stem cells (hiPSC) to dissect the pathomechanism of our unique BOFS patient. Importantly, we show that the inverted TFAP2A allele loses physical interaction with its cognate enhancers. This in turn leads to monoallelic expression of TFAP2A in human NCC and haploinsufficiency due to reduced TFAP2A binding to enhancers controlling the expression of genes involved in craniofacial morphogenesis and NCC migration. This demonstrates the power of combining patient-specific hiPSC differentiation with genomic and genetic engineering tools to overcome current limitations in elucidating long-range pathological consequences of human genetic structural variation. Overall, our results illustrate how TAD disruption can lead to a loss of enhancer gene-interactions and, consequently, to pathological changes in gene expression, thus highlighting a potential etiological mechanisms for other human congenital disorders.

P7

TNF and the clockwork: a reciprocal interplay

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A proper bidirectional crosstalk between the circadian network and key elements of immunity is vital for a functional biological clock and the immune system. In the present study, we used Hodgkin lymphoma as an experimental model for a type of cancer involving cells of the immune system. We identified this cancer type among haematological malignancies with a strong differential expression of core-clock elements. We used bioinformatics analyses and experimental procedures carried out in III- and IV-stage Hodgkin lymphoma cells and lymphoblastoid B cells to investigate this interplay and characterised diverse interacting partners of both systems. In particular, we assembled a wide-ranging network of clock-immune genes and identified TNF as a crucial intermediary player. A robust clock hallmarks III-stage lymphoma cells, differently from IV-stage Hodgkin lymphoma cells, which do not harbour a properly functioning clockwork. Based on our findings, TNF affected clock genes expression and altered phenotypic characteristics of Hodgkin lymphoma cells in regards to proliferation, apoptosis and migration, suggesting a key role for the physiopathological mechanisms hastening malignancy. Our results illustrate the necessity of a functional interplay between the core-clock and TNF and expand our understanding of the patho-biological properties of Hodgkin lymphoma.

P8

A nuclear RNA export factor variant licenses piRNA-guided co-transcriptional silencing

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The PIWI/piRNA pathway protects animal genome integrity in part through establishing heterochromatin at transposon loci. This process requires piRNA-guided targeting of nuclear PIWI proteins to nascent transcripts. The molecular events downstream of PIWI-target engagement are largely unclear. Here, we show that co-transcriptional silencing via Drosophila Piwi depends on a novel protein complex. It consists of the nuclear RNA export factor (NXF) variant Nxf2, the mRNA export cofactor Nxt1, and the Piwi-associated protein Panoramix/Silencio. In Nxf2 mutants, Panoramix is targeted for degradation, and piRNA-loaded Piwi is unable to establish heterochromatin. We further demonstrate that Panoramix links to the downstream heterochromatin machinery and Nxf2 enables Panoramix to repress target loci via the nascent RNA. Even though, originating from the core mRNA export machinery, the Nxf2-Nxt1 heterodimer has been coopted for co-transcriptional silencing at chromatin. Our data provide mechanistic insights how Nxf2 diverged from the canonical mRNA exporter, Nxf1. The unexpected link between nuclear small RNA and NXF biology highlights the molecular versatility of NXF variants, which are widespread in metazoans yet lack ascribed functions.

P9

Role of mitotic bookmarking in transcription dynamics

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During embryonic development, transcriptional properties of progenitor cells are stably propagated across multiple cellular divisions. Yet, at each division, chromatin faces structural constraints imposed by the important nuclear re-organization operating during mitosis. It is now clear that not all transcriptional regulators are ejected during mitosis, but rather that a subset of transcription factors, chromatin regulators and epigenetic histone marks are able to 'bookmark' specific loci, thereby providing a mitotic memory.

To first find potential bookmarking factors in the Drosophila embryo, we used live-imaging and high-resolution microscopy to visualize proteins during the cell cycle and found four candidates that stay bound to the chromatin during mitosis. Among these putative mitotic bookmarkers, we found members of the Trithorax group (Fs(1)h and Ash1). We now aim to decode the functions of these mitotic bookmarker candidates on the control of gene expression during early embryogenesis.

To follow transcription activation during time, we used the MS2/MCP system where MS2 loops transcription is under the control of the snail gene promoter and a truncated version of the snail shadow enhancer previously described to show transcriptional memory in the Drosophila embryo.

Using an automatic tracking software, we are able to quantify the timing of activation in space in hundreds of nuclei and their progeny, allowing us to determine activation kinetics in nuclei coming from active or inactive nuclei.

By reducing maternal Ash1 and Fs(1)h expression using RNAi and mutants, we found that transcriptional synchrony is affected as well as the memory in a spatial dependent manner. To further validate these memory factors, we now want to identify mitotic targets of these factors by whole-genome approaches, characterize their dynamics during the cell cycle using live-imaging approaches and develop mathematical models of mitotic bookmarking.

P10

Functional analysis of PIEZO1 using genome edited hiPSC-derived cardiomyocytes as a model system

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The cardiac muscle has to adapt in a highly dynamic mechanical environment and stretch-activated channels have been known to play an important role in the mechanosensitive autoregulation of the heart. PIEZO1 is a stretch-activated channel previously characterized in neurons and endothelial cells. In the murine heart it has been involved in erythrocyte volume homeostasis. Genome editing technology in human induced pluripotent stem cells (hiPSCs) is a powerful approach to implement our knowledge on PIEZO1 in cardiac mechanical activity and its potential involvement in cardiac diseases.

To study the functional role of PIEZO1 in cardiomyocytes, we have generated knockout (KO) hiPSCs using genome editing technology. The mutated cells are then differentiated into viable, beating cardiomyocytes. Currently, we are conducting phenotypic analyses including the evaluation of electrophysiological characteristics, observation of cell morphology and beating activity of the genome edited hiPSC-derived cardiomyocytes. With this approach we aim to gain more insight into PIEZO1 function in cardiomyocytes using a reliable, efficient and reproducible human cellular model system.

P11

Multiscale 3D genome rewiring during neural development

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3D nuclear architecture can be causal to gene expression and disruptions in topology often result in pathogenic phenotypes. However, how chromatin contacts change during development and what are the consequences for cell fate remains unclear.

Using ultra-high resolution Hi-C, we mapped comprehensively 3D chromatin organization during mouse neural development, both *in vitro* and *in vivo*. We discovered that transcription is highly correlated with chromatin insulation, as well as promoter-promoter interactions, but dCas9-mediated activation is insufficient to create such topology de novo. Surprisingly, we found that highly transcribed, exon-rich genes frequently engage in long-range contacts both in pluripotent and differentiated cells.

In addition to stable loops, we found dynamic rearrangement of chromatin interactions at various scales during neural differentiation. An extensive Polycomb-linked contact network in stem cells was disrupted independently of H3K27me3, while novel interactions driven by the transcription factors Pax6, NeuroD2 and Tbr1 were established *in vivo*. Finally, most of the enhancer-promoter interactions appear to be dynamically rewired and correlated with gene expression.
Collectively, this work suggests that 3D nuclear architecture represents an additional layer in the regulation of gene expression and cell fate during development and is deeply linked to the normal physiological and pathological function of the brain.

P12

ATAC-seq reveals regional differences in enhancer accessibility during the establishment of spatial coordinates in the Drosophila blastoderm

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Establishment of spatial coordinates during early Drosophila embryogenesis relies on differential regulatory activity of axis patterning enhancers. Concentration gradients along the embryonic axes of activator and repressor transcription factors (TFs) provide positional information to each enhancer, which in turn promotes transcription of a target gene in a specific spatial pattern. In order to receive the TF input, an enhancer must be accessible. However, the interplay between an enhancer regulatory activity and its accessibility as determined by local chromatin organization is not well understood. Notably, it is unclear whether chromatin organization of axis patterning enhancers is uniform across the embryo or varies regionally with their differential activity.

We profiled chromatin accessibility with ATAC-seq in narrow, genetically tagged domains along the antero-posterior axis in the Drosophila blastoderm. We demonstrate that one quarter of the accessible genome displays significant regional variation in its ATAC-seq signal immediately after zygotic genome activation. Axis patterning enhancers are enriched among the most variable regions and their accessibility changes correlate with their regulatory activity. When receiving a net activating TF input and promoting transcription, an enhancer displays elevated accessibility in comparison to the region of the embryo where it receives a net repressive input.

Chromatin context plays an integral role in the spatial regulation of axis patterning enhancers. We discuss potential mechanisms by which accessibility of enhancers may be modulated by interactions with activator and repressor TFs. We conclude that differential accessibility is a signature of differential regulatory activity and can potentially serve as a metric for de novo identification of enhancers patterning complex tissues.

P13

Characterising transcription regulation by dosage compensation in *C. elegans*

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Chromosome condensation facilitated by condensin complexes is a prerequisite for mitotic segregation of DNA. Recent data suggest that condensins not only function during mitosis but also affect gene regulation during interphase. However, the mechanistic foundation for this gene regulatory function of condensin remains poorly understood.

Studying condensin separate from its function in mitosis is required to understand its role in gene regulation. Most metazoans express two condensin isoforms, while *Caenorhabditis elegans* additionally expresses condensin DC (dosage compensation) that specifically binds to and represses transcription by half of both X chromosomes in XX hermaphrodites to equalize X expression between sexes. Therefore, studying condensin DC dynamics allows quantification of transcription in interphase without affecting mitotic condensin functions.

In our study, we aim to provide a quantitative and dynamic understanding of how chromosome condensation regulates transcription on a single-molecule level. Therefore, we combine live lightsheet microscopy with fixed single-molecule transcription imaging to elucidate the connection between the regulation of chromosome structure and transcription during development. To measure the effects of condensin DC-mediated regulation of transcription, we quantify expression of individual genes by single-molecule RNA fluorescence in situ hybridization (smFISH) and live transcription imaging and correlate these data with Pol II and condensin DC signals. We use the high spatio-temporal resolution of lightsheet acquisitions and the high degree of stereotypicity of *C. elegans* to map smFISH acquisitions to the precise developmental stage of each fixed sample.

Taken together, this approach allows us to investigate condensin-mediated transcriptional regulation at selected genes with single-cell resolution in hundreds of embryos and helps to reveal how chromosome condensation directly affects transcription.

P14

Functional sequestration of microRNA-122 from Hepatitis C Virus by circular RNA sponges

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Circular RNAs (circRNAs) have recently emerged as a new class of ubiquitously expressed non-coding RNA. They are created by the canonical splicing machinery in a process referred to as “back-splicing”, where a donor splice site is spliced to an upstream instead of a downstream acceptor site. Two endogenous circRNAs, CDR1as/ciRS-7 and SRY, have been shown to contain conserved microRNA (miRNA)-binding sites and function as miRNA sponges in vivo. Due to their high stability compared to linear RNA, circRNAs may be an interesting tool in molecular biology.

We have designed and produced artificial circRNAs in vitro that can be used as miRNA sponges to sequester miRNA-122 from Hepatitis C Virus (HCV) and thereby affect virus RNA stability and translation. Miravirsen, the first anti-miRNA drug, had been reported to functionally sequester miRNA-122 and decrease HCV virus titers to non-detectable levels in patients after a two-week subcutaneous injection. miRNA-122 is essential for HCV propagation and protects the single-stranded RNA genome from Xrn1-mediated exonucleolytic degradation and enhances HCV translation by binding to two adjacent sites at its 5’-end. We designed artificial miRNA-122 sponges that, if transfected as an in vitro transcribed and ligated circRNA, have a similar effect in HCV cell culture systems as a Miravirsen-like antisense oligonucleotide. These circRNAs are more stable in vivo compared to their linear counterparts and found both in the cytoplasm and in the nucleus. These results demonstrate that artificial circRNA sponges are a promising tool in molecular medicine and biology with a wide range of potential applications. We are currently developing several different circRNA sponges to sequester miRNAs and RNA-binding proteins linked to multiple human diseases.

P15

Artificial circular RNA sponges as a novel tool in molecular biology and medicine

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Circular RNAs (circRNAs) have recently become a focus of biomedical research. A cellular circRNA, CDR1as/ciRS-7 was identified as a neuronal microRNA (miRNA-) sponge that sequesters and functionally inhibits miRNA-7 function and regulates its homeostasis. CDR1as knockout mice display dysfunctional synaptic transmission, misregulation of miRNAs and a behavioral phenotype that is associated with human neuropsychiatric disorders.

Due to their elevated stability compared to linear RNA, circRNAs represent a promising tool in biotechnological applications. Many human diseases are associated with miRNAs that can be targeted by anti-miRNA drugs. The cellular miRNA-122 is hijacked by the Hepatitis C Virus (HCV) and appears essential for its propagation. Miravirsen, the first anti-miRNA drug has effectively inhibited HCV in patients. In an earlier proof-of-principle study, we demonstrated that in vitro transcribed and ligated circRNA sponges sequester miRNA-122 and impair HCV translation as effectively as Miravirsen in HCV cell culture model systems.

We are currently targeting miRNAs linked to other diseases, such as bacterial infection and cancer. Among promising targets are oncogenic miRNAs, which are upregulated in many tumor types and associated with poor prognosis in patients. We developed circRNA sponges targeting these miRNAs and assessed their effect on tumor growth in 3D cell culture systems. We observed a significant reduction in spheroid growth, spheroid invasion and anoikis resistance compared to control circRNAs lacking specific miRNA binding sites. In addition, we are applying strategies to bypass the innate immune response (foreign RNA recognition) of the cell. Overall, the described examples broaden the perspective on potential applications of artificial circRNA sponges in the field of molecular medicine.

P16

Sensitive mapping of regulatory variation in neuroblastoma tumor genomes

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Cancer genomes are characterised by aberrations, such as single nucleotide variants (SNVs), small insertions and deletions (indels), and somatic copy-number alterations (SCNAs). These acquired changes add to the underlying germline variation of the genome. While coding regions have been extensively studied, the non-coding part of the genome harbors the majority of variation. Non-coding single nucleotide polymorphisms (SNPs) in regulatory elements modulate gene expression differentially between tissues and individuals. Germline variation is of particular interest in neuroblastoma, which is characterised by a low overall somatic mutational burden. Germline variation has been linked to neuroblastoma susceptibility [1] but little is known about how it affects the regulatory program of neuroblastoma-associated genes.

We here integrate total expression and allele-specific expression (ASE) from tumor RNA-seq with germline and somatic genetic variation from whole genome sequencing (WGS) of neuroblastoma tumors and blood-derived normals [2]. We detect recurrent allelic expression imbalance (AEI) in genes with established roles in neuroblastoma such as MYCN, ALK, PHOX2B and LMO1. Using allele-specific copy-number profiling and cancer-specific eQTL maps we tease apart copy-number driven AEI from AEI driven by SNVs in regulatory regions. We identify genes with AEI independent of SCNAs and fine-map genetic effects in these candidates through QTL analysis of total and ASE. Our approach accounts for the contribution of SCNAs, captures known non-coding germline risk variants and identifies potential new regulatory variants active in neuroblastoma. The results shed light on the interplay between cancer gene regulation through SCNAs, SNVs and SNPs.

1. Wang, K. et al. Integrative genomics identifies LMO1 as a neuroblastoma oncogene. Nature 469, 216–20 (2011).
2. Peifer, M. et al. Telomerase activation by genomic rearrangements in high-risk neuroblastoma. Nature 526, 700–704 (2015).

P17

Increased quiescence and NSC division asymmetry during ageing, in the adult subependymal zone

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Neural stem cells in the adult murine brain have only a limited capacity to self-renew and the number of neurons they generate drastically declines with age. How cellular dynamics sustain neurogenesis and how alterations with age may result in this decline are both unresolved issues. Therefore, we clonally traced neural stem cell lineages using multicolor reporters in young and middle-aged adult mice. To understand the underlying mechanisms, we derived mathematical population models of adult neurogenesis that explain observed clonal cell type abundances. Models fitting the data best, consistently show self-renewal of transit amplifying progenitors and rapid neuroblast cell cycle exit, as expected. Most importantly, we identified an increased probability of asymmetric stem cell divisions at the expense of symmetric differentiation, accompanied by an extended persistence into quiescence between activation phases, with age. Our model explains existing longitudinal population data and identifies particular cellular strategies underlying adult neural stem cell homeostasis and the aging of this stem cell compartment. Moreover, we provide a quantitative framework to facilitate the interpretation of reported and novel neurogenesis-related phenotypes.

P18

Study of circDLC1 function in murine Motor Neurons differentiation

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Circular RNAs (circRNAs) have been suggested to regulate neuronal development and plasticity, though very little is known about their mechanism of action so far. By identifying the circRNAs expressed in in-vitro-derived motor neurons (MNs) knocked out for the FUS gene we have selected circDLC1 circRNA. We demonstrated that this circRNA is expressed at low levels in mouse embryonic stem cells (mESCs) while it is upregulated during MN differentiation. Notably its linear counterpart shows an opposite trend of expression. For functional characterization, we combined two strategies: over-expression of circDLC1 using an integrative construct under the control of a Dox-inducible promoter and down regulation by CRISPR/Cas9 mediated gene deletion.

Since the accumulation of circDLC1 from the transgene containing exon 2 and part of the upstream and downstream intron has been observed only in mESCs and not in MNs we speculate that circularization might be under a specific regulation. Thus, we will investigate the biogenesis of circDLC1 and the cis and trans-acting factors involved in the process.

By RT-PCR, we also found that circDLC1 is highly expressed in mice heart, muscle, lungs and cortex. In situ hybridization experiments will be performed on tissue sections in order to verify the specific localization of this RNA.

P19

Characterizing the expression of the circRNA Cdr1as and its regulatory network in mouse primary neurons

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Cdr1as is a highly expressed brain circRNA, relevant for synaptic neurotransmission and normal brain function. The molecular mechanism explaining this function is not understood. It has been hypothesized that Cdr1as expression is regulated by a complex network including miR-7, miR-671 and lncRNA Cyrano, but the exact interplay among them is unknown.

Here, we characterize and quantify expression and localization of Cdr1as and Cyrano in mouse primary cortical neurons from wildtype and Cdr1as-mutant animals using single-molecule imaging techniques. In addition, we analyzed the changes in Cdr1as and Cyrano upon perturbation of miR-7 expression. We show that both Cdr1as and Cyrano are broadly expressed in cortical neurons (soma and neurites), suggesting a possible role in transport of miR-7 to subcellular locations within neurons. By analyzing Cdr1as after miR-7 overexpression, we showed that Cdr1as its being downregulated and localized to soma/nucleus. This suggests a negative regulation of the circRNA when exposed to high concentrations of miR-7, adding an additional layer of complexity to Cdr1as/miR-7/miR-671/Cyrano network.

P20

High-resolution ribosome profiles for E. coli

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Ribosome profiling is the most advanced tool to study translational control of gene expression.

The potential of Ribo-seq to provide fundamental insights about the translational control of gene expression is supported by the long list of successful applications. Nevertheless, the exploitation of the full power of the technique is severely hampered by the low signal to noise ratio. These noise issues have a direct impact on the reproducibility of Ribo-seq. Indeed, while the total number of reads per mRNA correlates well across independent experiments, a growing body of evidence is suggesting that the experimental reproducibility of the ribosome profiles is, in general, poor.

To address the limitations of Ribo-seq and recover its full power, we identify highly reproducible ribosome profiles that emerge from the comparison of Ribo-seq experiments performed in different laboratories. The selected profiles will be characterised by an extremely high resolution because the underlying signal will be so sharp that it will overcome the biological noise. These highly reproducible profiles build a library that can be used as a reference for comparative experiments aimed at detecting differential translation events.

Instead of relying on more traditional noise-filtering approaches, we select the entries of the reference libraries through a data-driven statistical method that identifies the ribosome profiles bearing the sharpest signal. In the case-study presented in this work, out of 96 available samples for *E. coli* we found 11 genes whose ribosome profiles are highly reproducible under normal and non-stressed conditions. These genes represent a reliable workbench to evaluate the impact on the translation process of either the experimental conditions or the structural features of the template mRNA.

P21

Regulation and evolution of cardiopharyngeal fates in chordates

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How cells acquire their identity and behave accordingly remain outstanding questions in biology. In vertebrates, multipotent progenitors reside in the cardiopharyngeal mesoderm and produce second-heart-field-derived cardiomyocytes and branchiomeric skeletal head muscles. However, the cellular and molecular mechanisms underlying these early fate choices and the consequential morphogenetic behaviors remain largely elusive. The tunicate Ciona has emerged as an attractive model to study early cardiopharyngeal development at high spatial and temporal resolution: defined multipotent cardiopharyngeal progenitors migrate collectively and produce distinct first and second heart precursors, and pharyngeal muscle precursors through two stereotyped asymmetric and oriented cell divisions. We initially characterized the expression of Tbx1/10, a critical genetic determinant of the Di George/22q11 deletion syndrome in humans, and its role in early pharyngeal muscle specification. We performed extensive and lineage-specific analyses of transcriptome dynamics using single-cell RNA-seq and regulatory logics using chromatin profiling by ATAC-seq. We characterized molecular features of multipotent progenitors, as well as gene expression changes and regulatory mechanisms governing the transition to fate specification and commitment in the second heart lineage, and identified a new Tbx1-dependent regulatory pathway involved in cell type diversification in the heart. Finally, we extended the analysis to compare cardiopharyngeal development between Ciona and mammals, using novel methods to align single-cell RNA-seq datasets across species and identify shared molecular signatures for conserved developmental traits.

P22

Deconstructing enhancer-promoter communication in mammalian cells

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Transcriptional enhancers can regulate the expression of distally located target genes. However, while enhancer-promoter communication emerged as a critical aspect of cell-type-specific transcriptional programs, how enhancers 'select' their target promoter(s) remains poorly understood. Local chromatin contexts, chromatin architecture and biochemical compatibility between cis-regulatory elements are all thought to contribute to this selectivity. To tackle this question, here we developed a massively parallel reporter assay that allows to episomally measure selectivity and cooperativity of tens of thousands of enhancer-promoter pairs (EP) in mammalian cells. Our proof-of-concept design based on combinations of broad, cell-type-specific and inactive regulatory elements (192 enhancers x 192 promoters) from four human cancer cell lines suggests the absence of global promiscuity between enhancers and promoters. Rather, enhancer-promoter cooperativity appears to be selective, with some promoters exhibiting broad cooperativity profiles and with enhancers frequently increasing promoter activity but often not beyond additive cooperation. We will also discuss recent efforts towards the deconstruction of enhancer-promoter cooperativity within the naive pluripotency network in mouse embryonic stem cells.

