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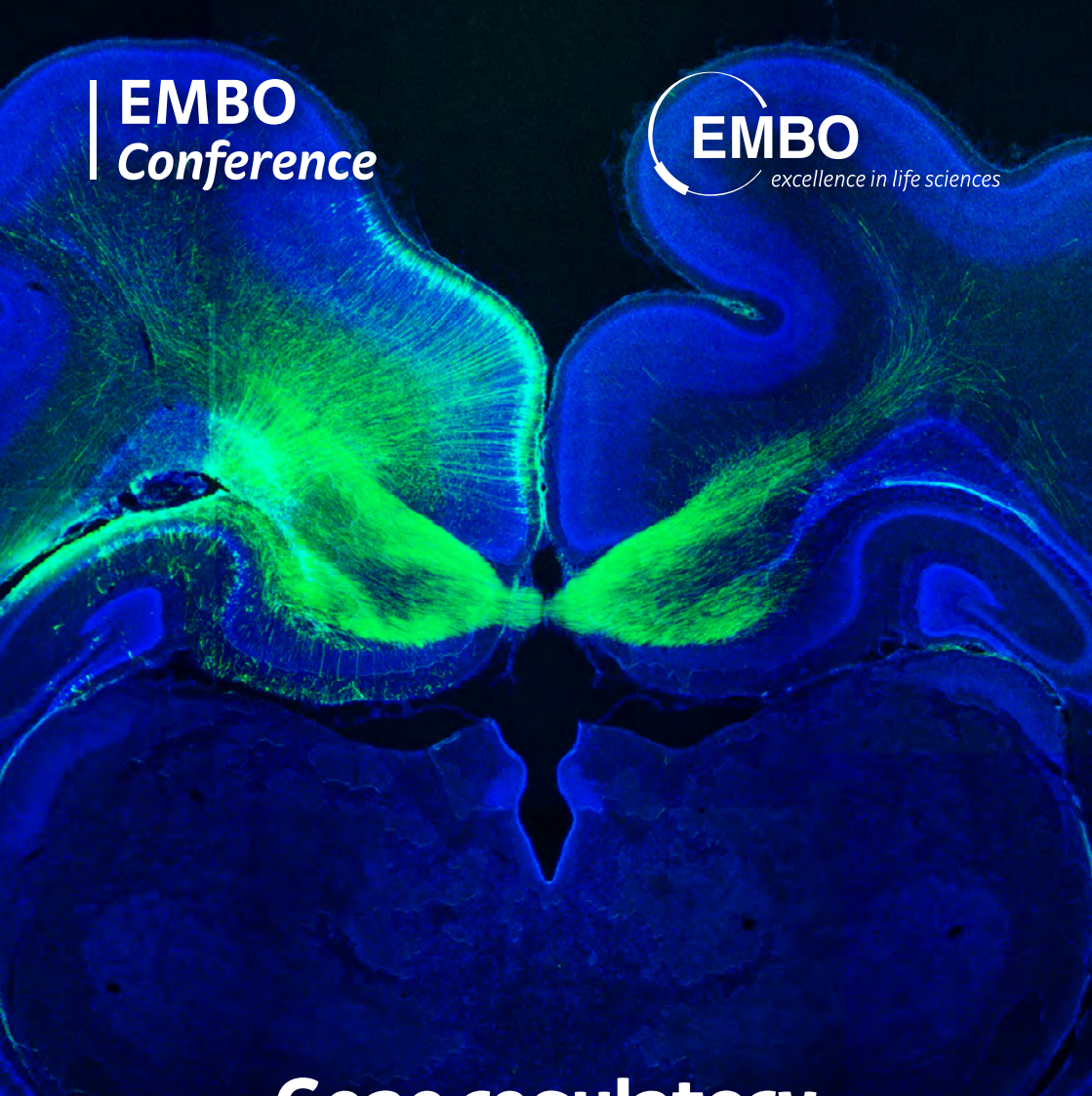