P23

Parallel bisulfite & transcriptome sequencing in single human haploid cells reveals epigenetic regulation with digital resolution

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Recent progress in sequencing technology and molecular techniques has shifted 'Genomics' to the single-cell level, enabling a better understanding of tissue complexity and the identification of disease causing cell populations. However, how locus-specific single-cell variations in chromatin structure, epigenetic state and transcriptional activity are causally linked during tissue homeostasis and malignancy is still poorly understood. DNA methylation at 5mCpG in promoter-linked CpG islands is well known for its repressive effect on transcription. At the same time, the role of 5mCpG at distal regulatory elements and in gene bodies, where intermediate levels of 5mCpG can show positive or negative associations with gene expression, remains largely elusive. The heterogeneity of bulk samples and the occlusion of allele- and strand-specific methylation information in bisulfite WGS data further complicate mechanistic studies using standard techniques. We have applied single-cell bisulfite sequencing coupled with transcriptome sequencing (scBS&T-seq) to obtain parallel genome-wide maps of 5mCpG and gene expression from individual haploid KBM7 leukemia cells in the G1 phase. Here, the presence of single copy chromosomes provides digital methylation information and avoids the otherwise prevalent ambiguity of allele- and strand-specific signals. Methylation heterogeneity between cells enables direct correlation of transcriptional output and mCpG at individual genomic features, revealing non-canonical mCpG functions that are masked in classical correlation analyses. Using time controlled inactivation of mCpG maintenance via the Auxin-inducible degron system, we introduce massive genome-wide mCpG heterogeneity, to strongly leverage the power of scBS&T-seq and investigate mCpG function on a genome-wide level.

P24

Homology Independent Replacement of Genomic Sequences

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Current strategies to specifically alter genomic DNA sequences mainly utilize the Homology Directed Repair (HDR) pathway. The requirements of the HDR pathway create experimental constraints. HDR can require long homology arms and can be inefficient in many targeting conditions or cell types. Notably, the pathway is completely off in G0/G1 stage of the cell cycle, making precise genomic changes in resting or slowly dividing cells difficult.

Recently studies have demonstrated the ability to insert linear DNA sequences into a genome using the Non-Homologous End Joining pathway (NHEJ). In this a genomic double strand break is created, into which a linear DNA sequence is ligated. This strategy allowed for high efficiency insertions in dividing and non-dividing cells of many types.

Our work investigates whether such a NHEJ based genome editing method can be extended beyond solely insertions. Here we investigated if the NHEJ pathway can allow for the replacement of a target sequence with a provided sequence, and thus open up a new pathway and strategy for precise mutagenesis. Using fluorescent reporters in human cell lines we were able to achieve sequence replacement with sequences containing no homology arms at a high frequency of 20-50%. This was done in multiple cell types and at multiple loci, with low INDEL formation at the ligated interfaces. Sequencing single-cell-derived colonies confirmed sequence replacement. Deep sequencing of the bulk population provided a more complete view of genetic outcomes and illuminated future design principles.

In short, this work provides a proof of principle for an alternative to HDR for precision mutagenesis. Using the NHEJ pathway one can now create deletions, insertions, or replace sequences to create precise mutations.

P25

The Chromatin Reader ZMYND8 Regulates Igh Enhancers to Promote Immunoglobulin Class Switch Recombination

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Class Switch Recombination (CSR) is a DNA recombination reaction that diversifies the effector component of antibody responses. CSR is initiated by activation-induced cytidine deaminase (AID), which targets transcriptionally active immunoglobulin heavy chain (Igh) switch donor and acceptor DNA. The 3' Igh super-enhancer, 3' Regulatory Region (3'RR), is essential for acceptor region transcription, but how this function is regulated is unknown. Here we identify the chromatin reader ZMYND8 as an essential regulator of the 3'RR. In B cells, ZMYND8 binds promoters and super-enhancers, including the Igh enhancers. ZMYND8 controls the 3'RR activity by modulating the enhancer transcriptional status. In its absence, there is increased 3'RR polymerase loading, and decreased acceptor region transcription and CSR. In addition to CSR, ZMYND8 deficiency impairs somatic hypermutation (SHM) of Igh, which is also dependent on the 3'RR. Thus ZMYND8 controls Igh diversification in mature B lymphocytes by regulating the activity of the 3' Igh super-enhancer.

P26

Regulation of spatial and temporal gene expression in an animal germline

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In animal germlines, regulation of cell proliferation and differentiation is particularly important but poorly understood. Here, using a cryo-cut approach, we mapped RNA expression along the *Caenorhabditis elegans* germline and, using mutants, dissected gene regulatory mechanisms that control spatio-temporal expression. We detected, at near single-cell resolution, > 10,000 mRNAs, > 300 miRNAs and numerous novel miRNAs. Most RNAs were organized in distinct spatial patterns. Germline-specific miRNAs and their targets were co-localized. Moreover, we observed differential 3' UTR isoform usage for hundreds of mRNAs. In tumorous gld-2 gld-1 mutants, gene expression was strongly perturbed. In particular, differential 3' UTR usage was significantly impaired. We propose that PIE-1, a transcriptional repressor, functions to maintain spatial gene expression. Our data also suggest that cpsf-4 and fipp-1 control differential 3' UTR usage for hundreds of genes. Finally, we constructed a "virtual gonad" enabling "virtual in situ hybridizations" and access to all data (https://shiny.mdc-berlin.de/spacegerm/).

P27

Analysis of factors and sequences determining circular RNA biogenesis

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Circular RNAs (circRNAs) are a newly discovered class of stable RNAs, generated by alternative splicing from normal pre-mRNAs by circularization of certain exons. Currently up to a forth of active protein coding genes (depending on the cell type) were reported to form circRNA products. The high stability and resistance to exonuclease digestion make ~10% of all circRNAs accumulate in amounts comparable to the corresponding linear transcripts. The most widely described circRNA functions are sponging of miRNA, regulation of RNA expression, and protein translation.

The generation of new and optimal expression vectors for transient and stable expression of circRNAs from any sequence and in a wide range of different lengths is the aim of the current project. By completing this, we should be able to express any designed circRNA, for example for antisense interference or protein sponging. Our study focuses on the development of optimal vector systems for circRNA biogenesis and overexpression, including a vector derived from the endogenous platelet-specific circRNA Plt-circR4.

In addition, we apply tRNA-expressing vectors for circRNA production. A number of archaeal and eukaryotic tRNA genes are known to produce circRNA from their introns. By cloning the sequences of interest into such tRNA introns, we assay for circRNA biogenesis and relative splice isoform abundance based on RT-PCR, RT-qPCR and Northern blotting.

P28

Comprehensive Analysis of Chromatin Modifications in HSV-1-Induced poly(A) Readthrough by ChIPmentation

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Lytic Herpes Simplex Virus type 1 (HSV-1) infection is characterized by a profound shut-off of host gene expression via targeting multiple steps of RNA metabolism. Recently, we identified disruption of transcription termination (DoTT) of most cellular but not viral genes, to contribute to this host shut-off. DoTT results in extensive transcription for tens of thousands of nucleotides beyond poly(A) sites and into downstream genes. Interestingly, we observed DoTT to be accompanied by a dramatic increase in chromatin accessibility downstream of the affected poly(A) sites. Large dOCRs are indicative of impaired histone repositioning when RNA polymerase II (Pol II) transcribes into downstream intergenic regions in HSV-1 infection. Therefore, the goal of this work is to investigate the molecular basis of DoTT and dOCRs regarding changes in (i) localization of different histone marks, (ii) chromatin remodeling factors as well as changes in (iii) total and phosphorylated RNA Pol II.

To achieve this, we established a rapid and low-input chromatin immuno-precipitation ChIP-seq approach, termed ChIPmentation. In contrast to the adaptor ligation commonly applied in ChIP-seq, ChIPmentation utilizes adaptor insertion by 'tagmentation' via Tn5 transposase directly on a bead-bound chromatin. We have successfully established ChIPmentation for H3K4me3 and found that it provides high quality data on genome-wide chromatin composition of histone marks in both uninfected and HSV-1 infected cells. Our preliminary results show selective loss or gain of certain histone marks during HSV-1 lytic infection. Altogether, we anticipate this work will provide important insights into the underlying molecular mechanisms involved in a DoTT and DoTT-associated dOCRs formation.

P29

Identification of RBP-RNA interaction sites from CLIP-Seq data

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All RNA molecules are subject to post-transcriptional gene regulation, including mechanisms such as splicing, cleavage and polyadenylation, editing, transport, stability, and translation.

These mechanisms rely on the specific recognition of functional RNA elements by RNA-binding proteins (RBPs) and a range of cross-linking and immunoprecipitation based sequencing-protocols (CLIP-Seq) have been developed to detect RBP target sites as well as RNA-modifications.

Therefore, it is possible to generate genome-wide maps of RNA binding protein -- RNA interaction sites and evaluate the contribution of these interactions to gene regulation in the context of organismal development or diseases. However, the analysis of CLIP-Seq data is challenging, as the reads are typically short and contain diagnostic events. Furthermore, many confounding factors need to be accounted for to control the false positive rate.

Here, we present omniCLIP, a probabilistic approach to identify RBB-RNA interaction sites from CLIP-data. Our model presents a principled framework for the analysis of CLIP-Seq assays and takes into account several important new aspects. First, we jointly model the CLIP-Seq data in all replicates and account for various confounding factors. Additionally, we use an empirical Bayesian approach to identify and model diagnostic events and sequencing errors. Finally, we model biological and technical variance in our model. Overall, jointly modelling all information and uncertainties allows determining an accurate picture of the RNA-RBP interaction landscape.

We show that omniCLIP can be applied to PAR-CLIP, HITS-CLIP, iCLIP and eCLIP data and that it outperforms each method that we have compared it against. This is insofar remarkable as most competitor methods are tuned for specific protocols. This shows, that omniCLIP greatly simplifies the CLIP-Seq data analysis, increases the reliability of results and can pave the way for integrative studies based on data from CLIP-Seq assays

P30

Temporal Lobe Epilepsy: how much can be explained by circular RNAs?

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Background: Circular RNAs (circRNAs) are a class of long non-coding RNAs produced as a result of mRNA back-splicing events. circRNAs are enriched in the brain and in specific subcellular compartments of neurons but their neuronal role remains largely unexplored. During the past couple of years, circRNAs have been linked to multiple brain diseases often via their function as microRNAs sponges. Temporal Lobe Epilepsy (TLE) is a chronic neurological disease characterized by the occurrence of spontaneous seizures initiated in the hippocampus and spreading to other brain regions. Despite the availability of anti-epileptic drugs (AEDs) approximately 30% of patients become pharmacoresistant, emphasizing the need for the discovery of new therapeutic targets and new drugs' design. Goal: To identify the role and mechanism-of-action of circRNAs in TLE.

Methods: Hippocampal tissue resected from TLE patients and postmortem control hippocampal tissue from control individuals was subjected to subcellular fractionation. RNA was isolated from nuclear and cytoplasmic fractions and analysed using high-throughput RNA sequencing. Bioinformatics pipelines were used to identify circRNA candidates and (semi-)quantitative PCR was performed to validate selected circRNAs.

Results: Transcriptome profiling identified a total 12986 circRNAs in the human hippocampus, most of these being exonic circRNAs (95%). 61 circRNAs were differentially expressed between TLE patients and control individuals (≥ 2 fold change). 36 circRNAs were upregulated and 25 downregulated as compared to the control group. Intriguingly, the majority of the 61 circRNAs was present in the nucleus (85%) while only a small fraction was cytoplasmic (15%). 10 circRNAs were selected for further validation using quantitative PCR. Conclusions: A selected set of circRNAs is deregulated in the hippocampus of TLE patients. Further studies will be aimed at characterizing a potential functional role for these circRNAs in TLE pathogenesis.

P31

Record-seq reveals core features of transcription dynamics in single cells

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Current single-cell RNA sequencing (scRNA-seq) approaches analyze total RNA profiles at a single time point but convey little information about the underlying temporal dynamics. They all share the major limitations that (i) responses to perturbations cannot be measured directly, (ii) kinetics of transcription (e.g. bursts) cannot be investigated, (iii) short-term changes due to a perturbation or stimulus within a timescale of a few hours are masked by pre-existing RNA and (iv) changes in RNA synthesis and decay cannot be differentiated. Here, we present Record-seq, which integrates metabolic RNA labeling, biochemical nucleoside conversion and scRNA-seq to directly record transcriptional activity in addition to transcript levels. Key to the analysis of Record-seq data was a new computational approach (Globally Refined Analysis of Newly transcribed RNA and Decay rates using SLAM-seq (GRAND-SLAM)). Based on a rigorous statistical model and Bayesian inference it enabled us to precisely quantify newly transcribed RNA for thousands of genes in hundreds of individual cells. We applied Record-seq to decipher intercellular heterogeneity in the initial cellular response to lytic cytomegalovirus (CMV) infection. Record-seq visualized gene-specific fluctuations in transcriptional activity, on-off dynamics and bursts at single-cell level. We show that heterogeneity is associated with promoter-intrinsic features (TATA-boxes, CpGs) indicating that DNA methylation renders promoters non-permissive in between transcriptional bursts. The non-permissiveness was resolved in infected cells for NF-κB and IFN induced genes. Thus, without increasing the maximal levels of newly synthesized RNA in individual cells, target genes are upregulated on population level, revealing a fundamental principle of their transcriptional regulation. This establishes Record-seq and GRAND-SLAM as powerful and broadly applicable tools to study transcriptional activity in single cells.

This poster will be presented to complement the short talk with the same title.

P32

Functional mapping of spontaneous and evoked neurotransmission reveals intersynaptic heterogeneity

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Neurotransmission in central synapses is primarily mediated by an action potential-evoked (AP-evoked) release of synaptic vesicles (SVs) from the presynaptic terminals. Alternatively, emerging evidence suggest that spontaneous fusion of SVs with the presynaptic membrane contributes substantially to synaptic transmission. There is large body of data showing that the postsynaptic targets of these two release modes are segregated. However, whether the same molecular machinery at the presynapse serve to both spontaneous and AP-evoked release or distinct molecular framework underlie the two forms of synaptic release has remained highly debated. In this study, we combined neurotransmission imaging with immunocytochemistry to first, perform a robust spatiotemporal characterization of spontaneous and AP-evoked release at the single vesicle level in cultured hippocampal neurons, and second, provide a detailed map of the molecular underpinning of the two synaptic release modes at the presynaptic terminals. We show that the two forms of synaptic transmission are poorly correlated at individual synapses, and that at synapses where one form of synaptic release is dominated there is a strong correlation between functional phenotype of the synapse with specific protein targets. With this approach, we shed light on an intersynaptic heterogeneity in presynaptic molecular composition including SV and plasma membrane-associated proteins as well as presynaptic soluble proteins, and demonstrate how the regulation of distinct forms of neurotransmission is coded by their corresponding molecular identifiers.

P33

Exploring RNA polymerase II role in circular RNA biogenesis

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Rpb1 is the largest subunit of RNA polymerase II (RNAPII) and it contains a long C-terminal domain (CTD) which is post-translationally modified at different stages of the transcription cycle. CTD modifications are essential for efficient capping, splicing and polyadenylation of nascent protein-coding transcripts. Circular RNAs (circRNAs) are a novel class of RNAs which are most abundant in neuronal cells and tissues, but their biogenesis remains ill understood. CircRNA formation starts by joining the 3' end of a downstream exon to an unspliced upstream exon junction, a process called backsplicing.

To investigate mechanisms that may interfere with recognition and splicing of introns in circRNA-producing genes, we identified circRNAs by RNA-seq and mapped the chromatin occupancy of modified forms of RNAPII by ChIP-seq in several stages of in vitro differentiation from mouse ESCs to dopaminergic and spinal motor neurons. CircRNAs were detected throughout differentiation, peaking at neuronal stages, as expected. We find that circRNAs tend to be produced from genes that are expressed at the mRNA level during differentiation. Interestingly, genes producing circRNAs have higher mRNA levels than genes not producing circRNAs at the corresponding stage. This suggests that circRNA formation may result from imbalances between RNAPII loading and recruitment of the splicing machinery. CircRNA-producing genes are also longer, and have more exons than genes not producing circRNAs. We further confirmed that exon 2 is the most frequently included in circRNAs irrespectively of differentiation stage, suggesting an early defect in the recruitment of the splicing machinery. We are currently exploring how RNAPII regulation affects circRNA formation to help clarify circRNA biogenesis mechanisms at different stages of neuronal maturation.

P34

From pathogenesis to therapy of triplet repeat expansion diseases

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The group of human neurological diseases caused by trinucleotide repeat expansions includes Friedreich's ataxia (FRDA), Huntington's disease (HD) and several spinocerebellar ataxias (SCAs). Our group studies pathological processes and develops therapeutic approaches in respect of these lethal diseases.

To precisely examine the contribution of mutant RNA to pathogenesis of polyglutamine (polyQ) diseases (namely HD and SCA3) we generated new cellular and mouse models allowing separation of transcript toxicity from protein toxicity. In our stable and isogenic neuroblastoma SCA3 cellular models (SH-SY5Y), expressing translated or non-translated full-length ataxin-3, we currently identify novel RNA- and protein-mediated pathogenic pathways by RNA-Seq and quantitative proteomics. Moreover, two HD transgenic mouse models expressing translated or non-translated human mutated cDNA huntingtin fragment were obtained by using knock-in strategy into Rosa26 locus.

Another aspect of our research is to determine the function of non-coding RNAs in selected diseases. So far, we have screened mRNA and miRNA pools in FRDA fibroblasts using RNA-Seq. We compared these two pools, identified and validated miRNA targets which could be essential for pathogenesis of FRDA.

It is an attractive option to target mutation site directly to therapeutically inhibit the expression of mutant gene in case of polyQ diseases. We use CAG repeat-targeting siRNAs with substitutions resulting in base mismatches with the target sequence. MiRNA-like activity of these siRNAs results in allele-selective downregulation of mutant protein. Currently, we investigate activity and mechanism of these siRNAs in inducible HEK293 Flp-In T-REx cell lines expressing huntingtin fragment and iPSC-derived human neural HD progenitors.

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P35

An integrative epigenomic analysis identifies SOX10 as a glioblastoma master regulator

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Cancer Master Regulators (MRs) are proteins that define the cell state of cancer cells via transcriptional regulation. As the epigenome of a cell is highly correlated with the transcriptome, it therefore follows that transcription factor (TF) proteins acting as cancer MRs are also likely to regulate the epigenome, which itself will affect the activity of TF MRs. Human adult glioblastoma (GB) consists of four methylation subtypes. However, the epigenetic landscape of each subtype remains largely uncharacterised, as well as the interplay between MR activity and the epigenome. We present an integrated epigenomic characterisation and MR identification of GB subtypes using a cohort of 60 deeply characterised adult GBs, identifying SOX10 as a master regulator of the RTK_I subtype, and whose loss causes a transition to the MES subtype. We describe differences between subtypes in their methylation and chromatin landscapes at base-pair resolution, including a novel CpG Island Methylator Phenotype (CIMP)-like pattern of hypermethylation targeting normal brain bivalent promoters in the RTK_II subtype. We identified 10 consensus MRs using orthogonal analyses of superenhancers and gene regulatory networks, and we show that the removal of SOX10 in an RTK_I cell line model results in MR activation, phenotypic, and epigenetic changes characteristic of the MES subtype. Our analysis is the first comprehensive comparative analysis of GB subtype epigenomes, and illustrates the interplay between epigenetics, gene expression and MR activity.

P36

The guardian on the move, between firing and tranquilized: From p53 dynamics to the regulation of stochastic bursting of target gene expression and cell fate.

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p53 is a main hub in the signaling network regulating the cellular stress response and orchestrates the conversion of incoming signals to alternate cell fate decisions by changing the expression of hundreds of target genes. Aberrations in p53's cellular activity are related to severe human malignancies such as cancer. Recently, it has been shown that variable dynamics of p53's nuclear accumulation enable the pathway to mediate distinct responses to cellular stress and resulting cell fate decisions. However, the molecular mechanisms translating time dependent changes of p53's nuclear accumulation to altered gene expression and cell fate remain elusive.

We now aim to understand how p53 activation influence target gene transcription mechanistically. As it has been shown that individual cells within a population react heterogeneously to incoming stimuli, we employ single-molecule FISH to quantify the number of target gene RNAs in single cells at defined time points and use the resulting data to inform a mathematical model of p53 dependent promoter activity. We find that absolute numbers and transcription properties of p53 targets are highly heterogeneous. Our data shows characteristic principles how p53 nuclear accumulation increases transcriptional bursting upon stimulation, but also highlights gene specific differences throughout the signaling response to DNA damage. To correlate nuclear p53 over time with the dynamics of target gene transcription in single cells we extend our results including time-resolved measurements in living cells using MS2 based live cell RNA imaging of p53 targets.

Our approach allows to following the information flow from a stress stimulus to p53 dynamics, target gene expression and cell fate and is the first attempt to characterize stochastic bursting of p53 targets on the level of individual transcription sites. This will not only extend our current mechanistic understanding of p53 as a major transcription factor but may also serve as a basis to enable more targeted cancer therapies in the future.

P37

Conditional, targeted and multiplexed mutagenesis of regulatory sequences in animals

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Regulatory genomic sequences are often redundant, fuzzy, and poorly understood, thus requiring many mutants to find regulatory mechanisms and phenotypes. Compared to the huge number of animal models with loss-of-function mutations in coding sequences, very few mutants for regulatory sequences are published. Here we use transient Cas9 induction and pooled guide RNAs in *C. elegans* to generate thousands of mutant animals. For example when we target a 250 bp region of interest, we detect hundreds of unique genotypes created by indel mutations. We apply this method to analyze 3'UTRs of more than a dozen genes and discover mutations which reduce fitness or lead to a very clear developmental phenotype. Our method is useful to understand genotype to phenotype relations and to discover functional elements in an animal.

P38

Single-molecule imaging of nuclear volume-enhanced transcription factor-chromatin associations during Zebrafish embryo development

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Zebrafish embryos start transcribing their genome after ten fast cell divisions. Before this major developmental step of zygotic genome activation (ZGA), they live on maternally provided mRNAs and proteins. How transcription factor-chromatin interactions evolve during development to support ZGA is largely unknown. We established single-molecule tracking in live developing Zebrafish embryos using reflected light-sheet microscopy to visualize two transcription factor (TF) species, the general TF TBP and the potential pioneering factor Sox19b. We further developed a novel data acquisition and analysis scheme to extract quantitative information on TF concentrations, binding kinetics and bound fractions during fast cell cycles. We observed that the percentage of chromatin-bound TFs increased for both TF species during early development. For TBP, the increase was as expected from a physical model of TF-chromatin interactions including the decreasing nuclear volume after each cell division and an increasing DNA accessibility. In contrast, for Sox19b, the increase in chromatin-bound fraction was predominantly due to the shrinking nucleus. Our quantitative single-molecule data suggests that the decreasing nuclear volume facilitates ZGA in Zebrafish embryos by enhancing TF-chromatin associations.

P39

Exon junction complexes suppress spurious splice sites to safeguard transcriptome integrity

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Productive splicing of human pre-mRNAs requires the correct selection of authentic splice sites from the large pool of potential splice sites. Although splice site consensus sequence and splicing regulatory proteins are known to influence splice site usage, the mechanisms ensuring the effective suppression of cryptic splice sites are insufficiently explored. We have recently discovered that many aberrant exonic splice sites are efficiently silenced by the exon junction complex (EJC), a multi-protein complex that is deposited on spliced mRNA near the exon-exon junction. Upon depletion of EJC proteins, cryptic splice sites are de-repressed, leading to the mis-splicing of a broad set of mRNAs. Mechanistically, the EJC-mediated recruitment of the splicing regulator RNPS1 inhibits cryptic 5' splice site usage, while the deposition of the EJC core directly masks reconstituted 3' splice sites, thereby precluding transcript disintegration. Thus, the EJC protects the transcriptome of mammalian cells from inadvertent loss of exonic sequences and safeguards the expression of intact, full length mRNAs.

P40

The non-canonical SMC protein SmcHD1 antagonises TAD formation on the inactive X chromosome

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The inactive X chromosome (Xi) in female mammals adopts an atypical higher-order chromatin structure, manifested as a global loss of local topologically associated domains (TADs), and formation of two mega-domains. In this study we demonstrate that the non-canonical SMC family protein, SmcHD1, which is important for gene silencing on Xi, contributes to this unique chromosome architecture. Specifically, allelic mapping of the transcriptome and epigenome in SmcHD1 null cells revealed the appearance of sub-megabase domains defined by gene activation, CpG hypermethylation and depletion of Polycomb-mediated H3K27me3. These domains, which correlate with sites of SmcHD1 enrichment on Xi in wild-type cells, additionally adopt features of active X chromosome higher-order chromosome architecture, including partial restoration of TAD boundaries. Xi chromosome architecture changes also occurred in an acute SmcHD1 knockout model, but in this case, independent of Xi gene de-repression. We conclude that SmcHD1 is a key factor in antagonising TAD formation on Xi.

P41

A multitask and multimodal deep neural network for characterizing RNA binding protein target preferences

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Deep learning has become a powerful method to analyze the binding sites of RNA-binding proteins (RBPs), owing to its strength to learn complex features from possibly multiple sources of raw data. However, interpretation of these models, which is crucial to improve our understanding of RBP binding preferences and functions, has not been investigated in depth.

We have designed a multitask and multimodal deep neural network for characterizing RBP binding preferences. The model incorporates not only the sequence but also the region type of the binding sites as input, which helps the model to boost the prediction performance. To interpret the model, we quantified the contribution of the input to the prediction of each RBP. Learning across multiple RBPs at once, we can identify the RNA sequence motifs and transcript context patterns that are the most important for the predictions of each individual RBP. Our findings are consistent with the known motifs and binding behaviors of RBPs and can provide new insights about the regulatory functions of RBPs.

P42

Tissue-specific chromatin dynamics reveal central processes in neuronal development

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We aim to understand the developmental trajectories naïve cells take to give rise to functional tissues during early Drosophila nervous system development. As the nervous system primordium first specifies, it is quickly subdivided into defined columnar domains that give rise to very distinct sets of neuronal stem cells. As neurogenic cell fate decisions intricately depend on in vivo context, we utilize and develop technologies that resolve regulatory information in developmental space and time. We are utilizing BiTS (Batch isolation of Tissue-Specific chromatin) to isolate nuclei from specific columnar cell populations at consecutive time points spanning formative events in neurogenesis. Use of this material in ChIP-Seq and ATAC-Seq allows assessment of genome-wide distributions of covalent histone modifications and chromatin accessibility which are predictive features of the regulatory genome. By profiling chromatin state dynamics with temporal and spatial resolution, we are mapping active and repressed genes, usage of alternate isoforms, and the neurogenic enhancers that drive these regulatory dynamics. Combining differential peak calling and chromatin state calling with Hidden Markov models allows de novo identification of differentially regulated CRMs providing novel insights into how the developmental trajectories of individual primordial regions differ, and allows to extract mechanisms that drive fate decisions.

P43

Patients' networks for clinical phenotype/outcome prediction

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Methods for phenotype and outcome prediction in bioinformatics usually rely on supervised models that employ set of biomarkers to discriminate between patients but do not directly consider the functional or genetic relationships between them. Instead, the field of "Network Medicine" adopts the whole set of connections between biomolecular components to e.g. ranking genes with respect to a given phenotype. Since our aim is to consider at the same time the relationships between individuals and their a priori available phenotypic information, we developed a network-based method called Sample-Net (S-Net) to rank patients with respect to an outcome of interest.

S-Net builds a network where nodes are samples/patients and edges similarities between them based on their clinical or genetic profiles (e.g. expression profiles). S-Net exploits the topological features of the net to assign a score to each individual and the application of a graph-kernel (e.g. random walk kernel) enriches the net with new informative edges. Then a semi-supervised method, a local learning strategy based on the guilt-by-association principle, assigns a score to each sample representing its odd to show the considered outcome and allowing its ranking.

We compared S-Net with supervised and semi-supervised methods on real datasets including Pancreatic, Melanoma, Ovarian, Breast, Colorectal and Colon cancer samples. S-Net not only is competitive with state-of-the-art methods but it can improve the predictions for patients difficult to classify with other methods. The graph of samples can be easily visualized to gain clues about the relationships between samples, considering the phenotype associated and predicted for each patient.

S-Net is able to effectively predict the phenotype, while the graphical representation of the patients' net provides significant insights into their biomolecular stratification. A fast implementation of S-Net is available online: https://github.com/GliozzoJ/S-Net.

P44

Genome-wide association study identifies the SERPINB gene cluster as a susceptibility locus for food allergy

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The genetic determinants underlying food allergy susceptibility remain largely unidentified to date. Due to the large spectrum of disease symptoms which may affect any organ system, a reliable diagnosis is often difficult to obtain. We performed a genome-wide association study on food allergy in patients in whom the diagnosis was based on oral food challenges. In total the discovery set comprised 497 cases and 2,387 controls. Another 1,050 cases and 2,510 controls were included in the replication. 5 loci reached the genome-wide significance threshold of $p < 5 \times 10\text{-}8$, the clade B serpin (SERPINB) gene cluster at 18q21.3, the cytokine gene cluster at 5q31.1, the filaggrin gene, the C11orf30/LRRC32 locus, and the human leukocyte

antigen (HLA) region. The HLA locus was identified as a peanut-specific locus after stratification of the results for the most common food allergens peanut, hen's egg and cow's milk. The other identified loci were associated with any food allergy.

Publically available databases such as the Ensembl Variant Effect Predictor and the GTEx Consortium Database, that harbours information on gene expression data in various tissues, were used to evaluate whether there is evidence for functionally relevant single nucleotide polymorphisms in the identified loci. Variants in the SERPINB gene cluster were identified as expression quantitative trait loci for SERPINB10 in leukocytes. GTEx expression data also showed high expression of SERPINB genes in the esophagus. As the five associated loci play a role in the regulation of the immune response or in the formation of an intact epithelial barrier, our results highlight the importance of both mechanisms in food allergy development.

P45

Robust network inference with a simple response logic

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Mechanistic understanding about a biological process is achieved once the interactions between its components are mapped out. Quite commonly though, there is no direct way of measuring e.g. effective binding between molecules that could reveal the regulatory network. Therefore various reverse-engineering methods attempt to infer causation from more indirect data such as differential gene expression. However most of these methods make assumptions on the underlying process that are rarely met in a realistic setting. To make network inference more robust, we propose a scheme that is based on a simple response logic with minimal presumptions: We require that we can experimentally observe whether or not some of the system's components respond to perturbations of some (other) components, and identify the network structure(s) that could most accurately reproduce such a response pattern, assuming that there must be a network path between a perturbed and all responding components and no path to any non-responding components. Since the number of possible network structures growth exponentially with size, this poses a considerable technical challenge as a naive search fails even for few nodes. Thus, we developed a constraint programming approach, which allows to analyze networks of hundreds of nodes. The method is robust to noisy, heterogeneous or missing data, allows to integrate prior network knowledge and outperforms existing approaches in DREAM-challenge competitions. We applied it to infer transcription factor networks from RT-PCR data in human hepatoma cells and from single-cell RNA profiles of mouse dendritic cells and confirmed the predictions by comparison to literature knowledge and ChIP data. Further, the method correctly identified the canonical mTOR and MAPK pathways in an adenocarcinoma cell line, and interestingly, indicated altered feedback mechanisms for different PI3K mutants which could explain their distinct sensitivity towards EGFR inhibitors.

P46

Mechanisms of neurogenic gene expression – Specific enhancer-regulator interactions with spatiotemporal resolution

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During organismal development, naive cells give rise to differentiated cell types as a result of a tightly regulated transcriptional program that allows distinct functional tissues to emerge. Although many aspects of this differentiation are still poorly understood, it is widely accepted that transcription factors (TFs) interacting with *cis* regulatory modules (CRMs), namely enhancers, are the major contributor to regulated gene expression. In nervous system development, for example, we have identified several tissue specific CRMs, but we cannot yet explain what regulates their specific spatio-temporal activities based on the known TF interactions.

I am using inSTEP to unravel the CRM-protein interactions on two major neurogenic (vnd and rho) and one mesodermal (1070) enhancers. inSTEP allows for the dissection of enhancer-regulator interactions with temporal and spatial resolution. Long term, this should result, not only in a better understanding of specific enhancer activity, but also in a more complete regulatory network driving early development.

One of the enhancers I am most interested in is the ventral neurogenic column (VC) enhancer for the gene ventral nervous system defective (vnd), which encodes a TF known to be a key regulator of VC fate. I have therefore expanded my project beyond the question of 'how' vnd expression is regulated, to also include the role Vnd itself plays in neurogenesis. I have conducted ChIP-seq experiments to elucidate the genome-wide binding profiles of Vnd.

Meanwhile, it became known that, although Vnd was assumed to be a dedicated transcriptional repressor, there is a second isoform with an alternate 1st exon, that lacks a repression domain. Thus the effect of both isoforms on target genes may well be drastically different. To elucidate the isoform-specific roles, we used CRISPR-Cas9, to tag the specific N-termini independently with a MIN-tag. These constructs should now allow for ChIP-seq, IP-MS, and further modification of the locus in an isoform-specific manner.

P47

Non-coding AUG circRNAs constitute an abundant and conserved subclass of circles

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Circular RNAs (circRNAs) are a subset of noncoding RNAs (ncRNAs) previously considered as products of missplicing. Now, circRNAs are considered functional molecules, although to date, only few functions have been experimentally validated, and therefore the vast majority of circRNAs are without known relevance. Here, based on RNA sequencing from the ENCODE consortium, we identify and characterize a subset of circRNAs, coined AUG circRNAs, defined by spanning the canonical translational start site in the protein-coding host genes. AUG circRNAs are more abundantly expressed and conserved than other groups of circRNAs, and they display an Alu-independent mechanism of biogenesis. The AUG circRNAs contain part of bona fide ORF, and in the recent years, several studies have reported cases of circRNA translation. However, using thorough cross-species analysis, extensive ribosome profiling analyses and experimental data on a selected panel of AUG circRNAs, we observe no indications of translation of AUG circRNAs or any other circRNAs. Our data provide a comprehensive classification of circRNAs and, collectively, the analyses suggest that the AUG circRNAs constitute an abundant subclass of circRNAs produced independently of primate-specific Alu elements. Moreover, AUG circRNAs exhibit high cross-species conservation and are therefore likely to be functionally relevant.

P48

Nanog induction dynamics are controlled by independent transcription factors and negative feedback

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Current pluripotency network models are based on snapshot or bulk average measurements, but gene regulation and cell fate decisions occur in single cells over time. Previous studies have measured the expression dynamics of one or two transcription factors (TFs) in mammalian cell lineage trees, but it remains elusive how they are regulated. Here, we quantified Nanog dynamics upon direct perturbations of the pluripotency TF-network by inducing Esrrb and/or Tfcp2l1 in mouse embryonic stem cells during live cell imaging. We found that both TFs induce Nanog in a dose-dependent way, but independently from each other. In combination, Esrrb and Tfcp2l1 cause robustly fast and strong Nanog inductions which are confined by negative feedback. We suggest that gene regulation by independent activators and negative feed-back stabilizes gene expression levels and functions as a noise filter during cell state transitions.

P49

A phase separation model for transcriptional control

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Phase-separated multi-molecular assemblies provide a general regulatory mechanism to compartmentalize biochemical reactions within cells. Recent work indicates that transcriptional co-activators can undergo liquid-liquid phase separation, and such phase separated condensates tend to occur at super-enhancers, which are clusters of transcriptional enhancers that drive robust expression of genes with prominent roles in cell identity. I will describe new insights into the formation and function of transcriptional condensates, and the roles they play during differentiation and disease.

P50

TAD structures are dispensable for developmental gene regulation but disruptive when reconfigured

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The genome is organized in megabase-sized 3D-units, called Topologically Associated Domains (TADs) that are thought to function as a scaffold for enhancer-promoter interactions. The importance of TADs for gene regulation has been implicated in studies using mostly correlative analyses. However, little experimental evidence exists that tests the role of TADs and its functional elements in vivo. In a developmental context, it remains unclear how TAD structures are formed, how the insulation between domains is achieved, and - importantly - how TADs affect gene regulation.

Here, we address this question by systematically editing the TADs at the *Sox9* locus. Through a series of genome-engineered mouse lines, we investigate the functional relevance of CTCF-anchored TAD structures for gene regulation. We correlate cHi-C, gene expression, and mouse phenotypes to understand how reconfiguration of TADs and the 3D genome architecture influences enhancer-promoter interactions.

We find that deletion of a TAD boundary alone is insufficient for TAD fusion. In contrast, disruption and fusion of neighbouring TADs is achieved only by deleting the TAD boundary in combination with five major CTCF sites shaping the intra-TAD structure. Surprisingly however, this fusion does not cause enhancer-promoter rewiring and pathogenic gene misexpression. Instead, we demonstrate through a series of targeted inversions and by repositioning an individual TAD boundary that effective enhancer-promoter rewiring requires the combinatorial effect of TAD boundaries and TAD sub-structure orientation. Collectively, we show that a refined TAD structure represents a layer of regulation that is not necessary to maintain enhancer-promoter interaction, but – if rearranged – is sufficient to force gene misexpression through enhancer-promoter rewiring.

P51

Inter-chromosomal hubs and disruption of chromatin organization in mouse model of Autism Spectrum Disorder (ASD)-linked 16p11.2 CNV

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Structural variants in the human 16p11.2 locus spanning a 600kb region with 29 genes are associated with early-onset autism spectrum disorder with variable degree of penetration. To investigate the changes in transcriptional regulation that accompany the complex phenotypes observed in the presence of 16p11.2 structural variants, we have measured gene expression and chromatin folding in mouse embryonic stem cells and during in vitro differentiation into neuronal progenitor cells and early dopaminergic neurons. In addition to the expected decrease in expression of the genes at the 600kb region, we also identified changes in expression in genes in different chromosomes which have known functions in neuronal biology. We are currently validating these changes by qPCR. We are also analyzing matched Hi-C data to ask whether the changes in expression are due to altered chromatin contacts. Using polymer physics and computer simulations, we connect our findings to topological features of 16p11.2 locus and its inter-chromosomal contacts. Our findings may provide novel insights into the critical, yet understudied mechanisms underlying the damaging role of the 16p11.2 variant for the neuronal development.

P52

Functional characterization of RNA fragments using high-throughput interactome screening

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Populations of small eukaryotic RNAs, in addition to relatively well recognized molecules such as miRNAs or siRNAs, also contain fragments derived from all classes of constitutively expressed non-coding RNAs. It has been recently demonstrated that the formation and accumulation of RNA fragments (RFs) is cell-/tissue-specific and depends on internal and external stimuli. Unfortunately, the mechanisms underlying RF biogenesis and function remain unclear. To better understand them, we employed RNA pull-down and mass spectrometry methods to characterize the interactions of seven RFs originating from tRNA, snoRNA and snRNA. By integrating our results with publicly available data on physical protein-protein interactions, we constructed an RF interactome network. We determined that the RF interactome comprises proteins generally different from those that interact with their parental full length RNAs. Proteins captured by the RFs were involved in mRNA splicing, tRNA processing, DNA recombination/replication, protein biosynthesis and carboxylic acid metabolism. Our data suggest that RFs can be endogenous aptamer-like molecules and potential players in recently revealed RNA-protein regulatory networks.

P53

Reconstructing physical properties of RNA-protein interactions from RNA Bind'n'Seq experiments

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RNA-binding proteins (RBPs) recognize RNA through specialized domains (RBDs). These interactions underlie gene expression, post-transcriptional regulation, and human diseases. However, detailed knowledge of RBP-binding is limited to a fraction of RBPs and/or substrates and often insufficient to explain *in vivo* binding. To elucidate RNA binding, the RNA Bind'n'Seq (RBNS) method employs high-throughput sequencing of RNAs that have been selected from a vast pool of randomized RNA by RBP-binding at different protein concentrations, and for 78 diverse RBPs. We have developed a thermodynamic-equilibrium model for RBNS, which allows to fit primary sequence affinities to the experimental data. Our model includes RNA secondary-structure accessibility, and saturation/titration effects due to finite amounts of RNA and protein, linking the model to the energy scale of RNA folding and known concentrations. The fitted affinities can therefore be interpreted as dissociation-constants. The results match known motifs and correlate with dissociation-constants from independent experiments. We then predict binding to longer, transcriptome-derived sequence fragments used in natural sequence RBNS (nsRBNS) experiments. Our method outperforms simpler models based on k-mer enrichment or RNAcompete data. We conclude that our model allows to extract important biophysical details of protein-RNA interactions from high-throughput experiments, bringing us closer to understanding RBPs in vivo.

P54

Gene regulation by PCF11-dependent alternative polyadenylation and transcription termination

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The pervasive nature of RNA polymerase II (Pol II) transcription requires efficient termination. A key player in this process is the cleavage and polyadenylation (CPA) factor PCF11, which directly binds to the Pol II C-terminal domain and dismantles elongating Pol II from DNA in vitro. However, PCF11 has been mainly studied in yeast and its vertebrate functions are unknown. We demonstrate that PCF11-mediated termination is essential for vertebrate development. A range of genomic analyses, including: mNET-seq, 3' mRNA-seq, chromatin RNA-seq and ChIP-seq, reveals that PCF11 enhances transcription termination and stimulates proximal alternative polyadenylation (APA) genome-wide. PCF11 binds preferentially between closely spaced genes, where it prevents transcriptional interference and downstream gene silencing. Notably, we show that PCF11 is sub-stoichiometric to the CPA complex and acts as a regulatory factor. Low levels of PCF11 are maintained by an auto-regulatory mechanism involving premature termination of its own transcript, and are important for normal development. Both in human cell culture and during zebrafish development, PCF11 selectively attenuates also the expression of other genes by premature CPA and termination. Strikingly, this PCF11-dependent attenuation targets preferentially transcriptional regulators, with the same genes prematurely terminated by PCF11 in human and zebrafish. The phenomenon of negative gene regulation by premature termination is well described in *S. cerevisiae,* where it plays a physiological role in response to changing growth conditions. We propose that premature termination is a fundamental gene regulatory mechanism operating also in vertebrates.

P55

Differential chromatin contacts between the parental alleles and their role in pluripotency and imprinting

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The folding of chromosomes and the structural organization of the genome impacts human health and disease. Regulatory regions influence the expression of their target genes through contacts in close spatial proximity. Thus, structural variability of the genome, leading to disruption of chromatin contacts or altered genome topology, can play important roles in gene regulation, especially in the context of differentiation and disease.

We study allelic chromatin contacts using Genome Architecture Mapping (GAM), an orthogonal method to 3C-based approaches. GAM extracts 3D genome folding information by sequencing DNA from a large collection of thin nuclear sections and quantifying the frequency of locus co-segregation. By applying GAM to hybrid mouse embryonic stem cells, we are able to detect chromatin contacts with allele specificity and at high resolution. Compared to other methods, GAM detects different contacts between the parental alleles, raising new questions about allele-specific gene regulation and imprinting in early development.

P56

CircRNA expression profile in neuronal activity

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Neuronal activity plays an essential role in synaptic plasticity and the electrochemical transmission efficacy of synapses. CircRNAs were previously shown to be highly expressed in the mammalian brain and enriched in synaptic terminals. The present study was conducted to identify the different subsets of circRNAs induced by different duration of neuronal activity. Expression profile of circRNAs was investigated in mouse cortical neurons upon stimulated neuronal activity by potassium chloride (KCl) treatment which controls the neuronal firing and calcium influx. Both brief and moderate duration of activity induced different circRNAs sets, which suggests that circRNA expression levels is coupled to neuronal activity patterns. Further study will be knock-down of circRNA candidates by CRISPR/Cas13 to conduct functional study in neuronal plasticity.

P57

Automated analysis and result reporting for targeted sequencing data of a hemophilia A & B patient cohort

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Targeted sequencing of genomic regions associated with Mendelian disease or somatic cancer variation is a cost- and time-efficient approach for screening patient cohorts in modern medicine. Here, we introduce a fast and efficient pipeline to analyze highly imbalanced, targeted next-generation sequencing (NGS) data sets generated using enrichment by molecular inversion probes (MIPs). MIPs are single-stranded DNA molecules that allow targeted amplification of genomic regions and enrichment using Capture by Circularization (Turner EH et al., Annu Rev Genom Hum Genet., 2009). By pooling multiple barcoded MIP reactions, cost-efficient multiplex sequencing can be performed and samples computationally separated using sequence barcodes. The proposed pipeline processes (MIP-arm trimming and overlap read merging), aligns and sorts MIP reads via burrows wheeler transform alignment (BWA), handles coverage imbalance and calls variants using either GATK v3 UnifiedGenotyper in combination with IndelRealigner or GATK v4 HaplotypeCaller. Further, the pipeline supports the analysis of MIPs specifically designed to capture certain structural variants and determines the genetic sex of patients using Y-chromosome-unique probes. In a user-friendly report, we summarize coverage information (incl. incompletely covered regions) as well as variant effect predictions (based on Ensembl VEP) and variant call qualities for SNVs and InDels of each patient and each targeted region. We developed and tested the pipeline using data for a MIP design of >450 probes targeting the Factor VIII (F8) and Factor IV (F9) genes in a hemophilia A & B cohort from the “My Life, Our Future” initiative (Johnson et al., Blood Advances, 2017). Our setup enables the screening of 384 patients on a single Illumina NextSeq run. The pipeline is available as a Snakemake implementation on GitHub (https://github.com/kircherlab/hemoMIPs).

P58

Systematic integration of models and data for yeast growth, division and stress response

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With the progress of genome-wide experimental approaches we witness the establishment of more and more libraries of genome-wide data for proteins or RNA or metabolites, especially for well-studied model organisms such as bakers' yeast. However, the separated consideration of metabolic networks or gene regulation networks does not tell us how these networks are integrated to allow a cell to grow, divide and respond to changing environments.

We use the yeast Saccharomyces cerevisiae as the model organism for eukaryotic cells allowing to comprehensively analyzing regulatory networks and their integration with cellular physiology. Here, we focus on processes during the cell division cycle and study the changes of signaling, metabolism, or ion transport during the growth of a single cell.

We use a modular and iterative approach that allows for a systematic integration of cellular functions into a comprehensive model allowing to connect processes that are strongly interlinked in cellular life, but measured separately. The modular concept also to zoom in and out of different aspects of regulation or dynamics become important.

P59

Circular RNAs contribute to the pathogenesis of neuroblastoma

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Circular RNAs (circRNAs) are a class of non-coding RNAs produced by backsplicing events. They are involved in gene expression regulation and are found to be highly abundant in neural tissues. This project investigated the role of circRNAs in neuroblastoma, the most common extracranial pediatric tumor. Whole transcriptome sequencing was performed on 69 primary neuroblastoma tumors from all risk groups. We identified 22,306 unique circRNA backsplicing events involving 5386 genes and 1049 intergenic regions. Hundreds of differentially expressed circRNAs were identified between risk groups. On average, we did not find circRNAs to be correlated with the corresponding gene mRNA. A biclustering method identified a subgroup of circRNAs that characterized the MYCN-amplified tumors and belonged to genes enriched for chromatin-remodeling functions. Several circRNAs were validated in a panel of neuroblastoma cell lines. Furthermore, we found circRNAs specifically upregulated in neuroblastoma when compared to healthy fetal brain tissue or to various other cancers. Among them was circARID1A. Knockdown of circARID1A, leaving the mRNA and protein levels of ARID1A unaffected, resulted in reduced cell numbers, viability, proliferation and in cell differentiation. Our preliminary results show that circRNAs play an important role in neuroblastoma and may help to elucidate the role of non-coding RNAs in neuroblastoma biology.

P60

The role of Dicer in shaping RNA landscape of human cells

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Endoribonuclease Dicer is best known for its pivotal role in the biogenesis of short regulatory RNAs; the enzyme processes precursor molecules to yield small interfering RNAs (siRNAs) and microRNAs (miRNAs). Extensive efforts over the last years have greatly expanded the functional repertoire of Dicer, highlighting the versatility of the enzyme. Dicer has been shown to serve a number of functions outside of the miRNA/siRNA pathways; it has been involved in processing of diverse RNA classes, including tRNA and snoRNA, detoxification of repeat-element-derived RNAs, and maintenance of genome integrity. Additionally, a caspase-processed form of Dicer has been reported to act as a deoxyribonuclease during apoptosis in *Caenorhabditis elegans*. Moreover, a recent discovery of Dicer passive binding to various RNAs pointed to a novel, catalysis-independent regulatory role of the enzyme.

Due to the essential function of Dicer in RNA silencing pathways, the profiling of RNA in Dicer-deficient cells have been mostly focused on RNA shorter than 30 nt, in particular miRNA. In this study, we performed next generation deep sequencing to characterize global RNA composition of wild-type and Dicer-knockout HEK293T cells. We characterized the pool of small RNAs (15–82 nt long), including stable RNA fragments (RFs), accumulating in the analyzed cell lines. We also determined changes in gene expression upon Dicer depletion and performed the enrichment analyses of processes and molecular functions associated with differentially expressed genes.

Altogether, our data reinforce the notion that Dicer regulates diverse aspects of RNA metabolism, and thus shapes mammalian transcriptome in a number of different ways.

P61

Quantitative analysis of circular RNA in *Arabidopsis thaliana*

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Circular RNAs (circRNAs) have recently emerged as a new large class of non-coding RNAs with unknown function. Up today, circRNAs abundance and evolutionary conservation have been shown across different species within animal and plant kingdoms. However, the more detailed studies of circRNAs have so far been performed mainly in human, leaving circRNAs in plants largely unexplored. Recently, a number of algorithms that enable circRNA finding based on back-splicing reads (reads overlapping circular splice junction formed by linking a downstream 5' splice site to an upstream 3' splice site) in total RNA sequencing data were developed. Unfortunately, most of these algorithms focus on the qualitative analysis of circRNA and neglects problems that are often encountered during the quantitative analysis.

In this study we focus on the quantitative analysis of circRNAs identified in flower, leaf, root and seedling of *Arabidopsis thaliana*. In total, we identified 2090 circRNAs supported by at least 2 back-splicing reads. To compare circRNAs abundances in different plant organs/seedling, we applied common normalization methods by the library size and total number of back-splicing reads in sample. Both of them gave false results because of differences in transcriptome composition and consequently in performance of rRNA removal kits. To solve this problem, we propose a pipeline which allows comparison of circRNA accumulation regardless of transcriptome composition. Differential analyses of the 168 most abundant circRNAs revealed organ-specific expression of circRNAs. Altogether, we identified 268 pairs of circRNAs differentially expressed (min. 2 times) in two organs or organ and seedling. Finally, for the selected circRNAs differential expression was confirmed using ddPCR.

P62

Enzyme-free digital counting of endogenous circular RNA molecules using NanoString technology

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Circular RNAs (circRNAs) are covalently closed endogenous molecules with tissue- and disease-specific expression patterns, which have potential as diagnostic and prognostic biomarkers. The molecules are formed by a back-splicing event and they exert diverse regulatory functions important in cellular differentiation and disease. The landscape of circRNA expression has not been characterized in several human diseases including psoriasis and B-cell malignancies, and current methods for circRNA quantification have several limitations that prevent development of clinically applicable assays. Here, we demonstrate that circRNAs can be accurately quantified without enzymatic reactions or bias using color-coded probes (NanoString technology). First, we performed high-throughput RNA sequencing (RNA-seq) to profile the genome-wide landscape of circRNA expression in psoriasis and in several B-cell malignancies. We detected circRNAs known to be deregulated in other human diseases and identified novel circRNAs. Based on these data, we selected more than 50 unique circRNAs for which we designed color-coded probes spanning their specific back-splicing junctions. These circRNAs were quantified in cell lines and patient samples simultaneously using the NanoString technology. The circRNA expression profiles obtained could distinguish different B-cell malignancies and lesional- and non-lesional skin from patients with psoriasis. The NanoString assays were specific for circRNA detection and data were more reproducible and quantitatively more accurate than RNA-seq data. In addition, we obtained high-quality data on severely degraded RNA samples from formalin-fixed, paraffin-embedded (FFPE) tissues. Together, we provide a map of circRNA expression in psoriasis and in B-cell malignancies and present an enzyme-free digital counting methodology, which has the potential to become a new gold standard for circRNA quantification.

P63

The role of 3D chromatin landscape in CA1 pyramidal neurons for high cognitive functions

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The regulation of genome architecture in neurons is crucial for synaptic plasticity and memory formation. SATB2 is a nuclear architectural protein which is a key determinant of memory consolidation in the adult hippocampus. Conditional deletion of Satb2 in the adult mouse forebrain prevents the stabilization of synaptic long-term potentiation and markedly impairs long-term memory formation (Jaitner et al., 2016).

The 3D chromatin topology of post-mitotic neurons has so far been studied only in cultured neuronal cells or in dissociated neural tissue. Genome Architecture Mapping (GAM) is a ligation-free approach to map chromatin contacts in single cells across cell populations (Beagrie et al., 2017). Here, we have developed a GAM pipeline that can be directly applied in intact tissues to map 3D contacts in rare cell types.

We have produced the first in-situ genome-wide chromatin contact maps in intact mouse brain tissue, for wildtype and Satb2(-/-) deficient CA1 pyramidal neurons. We identify cell-type specific patterns of chromatin contacts in CA1 pyramidal neurons and highly specific changes in chromatin contacts upon Satb2 depletion at relevant genes, both locally and across large genomic distances. We currently explore how the 3D neuronal chromatin landscape contributes to the physiology of CA1 pyramidal neurons in high cognitive functions.

References

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P64

Understanding zygotic genome activation in nuclear space.

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Zygotic genome activation is an important transition in the development of an embryo: after having been dependent on maternal factors during the first cell divisions, the zygote's own genome is activated for the first time. It is not fully understood how the transition from no transcription to global genome activation is controlled. Recent discoveries have highlighted nuclear organization as an important determinant of transcriptional regulation in both development and disease. Interestingly, in zebrafish, the canonical onset of transcription during genome activation is preceded by the appearance of two distinct areas of concentrated transcriptional activity. We propose that this organization of the transcriptional machinery is important for zygotic genome activation. To test this hypothesis, we will first identify the components that are brought together in these isolated foci. We have identified these foci based on their high concentration of elongating RNA polymerase II, but do not know what other proteins accumulate there. To identify such proteins, we will use proximity-based in vivo biotinylation. This will allow us to determine what the foci are comprised of, which proteins are required for their formation, and which ones are merely passengers. Next, we will visualize the different components of transcriptional hubs. We will start with RNA polymerase II and some candidate transcription factors and later extend our analysis to newly identified factors. This will reveal the order in which proteins assemble, clarify whether transcriptional activity is required, and uncover the sequence and dynamics with which the transcriptional activity spreads in nuclear space. Altogether, the project will broaden our understanding of the role of nuclear organization in genome activation. We will present our latest results.

P65

Probing the transcriptional regulatory landscape during zebrafish development

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Transcriptional enhancers and promoters dictate spatiotemporal patterns of gene expression thereby regulating cell differentiation and function. Determining the architectural features of such cis regulatory elements (CREs) has the bipartite benefit of increasing the accuracy of their identification while likely uncovering mechanisms for their mode of action. DNA accessibility, transcription factor binding, transcription initiation, and histone modification within adjacent nucleosomes are all architectural hallmarks of both enhancers and promoters, combinations of which reflect CRE class and activation state. Our recent analyses have uncovered an unexpected variation in CRE architecture between mammals and two other metazoan model systems *Drosophila melanogaster* and *Caenorhabditis elegans*. This led us to further investigate such architecture in the vertebrate zebrafish *Danio rerio,* a system that has emerged as a powerful in vivo model for development and disease. We have generated several genome-wide, next-generation sequencing datasets to assess CRE architecture in the whole 24hpf embryo, providing windows into hitherto unexamined aspects of transcription dynamics and chromatin structure. Both similarities and differences exist between zebrafish CRE architecture and that of other tested systems, begging the question of what underlying mechanisms lead to such variation. Future development of quantitative, tissue-specific assays for CRE architecture will enable spatiotemporal maps of the regulatory landscape during zebrafish development, with and without multiple modes of genetic perturbation, and allow us to computationally disentangle the underlying combinatorial DNA sequence grammar.

P66

Full-length mRNA sequencing reveals principles of poly(A) tail regulation

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Although isoform choice of eukaryotic mRNAs is highly diverse, functionally important and subject to complex regulation, there is little progress in obtaining isoform information from complex in vivo samples. In addition to the mRNA sequence complexity that is obtained by alternative transcription start, splicing and termination, further diversity resides in the poly(A) tail, which is known to play a role in most of the key steps of post-transcriptional regulation. Here we present a new method for sequencing full-length mRNAs, providing the first study where poly(A) tails were systematically sequenced.

Sequencing of a variety of biological samples revealed general principles of poly(A) tail length regulation. Moreover, for the first time we observed widespread differences in tail lengths associated with different mRNA isoforms of the same gene and the internal nucleotide composition of the tails.

P67

Rapid molecular evolution of pain insensitivity in multiple African rodents

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Noxious substances, called algogens, cause pain and are employed as defensive weapons by poisonous plants and stinging insects. But algogen-insensitivities have been reported, like the acid and capsaicin-insensitive African naked mole-rat. Here we identified four novel instances of algogen-insensitivity by screening nine related fossorial African rodent species for capsaicin, acid and Allyl isothiocyanate (AITC)-induced pain. RNA-sequencing allowed us to trace the emergence of sequence variants in sensory transduction channels, like TRPA1 and NaV1.7 that accompany algogen-insensitivity. We quantified changes in gene expression specifically associated with acid or AITC insensitivity. Only the AITC-insensitive Highveld mole-rat showed overexpression of the sodium leak channel NALCN which served to shut down AITC-detection by nociceptors. Highveld mole-rats are immune to the aggressive stinging Natal Droptail Ant, a likely driver of coordinated molecular changes in nociceptors. Our study reveals how evolution can be used as a discovery tool to find molecular mechanisms that shut down pain.

P68

Towards a Human Cardiac Cell Atlas

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Normal function of the four-chambered human heart and the interconnecting valves relies on highly heterogeneous cell populations with specialized functions that are governed by differential gene expression. However, the precise composition of the cardiac cell population is incompletely understood and little is known about the complex genomic architecture of individual cells. Rapidly developing methods of single-cell and single-nucleus RNA sequencing (scRNA-seq, sNuc-seq) allow for transcriptional profiling of individual cells/nuclei and to infer cell types, states and functional roles of individual cells from it. Of relevance to adult heart characterization, standard droplet based strategies are less suitable due to the large cardiac muscle cell size, and cardiomyocyte multinucleation may obscure sNuc-seq results. We therefore established reliable and robust advanced single-cell RNA sequencing techniques suitable for adult cardiac cell analysis. We furthermore compare sNuc-seq of cardiac tissue with scRNA-seq and show that sNuc-seq allows the analysis of very high numbers and thus is highly sensitive to comprehensively detect cardiac cell types including rare cell populations. To deconvolute the human heart cellular composition, we use IRB-approved cardiac tissue from unique clinical resources. We have the capacity to dissociate viable cells from these sources which allows us to apply scRNA-seq and sNuc-seq for the analysis of the different regions of healthy human hearts.

P69

Circular RNA polymorphism in neurodegenerative disorder

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Aging and related neurodegenerative disorders are important global societal and health challenges. Despite great advances in medicine and genetics, our etiological understanding of these complex diseases is still limited. Indeed, no effective treatments exist for most common ageing-related neurodegenerative disorders.

In recent years, genome-wide association studies (GWAS) have been successful in identifying genetic variants associated with disease phenotypes. As the majority of identified genetic variants discovered are located outside of the protein-coding regions, this implies that polymorphism of regulatory factors plays an important role in genetic mechanisms. Further integrative analysis of GWAS and high-throughput NGS data such as RNA-seq or chip-seq have identified polymorphisms of non-coding RNAs such as microRNAs and lincRNAs to be implicated in the etiology of complex disorders, including cancer, heart diseases, immunological diseases and neurodegenerative diseases.

As a recently characterized member of the non-coding RNA family, circular RNAs (circRNAs) are highly enriched in brain and globally accumulated during aging. Besides, many circRNAs are dysregulated in brain disorders, making these circular molecules potential biomarkers and treatment targets. This proposed study aims to find the genetic effect of circRNA polymorphisms in the context of human neurodegenerative disorders. Reanalyzing the public RNA-seq and single nucleotide polymorphisms (SNP) data with customized circRNA identification pipeline, the preliminary results show the allelic preference of some circRNAs, indicating the presence of cis-acting elements that affect circRNA synthesis and processing. Future work including eQTL analysis will not only provide new insights into the functional association of polymorphisms in neurodegenerative disorders, but will also be beneficial for early targeted intervention.

P70

Deciphering the role and dynamics of chromatin contacts in embryonic development using Genome Architecture Mapping

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Tightly controlled gene regulation is the key to normal metazoan embryonic development. The expression of cell-fate determining factors guides cells towards certain fates and orchestrates the establishment of the various lineages of the embryo. Contacts between cell-type specific enhancers and their target genes regulate gene expression. However, it remains a challenge to understand how lineage specification is regulated in early development due to the inaccessibility of small numbers of cells within the mammalian embryo, especially for high-throughput analysis of 3D chromatin organization of transient lineage commitment states.

To investigate the dynamic changes in chromatin contacts in distinct cell populations of the pre-implantation mouse embryo, we have combined Genome Architecture Mapping (GAM) with identification of cell-state markers using immunofluorescence. Immuno-GAM is a powerful new approach to study chromatin contacts that combines ultra-thin cryosectioning of nuclei, immunofluorescence and laser-microdissection, to isolate nuclear sections from stage-specific cell populations, without prior physical disruption of the embryo. After sequencing of the DNA content of the slices, we measure the co-segregation frequency of genomic loci, and quantify locus 3D interactions genome-wide. In our current analyses, we compare the genome architecture of the primitive endoderm and the epiblast of the blastocyst of embryonic day E3.5 and E4.5. With this study, we aim to dissect the mechanistic roles and the dynamics of chromatin contacts in early development.

P71

Nascent m6A regulates splicing efficiency

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Pre-mRNA splicing is a crucial step during gene expression and is highly controlled by several protein factors. The efficiency of splicing varies among transcripts and is important for coordinated gene expression. N-6-methyladenosine (m6A) is the most abundant RNA modification on the mRNA level and is involved in RNA biogenesis and RNA expression. Due to the nature of difficulties to evaluate the impact of m6A on splicing on mature RNA level, the direct role of m6A on splicing dynamics has not been investigated so far. Here, we provide the first time-resolved high-resolution assessment of m6A on nascent RNA transcripts and unveil its importance for the control of RNA splicing kinetics. We identify that early co-transcriptional m6A deposition near splice junctions promotes fast splicing, while m6A modification of intronic sequences is associated with long, slowly processed introns and alternative splicing events. In conclusion, by directly comparing the processing dynamics of individual transcripts in the methylated versus unmethylated state on a transcriptome-wide scale, we show that early m6A deposition marks transcripts for a fast-track processing.

P72

Dissecting common architecture of local transcriptome, translatome and proteome in Amyotrophic Lateral Sclerosis (ALS) using in vitro derived human motor neurons.

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In highly polarized cells like neurons, subcellular localization and local translation of mRNA are critical for establishment of the body axis and synaptic plasticity, providing foundation of learning and memory. RNA metabolism is tightly regulated by a combination of cis elements, zip-codes, and binding of trans-acting factors, RNA binding proteins (RBPs) and miRNAs. However, only a few of these factors are known, and little is understood about how they contribute to neuronal function. To identify these elements genome-wide, we designed a soma/neurite separation method followed by mass spectrometry, RNAseq and Ribo-Seq and demonstrated that mRNA localization accounts for more than a half of the neurite-localized proteome (Zappulo et al. 2017). Relying on this fractionation scheme, we will investigate architecture of local proteome, transcriptome and translatome in ALS, a degenerative disease that results in progressive loss of motor neurons (MNs). Despite heterogeneity, all mutations underlying ALS share common pathologic pathways, including defects in RNA metabolism. Therefore, we expect that across different ALS-mutations we will find similar compartment-specific changes in RNA and protein localization. To identify molecular mechanisms contributing to neurodegeneration in a model system most relevant to human disease, we will use MNs obtained by differentiation of human embryonic or induced pluripotent stem cells. As differentiation protocols vary in efficiency and our methodology requires high purity of neuronal cultures, we compared two induction methods: Maury et al. 2014, and Luisier et al. 2018. We evaluated the efficiency of MNs by expression of MN-specific transcription factors, ISL1/2 and HB9. To further aid MN generation and evaluation, we also tested a reporter system in which GFP expression is controlled by MN-specific HB9 promoter. We will use this system to investigate mechanisms of neurodegeneration and identify new approaches for ALS treatment.

P73

CRISPR-engineered Serial Inversions lead to Tissue-specific Architectural Stripes, Ectopic Gene Expression and Congenital Limb Malformations

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Balanced chromosomal rearrangements (BCRs) such as inversions and translocations are a common cause of congenital disease and cancer. BCRs can result in enhancer-promoter rewiring and gene misexpression by disrupting chromatin domains and inappropriately connecting regulatory regions with previously distal genes. However, it remains difficult to predict if and when aberrant gene activation takes place in the context of BCRs. We have addressed the effect of BCRs on gene expression in vivo by generating a series of genomic inversions that place an active limb enhancer cluster from the Epha4 regulatory domain at different positions within a neighboring gene-dense region. Expression studies in embryonic limb buds show that the inverted enhancer cluster was able to activate several genes downstream of its new position, resulting in gene misexpression and limb phenotypes. Capture Hi-C from mutant limb buds showed that the activated genes were located within a region with an asymmetric three-dimensional pattern, so called architectural stripes. Deletion of the CTCF binding site at the anchor of the stripe resulted in its disappearance, diminished ectopic gene expression, and a rescue of the skeletal phenotype. Moreover, unlike in the developing limb, inversion-derived stripes do not form in mouse embryonic stem cells (mESC) where the enhancers are inactive, indicating that its formation is likely to be dependent on enhancer activity. Based on Hi-C from limb buds we show that architectural stripes are a frequent feature of the chromatin structure in vivo and often associate with developmentally active enhancers. Thus, BCRs can induce ectopic gene expression and the formation of asymmetric chromatin contact patterns that are dependent on CTCF anchors and enhancer activity.

P74

Characterization of the histone chaperones LIN-53 during reprogramming and aging

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LIN-53 is a histone chaperone that has been identified as a barrier of reprogramming, as depletion of LIN-53 enables the direct conversion of germ cells into neurons in *C. elegans* (Tursun et al., 2011). Analogously, the mammalian counterpart of LIN-53, known as CAF-1, has been shown to act as a reprogramming barrier during reprogramming of mouse cells (Cheloufi et al., 2015).

Next to its role in reprogramming, LIN-53 plays a role in muscle maintenance and life span regulation in *C. elegans,* as lin-53 mutants show motility defects, impaired muscle structure and decreased lifespan compared to WT controls. Interestingly, muscle-specific rescue of LIN-53 could save the muscle but not the aging phenotype (Müthel et al., in preparation), indicating a distinct role of LIN-53 in specific tissues. So far, research on LIN-53 has mainly focused on the whole organism. The aim of this project is to study gene regulation and protein interaction networks of LIN-53 in a tissue specific-manner with a special focus on aging.

Using CRISPR/Cas9-mediated gene editing, I will tag the endogenous lin-53 locus with the auxin-inducible degron (AID) (Zhang et al., 2015) in order to knock down LIN-53 in a tissue-specific manner. Second, I aim to identify tissue-specific protein interactions of LIN-53 by using an in vivo biotinylation-based approach for the cell type-specific purification of protein complexes (Waaijers et al., 2016).

Importantly, recent experiments in the lab revealed that lin-53 depletion leads to the accumulation of the FOXO transcription factor DAF-16 in the cytoplasm instead of translocating to the nucleus. DAF-16 is a protein that is the central regulator of the insulin/IGF-1 signaling (IIS) pathway and the main downstream target of DAF-2, which plays a major role in lifespan regulation. To further investigate the potential mechanisms underlying the lifespan decrease upon lin-53 depletion I am investigating the potential link between DAF-16 and LIN-53.

P75

Phasing of Germline Variation using Genome Architecture Mapping

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The spatial organization of chromatin within the nucleus plays an essential role in organ development and tissue-specific gene expression regulation, by enabling or preventing functional contact between regulatory regions and gene promoters. Methods revealing chromatin contacts, such as all vs all chromatin conformation capture (Hi-C) rely on DNA fixation, digestion and re-ligation of co-located genomic regions. These methods are inherently biased, as they depend on the distribution of restriction sites and the efficiency of digestion and ligation. Recently, Beagrie et al. (2017, Nature volume 543, pages 519–524) developed Genome Architecture Mapping (GAM), a ligation-free method for detection of chromatin contacts through random nucleus cryosectioning followed by next-generation sequencing.

Motivated by observed spatial distances between homologous chromosome pairs in the nucleus, we are investigating the efficacy of this method for simultaneous whole-genome phasing of heterozygous germline variants, haplotype-specific chromatin contact point assembly and the potential for allele-specific detection of copy-number changes. As a gold standard for developing a chromosomal phasing approach from GAM data, we have used a large GAM dataset produced from a heterozygous mouse embryonic stem cell line, obtained from a cross between two genetically divergent, homozygous mouse strains. We employ generative probabilistic models on GAM data to infer the most likely whole-genome phasing of germline variants from sequencing data. By considering higher-order Markov models, we aim at predicting haplotype-specific chromatin contacts. In the future, we will investigate the efficiency of phased haplotype read ratios for the detection of allele-specific somatic copy-number aberrations in human cancers.

P76

Evolution of Replication Origins in Vertebrate Genomes: Rapid Turnover Despite Selective Constraints

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Background: The replication program of vertebrate genomes is driven by the chromosomal distribution and timing of activation of tens of thousands replication origins. Genome-wide studies have shown the frequent association of origins with promoters and CpG islands, and their enrichment in G-quadruplexes sequence motifs (G4). However, the genetic determinants driving their activity remain poorly understood. To gain insight on functional constraints operating on replication origins and on their spatial distribution, we conduct the first evolutionary comparison of genome-wide origins maps across vertebrates

Results: We generated a high-resolution genome-wide map of chicken replication origins (the first of a bird genome) and performed an extensive comparison with human and mouse maps. The analysis of intra-species polymorphism revealed a strong depletion of genetic diversity on a ~ 40bp region centered on the replication initiation loci. Surprisingly, this depletion in genetic diversity is not linked to the presence of G4 motifs, nor to the association with promoters or CpG islands. In contrast, we also show that origins have experienced a rapid turnover during vertebrates evolution, since pairwise comparisons of origin maps revealed that only 4 to 24% of them are conserved between any two species.

Conclusions: This study unravels the existence of a novel genetic determinant of replication origins, whose precise functional role remains to be determined. Despite the importance of replication initiation activity for the fitness of organisms, the distribution of replication origins along vertebrate chromosomes is highly flexible.

P77

Regulatory strategies of transcriptional reprogramming in human cells

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Living cells encounter stress conditions that disrupt homeostasis, such as elevated temperatures, cancer, or ageing. Thus, rapid global reprogramming of transcription is a crucial step to combat stress. The current view of transcriptional reprogramming postulates that promoter-proximal pausing is the major step at which global reprogramming is defined. However, the molecular mechanisms that specifically and simultaneously repress, or activate gene and enhancer transcription remain to be elucidated.

We recently solved a long-standing question in the field of gene regulation, namely how RNA polymerase (Pol) II near gene promoters can control the frequency of transcription initiation and thus, the amount of RNA synthesized per time (Gressel, Schwalb et al., Elife 2017). We demonstrated that the duration of Pol II pausing generally limits the productive frequency of transcription initiation ('pause-initiation limit'). Based on these insights gathered from 'multi-omics' data, our current efforts aim to disentangle the regulatory strategies of global reprogramming. To this end, we measured nascent RNA synthesis by Transient Transcriptome sequencing (TT-seq), and profiled Pol II occupancy at nucleotide-resolution by mammalian Native Elongation Transcript sequencing (mNET-seq). Our work makes use of human leukemia cells encountering acute heat shock which is a widely accepted model to examine the transcriptional response to many relevant physiological (e.g. heat stress, or exercise) and pathological (e.g. viral and bacterial infections) conditions. Our data provide novel insights into the regulation of Pol II transcription, i.e. temporal changes in initiation frequency, Pol II pausing, and elongation velocity.

P78

Understanding the local transcriptome and proteome of Amyotrophic Lateral Sclerosis (ALS) in in vitro derived mouse spinal motor neurons

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ALS is an adult-onset neurodegenerative disorder affecting motor neurons (MNs) in the brain and spinal cord. More than 20 genes that contribute to ALS have been identified in patients, yet the specific molecular mechanisms underlying neuronal decay remain elusive. Thus, it is critical to identify the exact molecular and cellular basis for MN death as more than 5,000 people are diagnosed each year with this fatal disease. RNA binding proteins, fused in sarcoma (FUS) and TAR DNA binding protein (TDP43) form protein-RNA aggregates in MNs of ~90% of ALS patients suggesting RNA misregulation underlies ALS pathology. We are interested in understanding ALS from the perspective of RNA metabolism, focusing on changes in localization and translation in in vitro disease models. Therefore, we generated mouse embryonic stem cell lines expressing GFP tagged proteins with mutations commonly found in ALS patients: superoxide dismutase (SOD1; G93A), FUS (R495X), and TDP43 (Q331K; M337V), or corresponding WT proteins. These cell lines were differentiated into spinal MNs using a doxycycline inducible cassette containing Ngn2-Isl1-Lhx3 transcription factors. (Mazzoni et al. 2013) These MNs recapitulate phenotypes of neurodegeneration, for example, SOD1 (G93A) increases MN apoptosis, while FUS (R495X) increases DNA damage as visualized by increased levels of γH2AX. To investigate how ALS-associated mutations change RNA localization and local translation we will perform spatial transcriptomic and proteomic analysis in induced MNs. To validate these results in a primary system, we isolated mouse adult spinal MNs. For spatial analysis, we use a method developed by the lab wherein the soma and neurite compartments of neurons are separated. This research has the potential to provide common molecular mechanisms in neurodegeneration across different genetic backgrounds, while affirming the use of in vitro models to study diseases.

P79

Mutations in disordered regions can cause disease by creating dileucine motifs

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Many disease-causing missense mutations affect intrinsically disordered regions (IDRs) of proteins. Since these mutations do not affect protein structure, the molecular mechanism of their pathogenicity is enigmatic. Here, we employ a peptide-based proteomic screen to investigate the impact of mutations in IDRs on protein-protein interactions. We find that mutations in disordered cytosolic regions of three transmembrane proteins (GLUT1, ITPR1 and CACNA1H) lead to an increased binding of clathrin. In all three cases, the mutation creates a dileucine motif known to mediate clathrin-dependent trafficking. Follow-up experiments on GLUT1 (SLC2A1), the glucose transporter causative of GLUT1 deficiency syndrome, revealed that the mutated protein mislocalizes to intracellular compartments in a model cell line and in patient-derived induced pluripotent stem cells. Mutant GLUT1 interacts with adaptor proteins (APs) in vitro, and knocking-down AP-2 reverts the cellular mislocalization and restores glucose transport. A systematic analysis of other known disease-causing variants revealed a significant and specific overrepresentation of gained dileucine motifs in structurally disordered cytosolic domains of transmembrane proteins. Thus, several mutations in disordered regions appear to cause “dileucineopathies”.

P80

A simple and cost-effective method for in situ visualization of short genomic sequences

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Fluorescent in situ hybridization (FISH) is one of the main methods used in cytogenetic studies. Conventional FISH relies on hybridization of several hundred nucleotide long DNA probes and is not well suited for detection of short sequences of chromosomal DNA. One of the solutions to overcome this problem is applying DNA padlock oligonucleotides, which specifically bind to short (20-30 nt) DNA sequences. However, this technique may be challenging to use due to the necessity of making chromatin accessible for hybridization. Therefore, the DNA padlock technique requires an application of a complex set of oligonucleotides that enhance padlock interaction with the sequence of interest and minimize the cross-interactions between oligonucleotides.

Here, we present a new approach for DNA padlock experiments, in which we applied short “opener” oligomers, composed of 2’O-methyl-RNA and LNA nucleotides. These oligomers hybridize firmly to one strand of chromosomal DNA making the other available for binding with the padlock oligonucleotide. The application of 2’O-methyl-RNA and LNA nucleotides, instead of the originally used PNA oligonucleotides, did not affect “opener” capability for mediating chromatin-opening and additionally significantly reduced the cost of our analyses.

The design of the oligonucleotides for the padlock-based experiment is a relatively complex, multi-step process including the selection of target sites and probe sequence optimization. To cope with this problem, we developed user-friendly software and showed that its application assures the high specificity and selectivity of the designed oligonucleotides.

Finally, we demonstrated the usefulness of our approach by in situ visualization of the selected genes in mouse brain cells.

P81

Single-cell transcriptomics characterizes cell types in the subventricular zone and uncovers molecular defects underlying impaired adult neurogenesis

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Neural stem cells (NSCs) contribute to plasticity and repair of the adult brain. Niches harboring NSCs are crucial for regulating stem cell self-renewal and differentiation. We used single-cell RNA profiling to generate an unbiased molecular atlas of all cell types in the largest neurogenic niche of the adult mouse brain, the subventricular zone (SVZ). We characterized > 20 neural and non-neural cell types and gained insights into the dynamics of neurogenesis by predicting future cell states based on computational analysis of RNA kinetics. Furthermore, we apply our single-cell approach to mice lacking LRP2, an endocytic receptor required for SVZ maintenance. The number of NSCs and proliferating progenitors was significantly reduced. Moreover, Wnt and BMP4 signaling was perturbed. We provide a valuable resource for adult neurogenesis, insights into SVZ neurogenesis regulation by LRP2, and a proof-of-principle demonstrating the power of single-cell RNA-seq in pinpointing neural cell type-specific functions in loss-of-function models.

P82

Massively parallel lineage tracing during adult neurogenesis and brain regeneration in zebrafish

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The zebrafish is a well-established model organism for studies of adult neurogenesis. In addition to high rates of ongoing neurogenesis in the healthy brain, there is a considerable increase in production of neurons after a traumatic injury, which results in complete regeneration. The main source of new neurons are adult stem cells (radial glia) localized in specialized niches across the brain. Although there is evidence of different modes of neurogenesis in terms of cell division patterns and proliferative capacity, the underlying stem cell diversity and potential lineage restrictions in the system remain unknown.

We aim to apply the massively parallel lineage tracing method LINNAEUS (LINeage tracing by Nuclease-Activated Editing of Ubiquitous Sequences) to the zebrafish brain in order to understand the lineage commitment of stem cells during healthy neurogenesis in comparison to regeneration occurring after injury. LINNAEUS relies on creation of unique and heritable cellular barcodes by Cas9-directed editing of multiple genomic targets. The barcodes are expressed and can be recovered from single-cell transcriptomic data, allowing us to couple information on cellular identity with their lineage history.

Here we report a single-cell transcriptomic profile of selected neurogenic areas of the zebrafish brain, including the telencephalon and the cerebellum. Several populations of radial glia cells can be distinguished, including globally distributed as well as some niche-specific subtypes. Additionally, lineage trees were generated from adult fish with barcodes created by Cas9 injection in early development, which show that lineage separation between radial glia and mature neurons happens after the current time window of cell labelling (0-10 hours after fertilization). In the next stage, a strategy for delayed labelling will be established, which will enable us to probe lineage relationships in the adult brain with increased temporal specificity.

P83

Characterizing the role of circular RNA molecules in Psoriasis

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Aims of the study

The proposed project aim is to investigate the potential role circRNAs play in Psoriasis, to gain further insight into the pathology of these diseases and to identify specific circRNAs, which can be used as diagnostic- and prognostic biomarkers.

Methods

Collection of patient samples and extraction of RNA

Skin samples from patients with Psoriasis have been collected. The biopsies from lesional and nonlesional skin were provided by the Department of Dermatology, Aarhus University Hospital.

Genome-wide characterization of circRNA expression patterns

The initial screening for differential expression patterns of circRNAs was performed using next-generation sequencing (NGS) of total RNA.

Validation of the most promising circRNAs in a large cohort of patients

By using the NanoString technology (http://www.nanostring.com/), we investigated the expression of the top 50 candidates

Integrated data- and pathway analyses

Differentially expressed circRNAs were identified using a Mann-Whitney U test with a Benjamini and Hochberg correction for multiple testing. Volcano plots of the data were generated using GraphPad (Prism). Heatmaps and cluster analyses, and principal component analyses were performed using the R software. CircRNAs and miRNAs in Psoriasis samples were scored as either up- or downregulated when being two standard deviations above or below the mean of the controls, respectively.

Functional characterization of the most interesting circRNAs

In situ hybridization (ISH) will be performed using highly specific probes provided by the RNAscope® Technology on skin biopsies from patients with Psoriasis.

Results

We will present the full outcome and future plans of the study.

P84

New motif analysis methods define a landscape of 3'UTR motifs associated with expression perturbation across thousands of cancer samples

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We developed a method for motif enrichment evaluation, and applied it systematically to characterize the transcriptional landscape of 7-mer motifs in 3'UTR regions in cancer.

To this end, we use the concept of a motif's differential expression, meaning a measure of the tendency of genes with a given motif in their 3'UTR sequences to be up- or down-regulated in individual cancer patients.

We analyzed 32 cancer types and 9,679 patients' gene expression data for the differential expression of all 16,384 7-mer motifs.

We show that 7-mer motifs complimentary to miRNA seed sites are more differentially expressed, as expected given the function of miRNAs.

Using miRNA expression data from the same patients, we further show overall correlation between a motifs' differential expression and paired miRNA expression across patients.

We have thus demonstrated, using gene expression, that differential expression of miRNA seed site target motifs in genes' 3'UTRs can serve as a proxy for miRNA expression.

This allow us to point to both known and novel perturbed miRNAs in a number of cancer types.

The method also allows for estimation of which strand(s) of an expressed miRNA may be active.

In addition, a number of motifs not complementary to miRNA seed sites showed differential expression.

This holds promise for new discoveries of motif binding factors with perturbed expression in cancer, which is currently under investigation.

P85

Global characterization of dynamic post-transcriptional gene regulation at the onset of neuronal differentiation

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Regulatory networks and molecular mechanisms regulating pluripotency and differentiation have been well characterized on the transcriptional, signaling and epigenetic level. However, a comprehensive understanding of post-transcriptional control is still missing. Here, we perform transcriptome-wide assays on multiple layers of gene expression control during the differentiation of mouse embryonic stem cells towards a neuronal fate. We then investigate dynamic post-transcriptional regulation using newly developed computational analysis tools. First, we develop RNA KInetic Rate Analysis (RNAkira), a probabilistic framework to characterize mRNA synthesis, processing, turnover and translation from metabolic labeling (4sU-seq) and ribosome profiling data. By means of hierarchical model selection, we find that gene expression during the exit from pluripotency is primarily driven by transcriptional inputs and largely unaffected by translational control at this early stage. Second, we develop Motif-Informed Read Coverage Analysis (MIRCA) to identify differentially bound regions for RNA binding proteins from protein occupancy profiling data. This allows us to characterize physiologically relevant and dynamic, rather than constitutive, protein-RNA interactions. These dynamic regulatory inputs induce a clustering of network modules with different regulatory outputs (metabolic strategies) and are predictive for post-transcriptional regulatory dynamics. By means of an integrative computational analysis focused on identifying significant changes in regulatory links, we thus provide a comprehensive picture of post-transcriptional dynamics at the onset of neuronal differentiation.

P86

The identification and characterization of chromatin regulators involved in coelomocyte to neuron conversion in *C. elegans*

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Reprogramming differentiated somatic cells into neurons is one of the frontiers of current neuroscience research. We have developed a new system to understand the mechanisms of transcription factor (TF)-induced transdifferentiation (Td) to neurons based on the zinc finger TF CHE-1 that specifies the identity of gustatory ASE neurons in *C. elegans* (Tursun et al., Science, 2011). Using this system, we discovered that ectopic expression of the TF CHE-1 directly reprograms coelomocytes (CCs), which are mesodermal cells with scavenging and hepatic functions, into ASE neuron-like cells. Notably, differentiated CCs express an ASE neuron-specific reporter, show neuron-like cell morphogenesis such as neuron-like projections, and, in addition, express a pan-neuronal marker and stain for the synaptic protein UNC-10 (Rim1 homolog). Individual animals show varying degrees of CC to ASE neuron conversion with converted CCs displaying neuronal morphologies or partially converted CCs. Interestingly, a portion of the population, approximately 40%, demonstrate no CC to neuron-like cell conversion, indicating that inhibitory mechanisms or barriers are restricting Td. To uncover the mechanisms behind CC Td at the chromatin level, we performed a chromatin sub-library RNAi screen. We have identified several candidates that, when knocked down, enhance or suppress conversion and may, therefore, play an important role during Td. Among our candidate barrier factors, we have observed an increase in conversion efficiency from 60% up to 82%. In addition, functional enrichment analysis indicates that our putative enhancers and suppressors show molecular interactions. By exploring the role of our candidate enhancers and suppressors in Td we aim to tease apart the mechanisms by which they function, which may contribute to our understanding of how neurons can be generated by Td from other cells and tissues.

P87

Introducing the PiGx Set of Bioinformatics Pipelines

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Computationally-intensive research fields, such as bioinformatics, require workflows that can reliably produce consistent, traceable output, from known software sources. Satisfying this constraint, however, is often complicated by the myriad dependencies and versions that make up a typical workflow, many of which are continually changing due to rapidly evolving technologies. Moreover, as bioinformatics tools are being increasingly applied to privacy-sensitive data, there is an increasing need for referential transparency in bioinformatics tools, so that developers can track down and eliminate potential security vulnerabilities.

For these reasons, we present a set of pipelines called “PiGx”, for the processing and analysis of RNA-seq, ChIP-seq, Bisulfite-seq, and single-cell RNA-seq data. These pipelines require no significant computational expertise to use, and are suitable for wet-lab researchers who wish to process their own results from raw data directly to publication-ready plots and reports. The pipelines are constructed using GNU guix, with the full dependency tree of tools used in the process explicitly declared.

We will describe the methodology and benefits of this approach, along with examples of recent applications and results that have been obtained by our group using this set of tools.

P88

Transcriptome analysis of *D. melanogaster* brain at Single-Cell level: Deconstructing-Reconstructing circRNA function.

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Circular RNAs (circRNAs) are a particular type of RNA in which the 5` end of the molecules is covalently bound to the 3` end. They are generated through a process named backsplicing and are mostly derived from exons and 5` UTR regions of coding genes. For some years they were seen as non-functional by-products of regular gene splicing. Nevertheless, during the last years many works have shown that they are highly abundant, conserved and have functions in cis and in trans. Moreover, a subset of them encode for protein. Interestingly, circRNAs are highly abundant in brain - specifically in synapses - and accumulate with age. Our lab recently generated more than one hundred fly lines that express short hairpin RNAs (shRNAs) against specific circRNAs to knock them down (KD). cirMbl is the most abundant circRNA in fly heads and its KD presents male developmental lethality, defects in synaptic function, flight defects and a wing posture phenotype. However, downregulation of specific circRNAs, including circMbl, provokes small changes in the transcriptome. Therefore, we hypothesize these expression changes can be either in a small portion of the brain at a high level or all over the brain at a low level. To tackle this question, we analyze the transcriptome at a single-cell level of both circMbl KD and control fly strain. So far, we saw changes in the expression of Insulin-like-peptide (ILP) neurons and in Dopaminergic Neurons. Interestingly, these changes correlate with the results obtained from the whole head transcriptome analysis. We are currently purifying these neurons and doing a more focused transcriptome analysis. In addition, we are performing additional physiological and behavioral experiments.

P89

scRNAseq analysis of brain organoids to study molecular mechanism of Leigh syndrome

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Leigh syndrome (LS) is incurable and early-onset neurodegenerative disorder is associated with defects in mitochondrial enzymes. The disease mechanisms remain elusive because of the lack of an experimental model that accurately recapitulates the human condition.

To create an experimental model which captures patient genetics and allows the study of numerous CNS cells types in 3D brain tissue-like microenvironment, we generated LS patient-derived brain organoids.

Modifying a protocol originally published by Lancaster et al., 2013, we could generate early-stage forebrain organoids from iPSC lines carrying SURF1 mutation and compared them to CRISPR-corrected isogenic iPSC lines. Combining the latest cutting-edge technologies such as single-cell RNA sequencing (scRNA-Seq) and total/circRNA RNA sequencing with immunohistochemistry, we identified novel disease-associated changes in cell type composition and global alterations in proliferation and differentiation signaling pathways.

Preliminary data and approaches to translate these data into new therapeutic strategies will be presented.

P90

Reconstructing the evolutionary history of cancer from allele-specific copy-number profiles

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Intra-tumour heterogeneity is one of the leading causes of drug resistance in cancer treatments. Hence, it is important to understand and model tumour evolution of cancer within a single patient.

Previous work has failed to successfully address and explore the evolution of tumours whose drivers are chromosomal rearrangements and somatic copy-number alterations. Here, we introduce a novel version of MEDICC, that analyzes high copy-number states and uses a new phasing method to reconstruct cancer evolution.

The novelty comes in two parts. First, we successfully adapted a finite-state-transducer-based approach employed in MEDICC to calculate a minimum-event distance between two copy-number profiles with an extended range of permissible copy-number states. This makes the computation more lightweight and efficient, which we demonstrate with systematic tests and evaluations. Second, we implemented a new method for phasing, i.e. assignment to the parental haplotypes, within a single patient that allows us to detect new MSAI (Mirror Subclonal Allelic Imbalance) events during cancer evolution.

By applying the novel version of the algorithm to the clinical data from a large pan-cancer cohort, and comparing phased with traditional unphased trees, we observe increased heterogeneity and explore changes in tree topology caused by MSAI events.

P91

Identification of Active Mutational Signatures with decompTumor2Sig

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In many cases, the somatic mutations of a tumor stem from multiple mutational processes such as cigarette smoke or age-related spontaneous deamination of 5-methylcytosine. The identification of the processes that have contributed to an individual tumor genome is an emerging research question in cancer genomics and may be of clinical relevance, e.g., when it highlights a DNA repair deficiency which may impact the response to cytotoxic treatments.

Recently, two different mathematical models of mutational processes have been developed, describing them as “mutational signatures” in terms of mutation frequencies of altered bases within their immediate sequence context. These two models differ with respect to their accuracy of describing mutational events and the number of parameters required to capture the mutation frequencies of specific sequence patterns.

The de novo establishment of mutational signatures requires a large number of tumor genomes to decompose them into (i) a set of signatures that reflect the processes driving the somatic mutations, and (ii) a set of “exposures” or contributions of the signatures to the single tumors. However, once accurate signatures have been defined, they can be used to estimate their contributions to the overall mutational load of an individual tumor sample.

We illustrate how quadratic programming can be used for this so-called “signature refitting”. The basic idea of this optimization technique is to determine the contributions in such a way that the differences between the observed mutation frequencies in the tumor genome and the frequencies predicted by the signatures’ contributions are minimal.

We present the novel R package “decompTumor2Sig”, the first tool that can seamlessly be used for signature refitting with both types of mutational signatures. We show the application of our tool to examples of both signature models and illustrate its effectiveness to dissect tumor genomes into a given set of mutational signatures.

P92

Alternative polyadenylation survey in single cells identifies 3'UTR changes regulating gene expression during cell cycle progression

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During cell cycle progression the expression of thousands of genes oscillate. Generally, these oscillations are caused by the sequential activation of specific transcription factors. However, most periodically expressed genes also show periodic changes in mRNA degradation rates, indicating that they are regulated both at the transcriptional and post-transcriptional level. A key post-transcriptional mechanism that could impact mRNA stability during cell cycle progression is alternative polyadenylation (APA) since it modulates mRNA stability by adding or removing regulatory elements in the 3'UTR. Here, we apply single-cell transcriptomics to investigate the role of APA in the regulation of gene expression during cell cycle progression. By computationally sorting cells along the cell cycle, we study the expression dynamics of individual isoforms. Our preliminary results suggest that the choice of 3'UTRs is cell cycle dependent.

P93

Epigenetics in T cell differentiation: From genome-wide signatures to local regulators and their targeted manipulation.

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CD4+ T cells shape the type, intensity and duration of most effective adaptive immune responses and contribute significantly to protective immunity. These features make them promising effectors for adoptive T cell therapies in various clinical settings. At the same time, CD4+ T cells are key effectors in chronic (auto-immune) inflammatory diseases when the physiological immune-regulation fails. It is therefore of utmost importance to understand the normal, but also the disease-associated altered differentiation pathways and survival requirements for human CD4+ T cells.

We approach this task by analyzing the regulatory impact of epigenetics on T cell differentiation. For this, we are generating genome-wide epigenetic maps of human T cell populations from healthy and diseased conditions and use these data for the identification of key epigenetic regulators, which are functionally involved in T cell differentiation, function and survival. Such epigenetic switch regions are also interesting new targets for therapeutic interventions. For their directed modulation, we are developing tools of site-specific ‘epigenetic editing’, which might be used for their functional analysis, but also for the fine-tuning of therapeutic T cell products in the future.

P94

Investigating role of SRRM4 in circRNA formation

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Serine/arginine repetitive matrix protein 4 (nSR100/SRRM4) is a neuronal-specific splicing factor, required for proper neural differentiation. Downregulation of SRRM4 has been observed in the brains of individuals with autism spectrum disorder. Homozygotic Knock-Out (KO) of Srrm4 in mice has been proven largely lethal, whereas heterozygotic mice, although viable, show multiple autistic-like features reaffirming previous observations.

So far, the main role of SRRM4 has been related to the neural-specific inclusion of microexons (3-27nt exons) and other short exons into mature mRNA transcripts. Here we investigated a potential novel function for SRRM4, related to the regulation of back-splicing events that result in circRNA formation. Based on initial analyses, we identified 38 circRNA candidates misregulated due to SRRM4 overexpression in 293T cells.

We will now use a highly reproducible protocol of mESC differentiation to glutamatergic neurons to explore the effect that Srrm4 KO has on circRNA formation during neuronal maturation.

P95

Dissecting cellular heterogeneity in a solid tumor model by single-cell transcriptomic analysis

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Characterizing the heterogeneous composition in solid tumors is fundamental for understanding carcinogenesis and tumor progression. Using a genetically-controlled model with combined gain- and loss-of-function mutations of β-catenin and Bmpr1a, respectively, we profiled ~23,000 cells from salivary glands of male and female tumor-bearing and control mice across two stages. We provide a comprehensive cell atlas and show that the hallmarks of the tumor are restricted to a subset of cells. Specifically, we identified previously uncharacterized tumor-specific luminal- and basal-like cells, and a small, but distinct cancer stem cell (CSC) population in which Wnt-specific genes are activated. Computational modeling of the data indicates that CSCs are at the center of a continuous three-branched epithelial cell differentiation hierarchy and represent a surprisingly heterogeneous cell population. Our work provides unbiased insights into tumor-specific cellular contexts in a controlled whole tissue environment.

P96

Characterizing effects and mechanisms of neuronal activity at single-cell resolution in the dauer-exiting *Caenorhabditis elegans* larva

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Upon unfavourable conditions during its L1 stage, *C. elegans* can develop into the dauer diapause larva, an alternative developmental stage characterized by distinct morphology and behaviour. Once conditions improve, larvae actively exit dauer stage and resume reproductive development. The neuronal dynamics during dauer exit as well as resulting transcriptional changes remain unknown at the single-cell level. Our project aims at deciphering the precise neuronal activity patterns that drive the dauer exit decision, focussing on amphid neurons capable of sensing improved environmental conditions. To characterize the behavioural phenotype of dauer larvae in comparison to non-dauer larvae as well as to identify behavioural changes that succeed dauer exit, we developed a custom, easy-to-use imaging tool that allows tracking of larvae exiting the dauer stage over several hours. To further understand how such changes in behaviour are recapitulated by a change in neuronal activity patterns, we are employing lines expressing pan-neuronal GCaMP fusion proteins as well as three-dimensional fluorescence microscopy. Hence, we track the activity of individual neurons over the time course of dauer exit in response to dauer-exit inducing stimuli. To reproducibly characterize neuronal activity, we are developing custom micro-fluidic devices, which allow for precise delivery of these stimuli in a controlled environment. Furthermore, graph-matching algorithms developed in our group will allow us to map all light-microscopy acquisitions of dauer larvae to a previously generated transmission electron microscopy dataset, which will significantly facilitate neuron identification and give important insights into the dauer larvae connectome. Taken together, we are aiming at an in toto description of the dauer exit at the single-cell level, focussing on how changing neuronal activity patterns are able to robustly drive complex developmental plasticity in a multicellular organism.

P97

In vivo reprogramming of coelomocytes into ASE neurons in *C. elegans*

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Understanding how cells maintain and safeguard their fate in vivo is a crucial step towards direct reprogramming of differentiated cells into other cell types. The zinc finger transcription factor (TF) CHE-1 specifies the fate of gustatory ASE neurons in *C. elegans*. Ectopic CHE-1 expression, together with knockdown of the reprogramming barrier *lin-53* (homolog of human CAF-1p48 (RBBP4/7)), leads to conversion of germ cells into neuron-like cells (Tursun et al., Science, 2011). We developed a complementary system to study induced transdifferentiation in *C. elegans*. For this, we utilize the mesodermal coelomocytes, which have scavenging and hepatic functions in the animals. Ectopic CHE-1 expression reprograms differentiated coelomocytes, which acquire neuron-like cell morphologies and express an ASE neuron-specific reporter in addition to a pan-neuronal marker. Furthermore, immunostaining showed that the synaptic protein UNC-10 (Rim1 homolog) was expressed in ASE marker-positive coelomocytes. Importantly, a portion of coelomocytes show growth of neuron-like projections coupled with strong repression of the coelomocyte fate. To probe the mechanisms by which coelomocytes convert into neuron-like cells, we knocked down a set of genes involved in chromatin regulation and have identified a number of putative enhancers and suppressors of reprogramming. Additionally, we are applying single-cell RNA and ATAC sequencing to the conversion of coelomocytes into neurons to identify transcriptome and chromatin dynamics during reprogramming in vivo. Using our novel system, we aim to gain insight into the mechanisms by which cells are amenable to TF-mediated reprogramming into neuron-like cells.

P98

CADD v1.4 – variant effect scoring on GRCh37 and GRCh38

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Recognizing disease causing genetic variants is one of the main challenges of personalized medicine. While modern sequencing technologies enable the rapid identification of variants in patient genomes, interpreting thousands of new or very rare variants remains an unsolved problem. Computational prioritization can support variant interpretation, specifically with genome-wide scores available across variant types. Such scores integrate diverse types of data including functional annotations, sequence conservation, and biochemical activity read-outs to a measure of variant effect.

Combined Annotation Dependent Depletion (CADD) is a method for genome-wide deleteriousness scoring of single nucleotide variants (SNVs) and short insertion/deletion events (InDels). CADD uses machine learning techniques to separate simulated de novo variants (proxy-deleterious) from sequence changes since the common ancestor of human and chimpanzee (proxy-benign). Since CADD's publication on human genome build GRCh37 in 2014, some studies suggested that novel annotations are available and that tweaks in model training may improve performance of variant scoring. Additionally, the latest human genome build, GRCh38, became more broadly adopted by the community.

Here, we revised CADD's source code to allow easy integration and preprocessing of new annotations as well as model training by the open source, machine learning library scikit-learn. Using the new code base, we integrated additional annotations (e.g. a splice effect score), updated genomic annotations and trained new models for GRCh37 and GRCh38. We show that CADD v1.4 has similar performance on both genome builds, outperforms previous versions in separating sets of known benign and pathogenic variants, and better predicts the effect size of multiplexed molecular assays. With this new version, we are able to offer CADD scoring for the latest genome build, providing an important tool for applications in personalized medicine.

P99

Expression profiles and functional analysis of circRNAs in primary and secondary gliomas

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Gliomas are a special group of malignancies due to their location, significantly impending the use of conventional methods of diagnosis and treatment. Molecular biology advances in medicine emphasize new, more sensitive methods of diagnosis, involving a variety of factors with an exceptionally wide range of changes appearing throughout neoplasia. A newly emerging class of endogenous, noncoding RNAs – circRNAs – have been reported as one of the most significant regulatory molecules, involved in maintenance of cells in the physiological state, but also contributing to tumorigenesis.

Based on an analysis of RNA-seq and meta-analysis of literature data, we identified at least 15 circRNAs upregulated in GBM tissue. Global analysis reveals the presence of circRNAs with positive impact on GBM onset such as e.g. ARAP2, CLIP2, and VCAN genes transcripts, increased 18-fold, 9-fold and 8-fold, respectively, in comparison to their linear counterparts. Additional analysis shows also the overexpression of given circRNAs in secondary (reccurence) GBM tumors, resulting in significant upregulation of ARAP2 and CLIP2 circRNAs in these samples regrading to the primary tumors. Given circular transcripts have been proved to be prominent candidates for loss-of-function approaches with siRNA, which allows us to explore their impact on basic events within the cell. A decreased migration potential of cells with simultaneous enhanced adhesion has been found, with exceptionally satisfying results for VCAN circRNA. We have also confirmed that circVCAN knockdown accelerates the number of cells entering S phase and partially arrests the cell cycle at this stage. As a step forward, we intent to analyze the selected circRNA variety in the population of neurospheres cultured from patient-derived GBM tissue, which states the most accurate, 3-dimensional model for GBM research, in order to characterize the function of circRNAs in glioblastoma stem cells (GSC).

P100

Deep learning on multi-omics data reveals cancer sub-types and helps select optimal in vitro cancer models

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The simultaneous study of multi-omics data promises to uncover new cancer subtypes with implications for prognosis and therapeutic choices, as well as selection for clinical trials. Additionally, all drug discovery programs use cell lines as a proxy for human cancer models to characterize their compounds, identify relevant indications and discover biomarkers. In order to maximize the translatability and the clinical relevance of in vitro studies, selection of optimal cancer models is imperative. We present a computational method based on deep learning to integrate copy number alterations, gene-expression and mutations data in order to identify cancer sub-types and estimate the similarity of tumors and cancer cell lines from publicly available resources such as TCGA and CCLE.

We demonstrate the utility of our method using a cohort of colorectal cancers from the TCGA, show that tumors and cell lines can be assigned to clinically relevant groups, and investigate the biological drivers of the different subtypes based on the latent variables derived from the deep learning framework. Such insights will prove invaluable to the cancer medicine of the future in terms of prognosis and therapeutic choices, in the design and selection of early-stage clinical trials, and in the discovery of biomarkers and drug targets. Our flexible approach can be used to query best matching cell lines based on desirable genomic properties such as mutations in a single gene or a pathway. In summary, our method can also be used to identify the best representative cell line panel for a given cancer sub-type or a metastatic cancer cohort. Eventually, the clinicians will be able to upload their omics data from new tumors and search the database for molecularly similar tumors and cancer cell lines. Our framework will present them the predicted clinical outcomes and drug response data based on matched cell lines.

P101

circRNAs in developing midbrain dopamine neurons

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Circular RNAs (circRNAs) are endogenous products of the eukaryotic splicing machinery. By covalent linkage of 5' and 3' splice sites of pre-mRNA segments a unique back-splice junction is formed, resulting in the formation of a circular RNA.

Many different genes can give rise to this novel class of non-coding RNA, while circRNA expression levels are tissue- cell type- and developmental stage-dependent. The mammalian brain is particularly rich in circRNAs and in neurons, circRNAs have been proposed to regulate processes such as synaptogenesis. Although the mechanism-of-action of circRNAs remains poorly understood, they serve as scaffolds for other molecules including miRNAs and RNA binding proteins while some circRNAs can be translated into protein fragments. Here, we study the spatio-temporal expression and function of circRNAs during the embryonic and postnatal development of the midbrain dopamine (mDA) system. mDA neurons mediate complex motor and cognitive functions and have been linked to disorders such as schizophrenia and Parkinson disease. Using novel mouse reporter lines and detailed immunohistochemical and microscopic analyses we generated a detailed map of mDA neuron development at the cellular level. At a select number of developmental timepoints, mDA neurons will be isolated using FACS and subjected to RNAseq and circRNA prediction. Subsequently, circRNAs will be visualised in mDA neurons in vitro and in vivo using single-molecule in situ hybridization followed by knockdown or knockout approaches to dissect the functional role of circRNAs during mDA neuron development.

P102

Regulation of spatial and temporal gene expression in an animal germline

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In animal germlines, regulation of cell proliferation and differentiation is particularly important but poorly understood. Here, using a cryo-cut approach, we mapped RNA expression along the *Caenorhabditis elegans* germline and, using mutants, dissected gene regulatory mechanisms that control spatio-temporal expression. We detected, at near single-cell resolution, > 10,000 mRNAs, > 300 miRNAs and numerous novel miRNAs. Most RNAs were organized in distinct spatial patterns. Germline-specific miRNAs and their targets were co-localized. Moreover, we observed differential 3' UTR isoform usage for hundreds of mRNAs. In tumorous gld-2 gld-1 mutants, gene expression was strongly perturbed. In particular, differential 3' UTR usage was significantly impaired. We propose that PIE-1, a transcriptional repressor, functions to maintain spatial gene expression. Our data also suggest that cpsf-4 and fipp-1 control differential 3' UTR usage for hundreds of genes. Finally, we constructed a "virtual gonad" enabling "virtual in situ hybridizations" and access to all data (https://shiny.mdc-berlin.de/spacegerm/).

P103

Impact of genetic variation on circRNA expression regulation in the human and rat heart.

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Circular RNAs are an abundant class of RNAs to which a growing variety of functions have been assigned. However, various aspects surrounding the regulation of circRNA production and the impact of genetic variation on the backsplicing process have remained unclear, especially in adult tissue samples. Here, we study circRNA expression regulation in a cardiac disease context across 30 recombinant inbred lines of the rat HXB/BXH panel and a large cohort of 80 human heart samples.

Using a genome-wide coregulation analysis of 8,697 human heart circRNAs, we find that steady state circRNA and host gene mRNA levels are generally not correlated. In diseased hearts, of the 478 differentially expressed circRNAs 82% is independent of the host mRNA, indicating circRNA-specific regulation. For a minority of 29 circRNAs we detect expression differences opposite to and anticorrelated with host gene expression, indicating mutually exclusive transcript production. To define the impact of naturally occuring genetic variation on differential circRNA expression, we next tested for local associations between genetic variants and circRNA levels in human hearts and performed local (cis) and distant (trans) circRNA-QTL mapping in the rat recombinant inbred panel. In total, we detect 46 local and 9 distant (eQTL-independent) circRNA-QTLs.

Our analyses provide a first systems-wide, integrated view of circRNA and mRNA expression regulation across a large number of healthy and diseased human and rat heart tissues, revealing the impact of genetic variation on circRNA expression in a complex disease context.

P104

Identification of novel microproteins in the human heart

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Many lncRNAs are of crucial importance to heart function and their dysregulation can contribute to heart failure, although only for a small number of cardiac lncRNAs the exact molecular mechanisms are clear. Some cardiac “noncoding” RNAs are, despite their name, translated into microproteins (e.g. modulators of muscle contractility: DWORF and MRLN). However, a global assessment of the coding potential of human cardiac lncRNAs is currently lacking.

Using RNA- and Ribo-Seq on left-ventricular heart tissue of 80 patients, we observe active translation of 169 presumed lncRNAs, showing that 22% of all 783 highly transcribed cardiac lncRNAs are translated into microproteins. We confirm microprotein production in vitro (73%) and in vivo (up to 60%) by i) in vitro translation assays on randomly selected translated human lncRNAs, ii) searching in human heart shotgun mass-spectrometry data and iii) a highly sensitive targeted mass-spectrometry approach on five human heart samples. To identify biological processes microproteins might be involved in, we perform gene ontology (GO) enrichment on genes that show high correlation with microproteins, resulting in GO terms for 42/169 microproteins. The biggest group (22/42) is enriched for mitochondrial processes and immunofluorescence, and immunoprecipitation experiments confirm mitochondrial localization for several candidates.

We conclude that a significant fraction of cardiac lncRNAs can produce small proteins in vivo and that their cellular localization and function can be inferred by expression coregulation analysis. Performed experiments, in line with computational findings, suggest that a subset of cardiac microproteins might act as novel regulators of mitochondrial processes.

P105

Single-Cell Transcriptomics in the Cell Cycle - Reconstructing Dynamic Behavior from a Snapshot

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The cell cycle is one of the most fundamental principles and comparatively an extremely well-studied object in biology. The recent advances in next-generation sequencing have enabled a completely new level of detail with which such complex and high-dimensional processes can be investigated. We have managed to visualize the cell cycle with the help of simple and linear computational methods. The introduction of a pseudotime on the basis of phase space density allows for tracking the progression of thousands of genes individually throughout the cell cycle despite the data being a snapshot. Additional considerations such as RNA velocity validate the obtained pseudotime remarkably well.

P106

Role of circRNAs in ALS and neuronal differentiation

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Amyotrophic lateral sclerosis (ALS) is a progressive degenerative disease of the upper and lower motor neurons in the motor cortex, brain stem, and spinal cord. ALS appears as the most common adult-onset motor neuron disease (MND), which typically leads to death due to respiratory failure within 3-4 years upon disease onset. As previous studies have shown that circular RNAs (circRNAs) accumulate with ageing and are differentially expressed in neurological diseases, it is of fundamental interest to investigate the circRNA levels in patients suffering from ALS.

CircRNAs are abundant, conserved, stable, and expressed in a cell-type as well as tissue-specific manner. Furthermore, circRNAs are a novel type of long non-coding RNAs, which are highly expressed within neuronal tissue and were shown to be differentially expressed throughout neuronal differentiation.

Therefore, the present study aims to identify circRNAs with altered expression in murine and human ALS models as well as characterize the mechanism of action of ALS-associated circRNAs. In addition, previous studies already identified several highly upregulated circRNA during neuronal differentiation. Thus, the validation of the functional role of the identified circRNAs in neuronal differentiation will be part of the present project.

P107

Identification of protein interactors at functionally distinct types of promoters

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Diverse and cell type specific gene expression patterns arise and are controlled by two main types of regulatory elements: enhancers and promoters. Enhancers activate transcription which initiates at promoter regions located upstream of genes. However, interestingly promoters cannot indiscriminately be activated by any enhancer, suggesting that a functional specificity exists. For example, housekeeping enhancers can only activate transcription from housekeeping promoters but not developmental promoters and vice versa, developmental enhancers can activate developmental but not housekeeping promoters. This mutual enhancer compatibility suggests that functionally distinct types of promoters assemble distinct (pre-) initiation complexes that respond differently to activating cues from enhancers.

To identify promoter bound proteins we implemented an immobilized DNA template assay that aims at enriching promoter-bound proteins from a crude nuclear extract and identify them by label free LC-MS. We were able to enrich at promoter DNA compared to control DNA RNA polymerase II and general transcription factors, which form the transcription pre-initiation complex commonly found at promoters. We are applying this approach to different types of developmental and housekeeping promoters containing unique sequence motifs such as the TATA box, Downstream Promoter Element (DPE), Ohler 1/6, or the TCT motif. Our approach should identify proteins common to all promoters or unique to only one promoter type, revealing factors which participate in transcription initiation at distinct functional classes of promoters.

P108

Activity-dependent gene regulation in induced neurons

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Recovery of brain function following damage depends on the functional integration of newly generated neurons and their ability to plastically contribute to pre-existing neuronal network activity. Direct astroglia-to-neuron conversion by ectopic expression of the neuronal fate determining transcription factor neurogenin-2 has emerged as a powerful tool to generate induced glutamatergic cortical neurons (iGluNs) in vitro as well as in vivo. However, the extent to which iNs may display long-term synaptic plasticity when compared to endogenous neurons has not yet been investigated. Thus, little evidence exists whether iNs possess the ability of functional and structural incorporation into pre-existing neuronal networks. In this study, we aim to compare activity-dependent transcriptional responses in developing glutamatergic iNs with those evoked in developing primary cortical neurons (endogenous neurons, eN) in vitro. Following electrophysiological or pharmacological induction of NMDA receptor-dependent long-term potentiation (LTP), we aim to study transcriptional responses in form of immediate-early gene (IEG) expression profiles as a first proxy for the ability to drive activity-dependent gene expression modulations, followed by examining genome-wide gene expression changes of iN and eN after LTP induction. Finally, existing bioinformatical tools will be crucial to perform unbiased mapping of iN phenotypes onto the highly diverse eNs landscapes. Until now, we investigated whether astroglia-to-neuron conversion enables the generation of glutamatergic iN of sufficient homogeneity and electrical maturity to enable the study of activity-dependent gene expression. Based on whole-cell patch clamp recordings, we found that iNs display a high functional heterogeneity at earlier time points, converging onto a homogenous phenotype at later developmental stages.

P109

circRNA in muscle

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Circular RNAs (circRNAs) are a subset of noncoding RNAs (ncRNAs) previously considered as products of missplicing. Now, circRNAs are considered functional molecules, although to date, only few functions have been experimentally validated, and therefore the vast majority of circRNAs are without known relevance. Here, based on RNA sequencing from the ENCODE consortium, we identify and characterize a subset of circRNAs, coined AUG circRNAs, defined by spanning the canonical translational start site in the protein-coding host genes. AUG circRNAs are more abundantly expressed and conserved than other groups of circRNAs, and they display an Alu-independent mechanism of biogenesis. The AUG circRNAs contain part of bona fide ORF, and in the recent years, several studies have reported cases of circRNA translation. However, using thorough cross-species analysis, extensive ribosome profiling analyses and experimental data on a selected panel of AUG circRNAs, we observe no indications of translation of AUG circRNAs or any other circRNAs. Our data provide a comprehensive classification of circRNAs and, collectively, the analyses suggest that the AUG circRNAs constitute an abundant subclass of circRNAs produced independently of primate-specific Alu elements. Moreover, AUG circRNAs exhibit high cross-species conservation and are therefore likely to be functionally relevant.

P110

From cells to communities: Modeling microbes and their interactions

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Microbial life is ubiquitous in all environments on Earth, and understanding the life and growth of microbes has outstanding relevance for biotechnology, biogeochemistry, and human health. Over the past two decades, numerous methods, algorithms, and software for the reconstruction and modeling of microbial metabolism have been established — including a tremendous increase in available online resources and data. Genome-scale metabolic reconstructions and their analysis using constraint-based methods are now standard tools to investigate microbial growth, genotype–phenotype relationships, microbe–microbe interactions, and host–microbe interactions.

The purpose of this contribution is to outline recent advances in modeling microbial growth, in particular in the context of cellular resource allocation. Going beyond traditional metabolic reconstructions, resource allocation models consider the "protein economy" of cellular growth and allow to predict how organisms allocate their (limited) resources to the diverse cellular processes required for survival and growth. As yet, however, such models have been primarily applied to single species. Extending models from cells to multi-cellular systems and communities is a natural next step. The poster will present examples and challenges of multi-species models, with application from cyanobacterial interactions to models of microbial consortia relevant to human health.

P111

Targeted removal of epigenetic barriers during transcriptional reprogramming

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Master transcription factors have the potency of directing and reverting cellular fates. Consequently, their endogenous gene copies have to be subject to particular transcriptional control. It is however unclear, which molecular processes are responsible for safeguarding cellular identities. Here we show that DNA-methylation has the potential to dictate the outcome of transactivation and thus to prevent the harmful activation of a foreign cell identity factor. By employing transcriptional engineering we demonstrate, that targeting of a transactivation domain to the promoter of the endogenous copy of the SRY (sex determining region Y)-box transcription factor Sox1 can result in a strong induction of transcript and protein. This gene induction restores neuronal differentiation potential of progenitor cells largely committed to a glial fate, substantiating the role of Sox1 as a master transcription factor. However, analysis of Sox1 expression on single-cellular level revealed that, despite efficient activator targeting, major proportions of progenitor cells are not responding to the incentive stimulus. This enabled us to investigate, which molecular barriers are interfering with cellular conversion and we found that among a series of euchromatic processes removal of DNA methylation has the most significant influence on releasing Sox1 induction.

P112

Systems biology model of the mucosal immune system in the context of inflammatory bowel disease

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Inflammatory bowel diseases are chronic diseases caused by autoimmunity of T cells against commensal bacteria in the gut. Monoclonal antibodies targeting the cytokine TNF-α are used to treat severe forms, but the therapeutic outcome differs highly between patients. Our long-term aim is to better understand the underlying mechanisms. To this end, we present a mathematical model describing the cellular and humoral processes of the intestinal immune system, to provide a basis for further analysis of drug effects and inter-individual variability in response.

Important processes of the mucosal immune system were identified by an in-depth literature query. These processes were described (i) in terms of parameter values from literature; (ii) by estimating parameters from literature data; or (iii) by assuming reasonable parameter ranges resulting in adequate model behaviour, if literature data were not available. We combined these processes into a systems biology model that described concentrations of several immune cell types in the gut lamina propria and lymph nodes.

The developed ODE model included different subpopulations of dendritic cells, T cells, B cells, macrophages, neutrophils and bacterial cells. Simulations were able to reflect the main characteristics of the mucosal immune system: Following bacterial stimulation, neutrophils infiltrated the lamina propria, further recruiting dendritic cells and macrophages, which shifted to an inflammatory state upon activation. With a time delay, effector T cell concentrations increased. After acute inflammation all concentrations returned to baseline.

The next steps will be to include IBD triggers, pharmacokinetics and drug effects, and to generate a virtual population of patients to analyse inter-individual variability.

The developed systems biology model was able to reflect the main characteristics of the mucosal immune system. This is a promising first step towards modelling the pathological processes and drug effects in IBD.

P113

RNAComposer - prototyping and tuning of RNA 3D structures

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RNAComposer is the world's most popular system for fully automated prediction of RNA 3D structures. Based on the user-provided information about RNA sequence and – optionally – the secondary structure topology encoded in dot-bracket notation, it calculates RNA molecule shape in the 3D space. The speed of computation and high quality of the results have made RNAComposer useful in a variety of applications. They are dedicated to both scientific and educational issues defined in the field of RNA structural bioinformatics. Recently, real-time modelling of new folds for biomedical and personalised medicine purposes has become one of the critical tasks for the system. The second, equally important field is the application of the system for prototyping the tertiary structure which becomes the object of experimental research, for example, X-ray or NMR.

New functions of RNAComposer also allow adjusting the predicted prototype model of an RNA molecule. The user can tune the three-dimensional shape by editing the structure on the level of secondary structure elements, like loops, stems and single-stranded fragments. Their substitution or *ab initio* modelling can be easily performed in the batch mode of the system.

The presentation aims to familiarise the audience with the advantages and limitations of the basic, interactive version of the system devoted to RNA 3D structure prototyping. Moreover, we will discuss the batch version which has been recently equipped with some functionalities. We will present how the preliminary RNA 3D model can be adjusted to meet the user requirements.

P114

Identification of ribosome-associated non-coding (circular) RNAs

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Circular RNAs (circRNAs) are an abundant class of RNAs which are present across different mammalian tissues. During splicing, instead of forming linear mRNAs, a downstream and an upstream splice site of a transcript are joined together during a so-called back-splicing event, which links the 3' end and the 5' end of a transcript in a circular manner. Previously it has been found that circRNAs are highly abundant in mammalian brain tissues, and that they can have regulatory potential, however there is little known about their function. Recent evidence suggests that some circRNAs have coding potential, and that they may produce proteins.

To study translation of circRNAs - and other non-coding RNAs - on a genome-wide scale, one possibility is to use a RiboTag mouse system, which allows cell-type specific ribosome-associated RNA pull-down of total RNA. However, identification of reliable candidates is complicated, mostly due to noise introduced by non-specific reactions occurring during the RiboTag pull-down. Moreover, finding a control sample for these experiments is also non-trivial which adds further complexity when identifying ribosome-associated transcripts. Here, we propose a mathematical model for pull-down experiments - with an emphasis on NEUROD6-positive RiboTag mouse - which tries to account for the above outlined problems. We validate our model using real and simulated data and were able to filter out most false-positive candidates (abundant, but likely not translated circRNAs), and minimise false negatives. The model also allows to study potential translation of other non-coding RNAs, for example lincRNAs.

P115

SPACE-seq: unbiased spatially resolved single-cell sequencing

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Obtaining transcriptome-wide and spatially resolved information from single cells has been proven to be a challenging task. Current state-of-the-art experimental methods are either limited by the number of genes that can be simultaneously detected within a single cell, or require preexisting spatial information. Here, we introduce SPACE-seq, a new experimental technique that allows for unbiased, high-throughput single-cell spatial transcriptomics. SPACE-seq combines a physical grid with combinatorial indexing to label single cells of any tissue in a unique way and thereby preserving the approximate spatial localization of any given cell. We developed SPACE-seq by applying it to slices of mouse brain. SPACE-seq is easy to adapt, can be applied to any tissue and can be combined with other technologies.

P116

Single-cell transcriptomics in brain organoids identifies a critical transcription factor whose dosage controls neuronal maturation

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We present new findings from our cell reprogramming-based brain organoids modeling platform of paradigmatic diseases affecting human cortical development. We established a new analytical pipeline that, adjusting for all main sources of variability, allows a meta-analysis of diverse datasets, enabling the first systematic comparison of transcriptomes from all the best established brain organoid protocols, benchmarking them to the in vivo developing human brain. We could thus extract the influence of patterned versus unpatterned conditions on the co-expression of gene modules that direct the specification of different brain regions. In particular, an in-depth characterization of the transcriptome of cortical organoids at bulk and single-cell levels revealed high concordance between different stages of cortical spheroid differentiation and fetal cortical tissues, with a faithful recapitulation of the defining signatures for the main cell populations across mid-fetal corticogenesis. On the basis of such analytical framework, we next analyzed cortical organoids from two disorders caused by genetic dosage imbalance of the 7q11.23 locus (hemideletion Williams-Beuren Syndrome, WBS and hemiduplication, 7dupASD), which display a paradigmatic symmetry of cognitive-behavioral features spanning autism spectrum disorder (ASD) and intellectual disability. Bulk and single-cell RNAseq uncovered the molecular phenotypes only detectable at single-cell resolution, revealing for the two conditions divergent and symmetrical dynamics of neuronal maturation that were confirmed through spatially resolved in situ approaches. Finally, we uncovered the basis of this phenotype by identifying one key transcription factor from the 7q11.23 region whose dosage rescue is alone sufficient to revert the neuronal maturation phenotype in organoids from 7dupASD patients, defining the mechanistic link between its established role in social behavior and cognition and the timing of cortical maturation.

P117

Comparing chromatin contacts mapped in mESCs by Genome Architecture Mapping and Hi-C

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Gene expression is functionally coupled with the spatial organization of the genome. Folding and topological organization of chromosomes establishes higher order structures such as topologically associated domains (TADs) and creates a spatial proximity between regulatory genomic elements and their target genes. Genome Architecture Mapping (GAM) is a novel genome-wide method which maps chromatin contacts in 3D. GAM is based on measuring the frequency of locus co-segregation in a large collection of ultra-thin nuclear sections.

Here, we present an extended GAM dataset obtained from mouse embryonic stem cells using a more efficient, multiplexed version of GAM. We devised a method to compare contact frequency matrices from GAM and Hi-C. After extracting the contacts detected most abundantly in GAM or Hi-C, we investigated the underlying genomic features in the contacting regions. We also detected TADs on the GAM and Hi-C datasets and found about 80% conservation of detected boundaries. By enrichment tests for epigenetic, genomic and gene expression features, we performed a functional characterization of the GAM- and Hi-C-specific TAD boundaries. Our analyses show that GAM and Hi-C are both powerful approaches to detect TADs, but our finer detailed analyses of pair-wise contacts show that the two approaches can also capture different contacts which we currently investigate.

P118

Quantitative mapping of human of human protein-protein interactions in health and disease

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Information on protein-protein interactions (PPIs) is of critical importance for studying complex biological systems and developing therapeutic strategies. Here, we have developed double-readout bioluminescence-based two-hybrid technology, termed LuTHy, which provides two quantitative scores in one experimental procedure when testing binary interactions. PPIs are first monitored in cells by quantification of bioluminescence resonance energy transfer (BRET) and, following cell lysis, are again quantitatively assessed by luminescence-based co-precipitation (LuC). The double-readout procedure detects interactions with higher sensitivity than traditional single-readout methods and is broadly applicable, e.g. for detecting the effects of small molecules or disease-causing mutations on PPIs. Applying LuTHy in a focused screen, we identified 42 interactions for the presynaptic chaperone CSP□, causative to adult-onset neuronal ceroid lipofuscinosis (ANCL), a progressive neurodegenerative disease. Nearly 50% of PPIs were found to be affected when studying the effect of the disease-causing missense mutations L115R and ΔL116 in CSP□ with LuTHy. Our method is a sensitive research tool with high utility for investigating the molecular mechanisms by which disease-associated mutations impair protein activity in biological systems.

P119

Differential activation of ERK by oncogenic KRAS versus BRAF revealed by single-cell RNA-seq

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The intestinal epithelium consists of multiple different cell types forming a well organised structure of crypts and villi. In colorectal cancer development a series of genomic alterations causes the malignant transformation of this structure. Here, we elucidate the cell type specific effects of KRAS(G12V) and BRAF(V600E) alterations in colorectal cancer by integrating single-cell protein assays and single-cell RNA-sequencing data obtained from oncogene-inducible intestinal organoids. We find that BRAF(V600E) induction triggers high and generalized ERK signalling in both undifferentiated and differentiated intestinal cells and disrupts organoid cellular organisation. In contrast, transgenic expression of KRAS(G12V) is well tolerated and induces cell type specific ERK signalling. Here, ERK-dependent reporter activity is induced in all crypt cells but only in a small subset of enterocytes, demonstrating the existence of cell-intrinsic mechanisms controlling oncogenic signal transduction from KRAS to ERK in the intestinal epithelium. Our experiments highlight divergent properties of oncogenes activating the ERK signalling pathway, and thus provide a rationale for the different placement of the KRAS and BRAF oncogenes in the conventional versus serrated progression pathways leading to colorectal cancer.

P120

Discovery of an evolutionarily conserved sORF regulating neurogenesis in vertebrates

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Despite their short length, small open reading frames (sORFs) can encode functional micropeptides and play important roles in development or physiology. Hundreds of such translated sORFs exist in the vertebrate transcriptome, but thus far, only few have been assigned a function. Here we focused on an evolutionarily conserved sORF, which we termed neuronal small ORF (nsorf). The encoded 25 AA hydrophobic transmembrane peptide localized to the endoplasmatic reticulum and highest nsorf expression was observed in nervous tissues in both mammals and zebrafish. Single-cell RNA sequencing (scRNA-seq) using zebrafish embryos and adult zebrafish brains identified particularly high nsorf expression in neural stem cells and neurons. CRISPR/Cas9-mediated genetic ablation of nsorf in zebrafish impaired neurogenesis. We observed premature neuronal differentiation resulting in a depletion of the proliferating stem cell/progenitor population, culminating in significantly fewer mature neurons in nsorf mutants. RNA-seq of neuronal organoids derived from human nsorf mutant induced pluripotent stem cells and scRNA-seq of zebrafish nsorf mutants furthermore identified reduced expression of neurotransmitters and hormones associated with tissue metabolism. Consequently, adult nsorf mutants showed reduced body weight, and were less active. We conclude that nsorf plays a key role in neurogenesis and metabolism essential for normal growth and homeostasis.

P121

coTRaCTE predicts co-occurring transcription factors within cell-type specific enhancers

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Cell-type specific gene expression is regulated by the combinatorial action of transcription factors (TFs). In this study, we predict transcription factor (TF) combinations that cooperatively bind in a cell-type specific manner. We first divide DNase hypersensitive sites into cell-type specifically open vs. ubiquitously open sites in 64 cell types to describe possible cell-type specific enhancers. Based on the pattern contrast between these two groups of sequences we develop “co-occurring TF predictor on Cell-Type specific Enhancers” (coTRaCTE) - a novel statistical method to determine regulatory TF co-occurrences. Contrasting the co-binding of TF pairs between cell-type specific and ubiquitously open chromatin guarantees the high cell-type specificity of the predictions. coTRaCTE predicts more than 2000 co-occurring TF pairs in 64 cell types. The large majority (70%) of these TF pairs is highly cell-type specific and overlaps in TF pair co-occurrence are highly consistent among related cell types. Furthermore, independently validated co-occurring and directly interacting TFs are significantly enriched in our predictions. Focusing on the regulatory network derived from the predicted co-occurring TF pairs in embryonic stem cells (ESCs) we find that it consists of three subnetworks with distinct functions: maintenance of pluripotency governed by OCT4, SOX2 and NANOG, regulation of early development governed by KLF4, STAT3, ZIC3 and ZNF148 and general functions governed by MYC, TCF3 and YY1.

In summary, coTRaCTE predicts highly cell-type specific co-occurring TFs which reveal new insights into transcriptional regulatory mechanisms.

P122

Neurogenic Lineage Decisions in the Developing Drosophila Embryo with Single-cell Resolution

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Early nervous system development is an intricate sequence of events that allows an undifferentiated primordium to specify and differentiate into diverse cell types, including neurons and glia. Cell fate decisions are coordinated in both space and time and depend on the immediate cellular environment. While decades of genetics have uncovered factors that are necessary for many of these differentiation decisions, a genome-level understanding of specific cell types as neurogenesis unfolds has been impossible in vivo until recently.

The aim of my project is to unravel the molecular mechanisms that drive fundamental cell fate decisions in neurogenesis, and to then test and verify my regulatory models in vivo. To understand the events modulating the major cell fate decisions of the early nervous system development, tightly staged embryos will be collected in order to capture the different waves of neuroblast delamination and their initial proliferation and differentiation trajectories. I am exploring their transcriptome dynamics with special focus on the different cell types that emerge and how spatial and temporal variation impacts these decisions.

My preliminary work has focused on method development, optimizing sequencing platform choice, native-vs-fixed and cell-vs-nuclear sequencing. Furthermore, I have optimized sorting techniques suitable for single-cell sequencing of specific, purified neurogenic sub-populations.

Using whole embryo data, I have already been able to identify different neuronal populations, genes expressed in specific populations and along differentiation trajectories. In fact, single-cell transcriptomics has allowed for the identification of specific individual neuroblasts, for which the eventual fates of emerging neurons and glia are known. By investigating their repertoire of, for example, cell adhesion molecules, I hope to understand their projection behavior, including the connections they make.

P123

Brainwide optical circuit interrogation at the cellular level guided by online analysis of neuronal function

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Whole-brain imaging allows comprehensive functional mapping of distributed neural pathways, but neuronal perturbation experiments are usually limited to targeting predefined regions or genetically-identifiable cell types. To complement whole-brain measures of activity with brainwide manipulations for testing causal interactions, we introduce a system that uses measured activity patterns to guide optical perturbations of any subset of neurons in the same fictively behaving larval zebrafish. First, a light-sheet microscope collects whole-brain data that are rapidly analyzed by a distributed computing system to generate functional brain maps. Based on these maps, the experimenter can then optically ablate any subset of neurons, and image activity changes across the brain. We applied this method to characterize contributions of behaviorally tuned populations to the optomotor response. We extended the system to optogenetically stimulate arbitrary subsets of neurons during whole-brain imaging. These open-source methods enable delineating the contributions of neurons to brainwide circuit dynamics and behavior in individual animals.

P124

Mapping of the zip-codes - determinants of sub-cellular mRNA localization in neurons

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The sub-cellular localisation and translation of RNA is a crucial element of post-transcriptional gene regulation. Especially for polarised cells like neurons, it is an essential mechanism to sustain growth and synaptic plasticity, which is the basis of learning and memory. RNA localization is mediated by so called zip-codes, *cis*-acting elements that are often located in the 3'UTR of mRNAs and are bound by *trans*-acting factors like RNA-binding proteins that drive the localisation. Recent research showed success in using massively parallel reporter assays (MPRA) to identify RNA *cis*-regulatory elements. Based on this, we are developing a method to identify zip-codes in neurons differentiated my mESC inducible expression of the neurogenic transcription factor Ascl1.

This method combines physical separation of neurons into the soma and neurites, which was established in the group [1], with an MPRA assay. By generating RNA sequencing libraries from these two compartments, our group has already identified the neurite enriched transcriptome of mESC-derived neurons. Now we have constructed a reporter library that contains small fragments spanning the 3'UTR sequences of 400 neurite-enriched mRNAs. These fragments are inserted into the 3'UTR of a reporter transgene and thereby control the localisation of this specific mRNA. By identifying the fragments and sequence elements in this library that are sufficient to drive mRNA localisation to neurites, we aim to identify neuronal zip-codes on a near genome-wide level.

[1] Zappulo A, et al. (2017). RNA localization is a key determinant of neurite-enriched proteome. Nature communications, 8(1), 583.

P125

Functional dissection of enhancers involved in neurogenesis using CRISPR/Cas9

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Enhancers are non-coding genetic elements that regulate gene expression in a temporal and tissue-specific manner. Although a plethora of computational methods for predicting enhancers based on sequence and chromatin features exist, it is still challenging to identify functional enhancers and examine their roles in different biological processes. In order to identify and functionally perturb enhancers at their endogenous sites we are utilizing CRISPR interference (CRISPRi) and activation (CRISPRa). By using several CRISPRa/i constructs in a pooled screen, we aim to comprehensively assess the genomic region surrounding master regulators of neuronal cell fate. We demonstrate that several different CRISPRa constructs can each be used to successfully activate enhancers in a highly specific manner allowing us to identify multiple functional enhancers of a master regulator of neuronal differentiation. In order to assess combinatorial effects between multiple enhancers, we simultaneously targeted multiple loci and obtained promising evidence for synergism between proximal enhancers. We will broaden our scope to study whether synergism occurs between specific subsets of the more distal enhancers for which we observe weaker individual effects. In addition to detecting the effect of the CRISPRi/a-mediated enhancer perturbation on selected targets (via FACS or qPCR), preliminary data suggests it may be feasible to detect the effect of an enhancer perturbation on multiple genes through single-cell RNA sequencing. This functional CRISPR enhancer screen will not only allow the identification of enhancers and their targets but will also enable improved computational predictions of enhancers by examining their features such as sequence, accessibility, histone modification status and transcription factor binding. Finally, our efforts should improve our understanding of the regulatory mechanisms adopted by living organisms to ensure an accurate control of their genetic program.

P126

Investigating the synaptic ultrastructure with multicolor superresolution microscopy

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We are interested in the **spatial distribution of proteins at the presynaptic terminal** and how the molecular composition of the terminal regulates the synaptic vesicle cycle. Typical dimensions of synaptic structures are however well below the diffraction limit of light.

Single-molecule localization microscopy methods such as **STORM** allow us to observe these structures on the nanoscale. Due to the high frame numbers required to achieve good localization precision, multi-colour experiments can be challenging and chromatic abberations between different colour channels or drift easily disturb these measurements.

Here, we present two methods that exploit the spectral information of single emission event to record up to four **standard organic fluorescent dyes** simultaneously with a **single excitation laser** over a **large field of view**. Because all emission events pass through the same optical path, their relative positions can be compared more reliably. The localization of a particular protein of interest are mapped against pre- and post-synaptic markers (Bassoon, PSD95) on hundreds of synapses in parallel.

We use a homogenous epi-illumination of the entire field of view. This ensures field-independent image resolution (Douglass et al. 2016). Stochastic emission events are detected through an image splitter on an sCMOS camera. For 3D localization, we introduce a depth-dependent astigmatism. The setup can be implemented on any inverted epi-fluorescence microscope. Microscope control and localization fitting are performed in open-source software µManger & Fiji.

For spectral demixing (**sdSTORM**, Lampe et al. 2015), emission photons are split by a dichroic filter into a short and long wavelength channel. Colour is assigned to individual localizations based on the intensity histogram from both image channels. Alternatively, a prism can split emission photons into spectra (**spectral STORM**). Colour is then assigned to each localization based on its spectroscopic signature.

P127

Novel lncRNAs involved in nervous system development of the fruit fly

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Across different metazoan species a staggering diversity of neurons is generated from a small pool of progenitor cells. Mapping out how many molecularly distinct neuron types exist and how they are specified from common precursors is an important undertaking. It is not only key to understanding hereditary disease of the nervous system, it also is crucial for developing regenerative approaches in medicine.

In vertebrates, neurons arise from the neural tube with different neuronal subtypes being specified along the dorsal-ventral axis in response to morphogen gradients. The relative position of a cell within the embryonic neural tube gives rise to distinct gene expression pattern, which in turn determine neuronal cell fate (e.g. motor neuron vs. interneuron). This process occurs in a remarkably similar fashion in *Drosophila melanogaster*. How such expression patterns are established, however, is insufficiently understood in any animal. We have generated a high resolution expression map of the developing nervous system in the Drosophila embryo. We detected a number of long non-coding RNAs (lncRNAs) with distinct temporal and cell type specific expression. Little is known about lncRNAs in the nervous system, but they have generally been implicated in establishing and fine-tuning of gene expression. We will use CRISPR to further investigate the role of selected candidates in establishing the Drosophila nervous system.

P128

Comprehensive Reannotation of the Herpesviral Transcriptome and Translatome

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To date, herpes simplex virus 1 (HSV-1) was thought to encode about 80 viral proteins as well as a set of non-coding RNAs during lytic infection. Here, we employed a broad range of systems biology approaches (4sU-seq, 5'-seq, PacBio-seq, Ribo-seq, translation start site profiling and total proteome analysis) to comprehensively re-annotate functional elements within the HSV-1 genome. This revealed dozens of new viral transcripts as well as >120 novel viral proteins and polypeptides. Combined analysis of data obtained by second- (5'-start site profiling) and third- (previously published PacBio data) generation sequencing provided a precise and validated map of the viral transcriptome. Similar to mammalian genes, we found prevalent translational regulation of viral gene expression by translation of short upstream open reading frames (uORFs) and alternative initiation sites (non-AUG start codons) upstream of the primary start codon. Translational regulation by viral uORFs was validated by dual luciferase reporters. In addition, translation of N-terminal peptide extensions was visualized in the context of infection for several key viral proteins, including the serine/threonine-protein kinase US3, major capsid protein UL19, and the transcriptional regulator UL54 (ICP27); with apparent effects on protein localization. These differential isoforms explain processes in virion assembly observed in a range of mammalian alphaherpesviruses. Furthermore, a novel peptide from the RL2 locus could be visualized in infected cells and an additional peptide from a uORF in the mRNA encoding the neurovirulence factor ICP34.5 could be exogenously expressed with ongoing work to examine production during in vivo. To visualize and study such large and mixed datasets, we have released a graphical user interface which can integrate an array of data and be analyzed on any computer. In summary, our data comprehensively revise the current annotation of an important human pathogen.

P129

Genome Architecture Mapping of Rare Cell Types in the Brain

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Four-dimensional (4D) interactions between genes and non-coding regulatory elements in space and time are fundamental elements of transcriptional control. In post-mitotic neurons, the regulation of 4D genome architecture is critical for synaptic plasticity and memory encoding, while defects in chromatin-associated processes are implicated in multiple neurodevelopmental, neurodegenerative and psychiatric disorders. Thus, a major challenge is to understand the relationship between the 4D genome and the aetiology and progression of neurological disease.

To date, chromatin contacts have only been mapped in cultured neuronal cell lines or in dissociated neural tissue. Genome Architecture Mapping (GAM) is a novel in-situ approach to map chromatin contacts from thin nuclear sections across cell populations. GAM is ideally suited to map contacts directly within a tissue of interest and with selective recovery of only rare cell populations or states. Thus, we have developed the application of GAM in mouse brain tissues to identify *in-situ* chromatin contacts in rare neuronal populations.

We have generated the first *in-situ* genome-wide chromatin contact maps directly from dopaminergic neurons (DNs) in the ventral tegmental area. We observe topologically associating domains (TADs) *in-situ*. We also detect cell-type specific chromatin contacts between biologically important loci (e.g. neurodevelopment, synaptic plasticity), which are separated by tens of Mb, revealing new aspects of 3D genome structure in neuronal function. We have also begun to explore changes in the genomic landscape following environmental exposure to drugs of abuse (e.g. cocaine). Our observations demonstrate that *in-situ* DNs form TADs and long-range chromatin contacts specific for neuronal specialization and cell state, and that loci relevant for neuronal functions can be dynamically rearranged at the onset of addiction.

P130

Dissecting the genetic basis of transcriptional and translational regulation in heart and liver

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Gene expression regulation is a multi-layered process and genetic variation can modulate expression levels at various stages. One layer of regulation that has not yet been fully assessed for genome-wide genetic effects is translational regulation

We perform a combinatorial RNA-Seq, Ribo-Seq and genotyping approach to liver and heart tissue of the well-established HxB/BxH rat recombinant inbred (RI) panel to elucidate the role of genetic variants on transcriptional and translational levels of gene expression. It consists of 30 lines that have been derived from a reciprocal cross of the spontaneously hypertensive rat (SHR.Ola) and the normotensive Brown Norway rat (BN.Lx/Cub). The RI lines, which have been previously comprehensively described on multiple levels, enable us to perform quantitative trait loci (QTL) mapping to link causal genetic variants to quantitative differences in both layers of expression.

We identify hundreds of associations on the transcriptional (eQTL) and the translational (riboQTL) level in each tissue. The majority of detected associations occurred between SNPs and genes in close proximity to each other (local QTL). However, we also detect genetic variants that affect gene expression levels over longer distances (distant QTL). Both types of QTL have been assessed in detail to understand the mechanistic and genetic basis of these QTL.

We observe 17 genes regulated by a single distant riboQTL on chromosome 3. To follow-up this finding, we performed an independent RNA-Seq and Ribo-Seq experiment in two congenic rat strains that differ specifically in this riboQTL region. A differential expression analysis of these strains reproduces the results and suggests that the chromosome 3 locus controls the translation of many additional genes. These global translatome changes appear to be absent from liver, so we hypothesize that the riboQTL hosts a genetic variant responsible for a cardiac-specific state of translational stress in strained hearts.

P131

Dissecting the dynamic transcriptional response in Herpes simplex virus 1 infection using single-cell sequencing

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The initial contact, entry, and intracellular establishment of a virus triggers a range of host cell responses. Using single-cell transcriptomics of HSV-1 infected primary human fibroblasts, we have explored the dynamic transcriptional responses to lytic infection in unprecedented detail. Our results precisely describe the onset of viral gene expression and reveal two surprising bifurcations therein. We show that two host cell Ras-related GTPases, not expressed in uninfected cells, are induced by viral factors and support the infection. Furthermore, we define a group of chemokines coordinately expressed in a subpopulation of cells. Using various trajectory algorithms, we provide evidence that Nrf2 activation temporarily protects cells from the infection, and that G1/S phase is a preferred starting point for productive infection. Our findings open new ways to understand HSV-1 infection, and the applied analysis approaches can serve as a methodological blueprint for investigating transcriptional cascades during viral infection and other stress conditions.

P132

Deeply Exploit The Expression and Function of Protein-Coding and Non-Coding RNAs in Single-Cell RNA-Seq Datasets

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Single-cell transcriptome sequencing technology has revealed significant differences in gene expression levels between different cell groups. However, the expression and function of non-coding RNAs (ncRNAs) in different cell types are still unclear. In this study, we developed a database to decode the expression profiles of both protein-coding and ncRNAs from multiple single-cell sequencing data sets. Our database performs the cell-cell similarities estimation by shared nearest neighbor (SNN) and Pearson correlation methods. Based on Principal Components Analysis (PCA) and T-distributed Stochastic Neighbor Embedding (t-SNE) algorithm, we show the classification of cells in two-dimensional (2D) and three-dimensional (3D) space. In addition, this database reveals the marker genes in each cell group and the high variable genes between different cell clusters. These genes include protein-coding genes and multiple types of non-coding RNAs. Some of the non-coding RNAs are significantly higher expressed in particular cell groups or states, which indicate their special regulatory role during cell differentiation. Through gene function enrichment analysis, we predict the function of genes in each group. By combining expression profiles and functional genomic annotations, we built protein-lncRNA co-expression networks to predict lncRNAs function in single-cell cluster. This database will provide insights into the coding and non-coding RNAs expression and their roles in single-cell levels.

P133

Recognizing native-like structure in the set of RNA 3D models

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Nowadays there are many tools available to predict RNA 3D structures from sequence with optional 2D structure input. Some of them generate up to thousands of models, which are later clustered into tens of representative predictions. When a reference 3D structure is present then one can assess the (dis)similarities of obtained RNA 3D models using any of available metrics. However, in most of the relevant cases where researchers use RNA 3D structure prediction methods, the reference structure is unknown. Adding to it the possible large number of results from different prediction tools, it is hard to select native-like RNA 3D models among them.

We address this problem by constructing a virtual approximation of the native structure interaction network. Each input model is analyzed and its canonical and non-canonical base pairs are treated as separate votes. Once all of the models are processed, a consensus is made. It consists of those base pairs which were voted for a certain number of times. The acceptance threshold is a parameter of the method, for which a sensible default value is used. We performed several computational experiments to optimize the parameter based on past RNA-Puzzles results.

The consensus interaction network allows to address the original problem in two ways. First, it enables to score each model (INF – Interaction Network Fidelity) against the virtual native structure and provide a ranking. Second, it allows to look at each model more carefully and check which of its base pairs are part of the consensus, which are not and which are missing (true positives, false positives and false negatives respectively). From these results, users can select a small subset of RNA 3D models most likely to be native-like.

We propose an automatic method to assess quality of a set of RNA 3D models when the reference structure is unknown. As a result, models are ranked and supplemented with details about the predicted correctness of their base pairing pattern.

P134

Characterizing circRNA distribution at single-molecule level in whole tissue

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Circular RNAs (circRNAs) are a large, newly discovered class of non-coding RNAs. Due to their specific circular structure, they display unusually high stability and can just recently be detected using new sequencing and computational technologies. Characterising the function of circRNAs has recently spiked very high interest in many fields including basic molecular biology, neurobiology, neurodegenerative diseases, aging, cancer, and biomarker research.

To analyze circRNAs, we are developing a protocol to quantify circRNA expression in terabyte-sized reconstructions of whole animal light-sheet microscopy acquisitions. Our project aims to localise circRNAs in tissues by combining high-resolution imaging of cleared samples with single-molecule imaging, thereby preserving the global context (mouse brains, organoids, ...). Subsequently, we compare localisation, distribution and quantity between mutants and wild-type animals.

Initially, we are clearing large tissues such as mouse brains. We start by polymerisation to stabilise the sample using hydrogel monomers, which still yields an opaque brain. We therefore remove lipids to create cleared tissues. Next, we aim to establish a single-molecule staining protocol FISH to visualise the circular RNA, which is a one-day staining protocol before imaging. After establishing the protocol, we image the cleared sample using light sheet microscopy and we reconstruct imaging tiles to produce one seamless, large, three-dimensional image. We start by selecting the best illumination direction between the right and the left illumination for each image tile and then stitch hundreds of large 3d tiles together using BigStitcher.

As the last step, we aim to design and implement algorithms using machine learning (e.g. Random Forest) and model-based approaches to measure differences in circRNA expression and localisation. To apply those to the whole tissue, we develop a processing software manager that allow us to handle big tasks using less local compute power and in less processing time by distributed computing using available resources (e.g. cluster, other workstations...).

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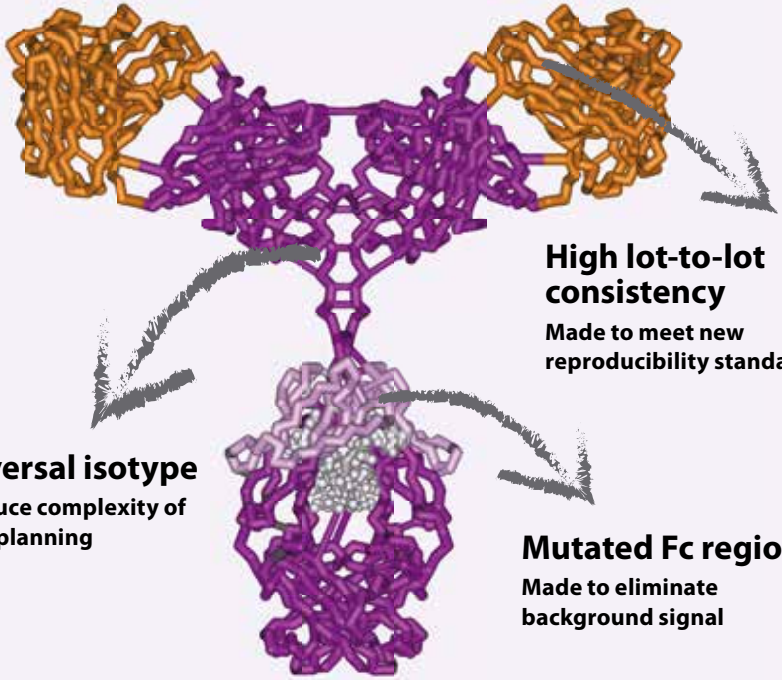

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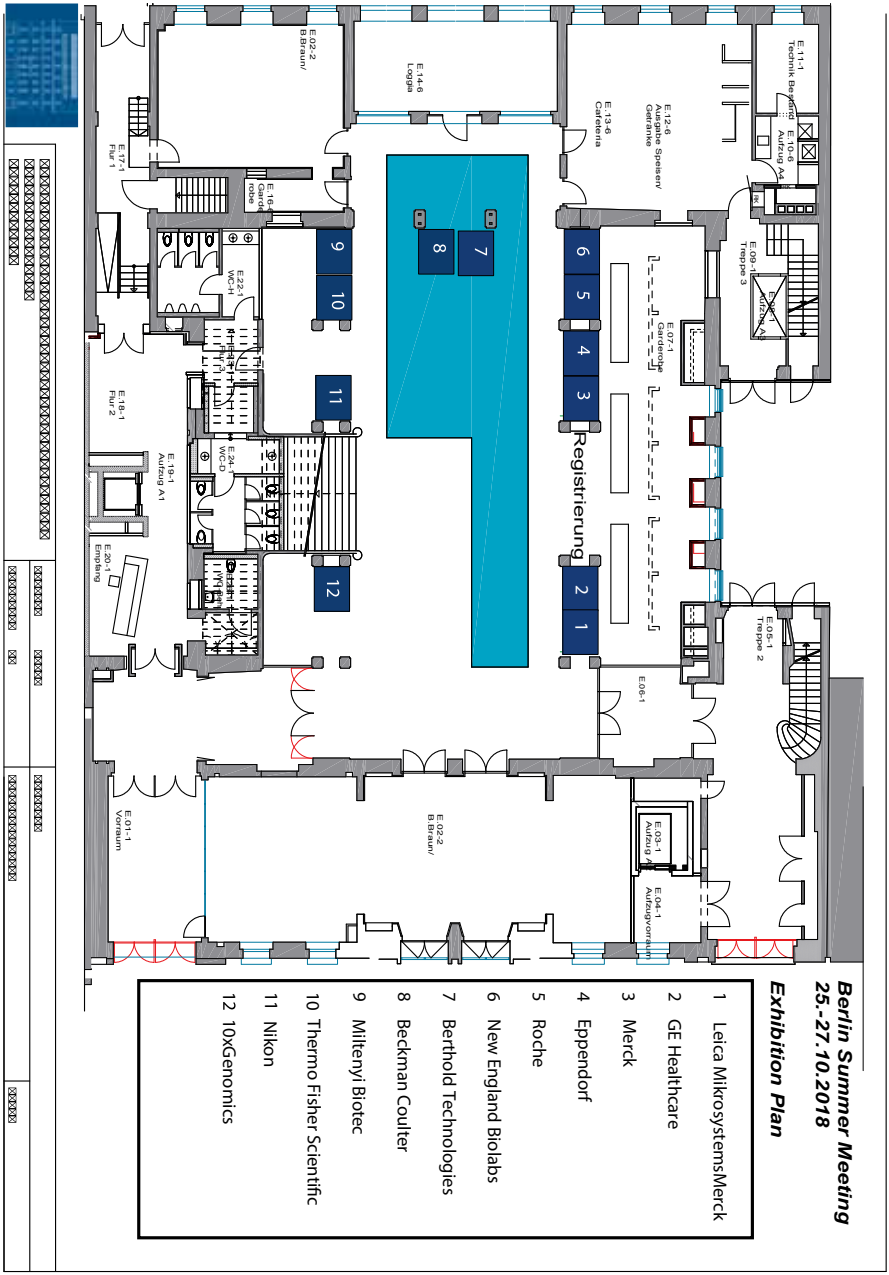

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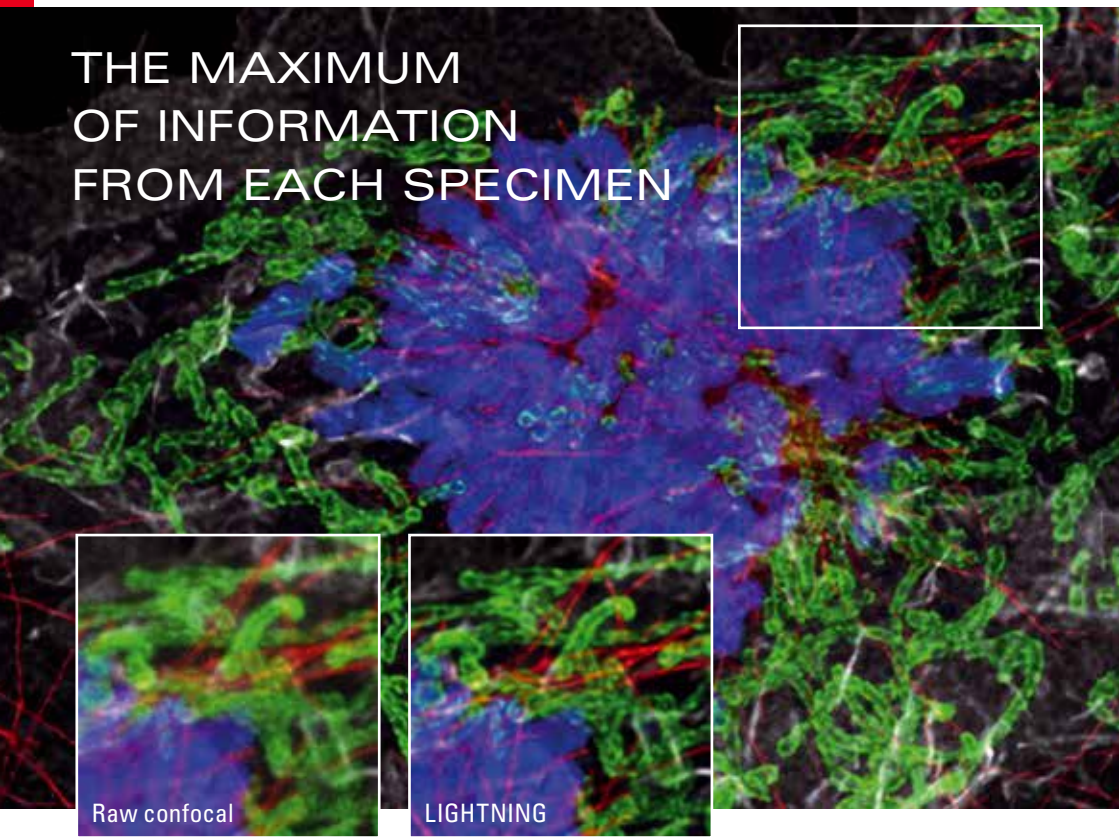

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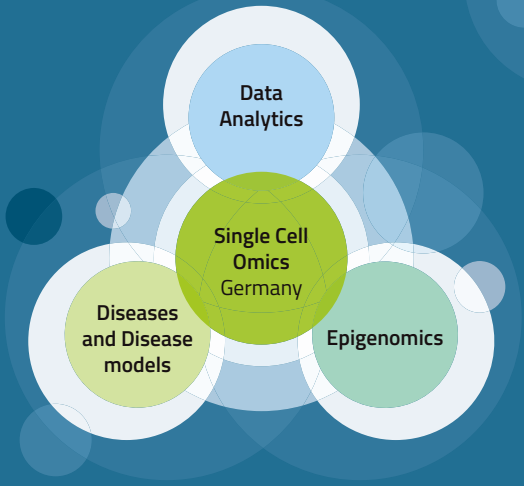
Connecting the German single-cell community with relevant international initiatives (e.g. the Human Cell Atlas and the International Human Epigenome Consortium), this network will help to address biomedical questions and foster translational research.



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