Gene regulatory mechanisms in neural fate decisions

San Juan de Alicante, Spain | 07 – 10 September 2017

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Organizers

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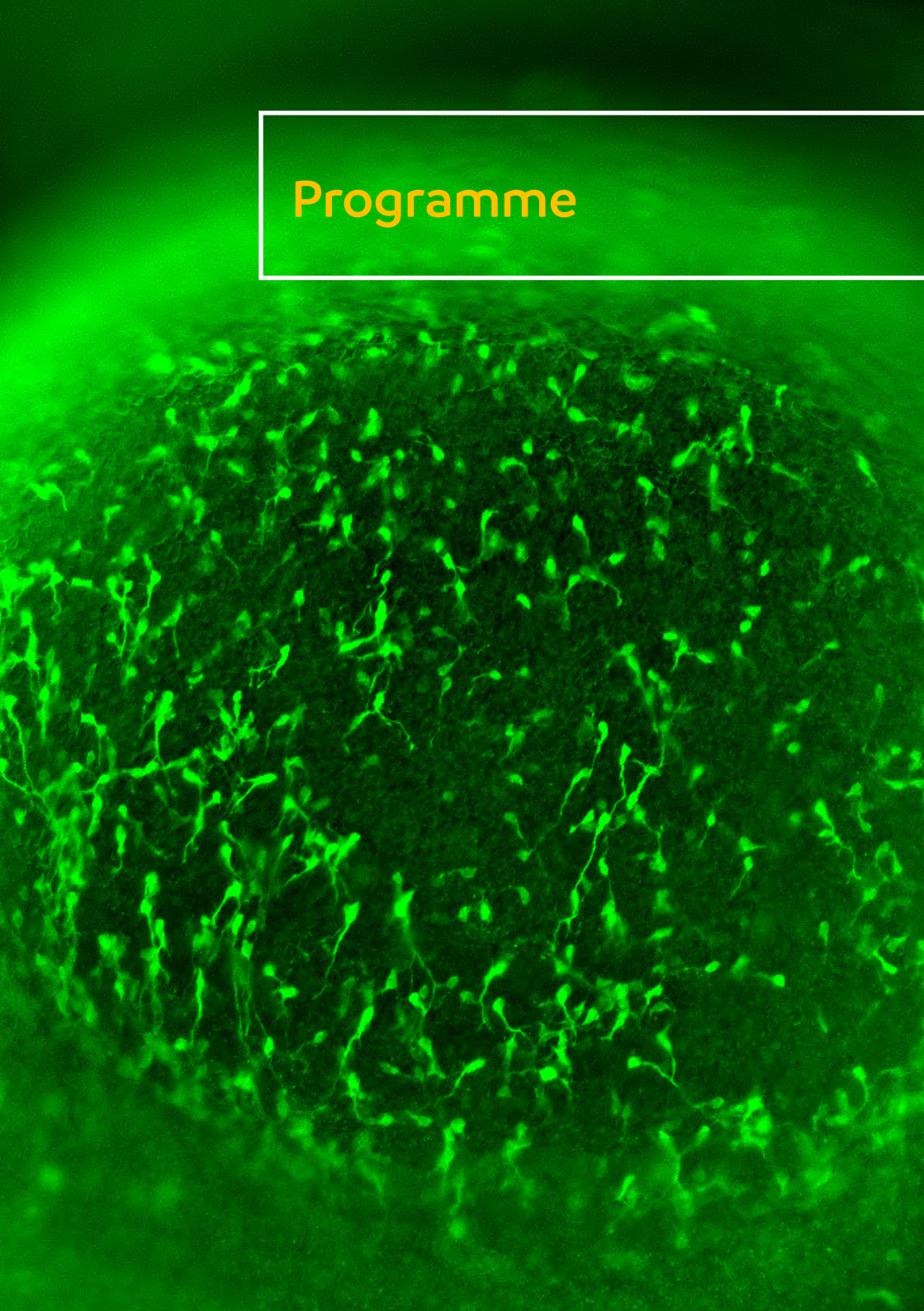
Programme



Programme

Day 1 – 7 September 2017

11:00 - 13:45 Registration

13:45 - 14:00 Welcome and Opening remarks

Session 1. Histone Modifications

Chair: Victor Borrell

14:00 - 14:30 *Epigenetic priming contributes to lineage-appropriate binding of transcription factors*
Gunnar Schotta, Biomedical Center, Munich, Germany

14:30 - 15:00 *Mechanisms regulating synapse-specific gene expression by HDAC2 in neurons*
Li-Huei Tsai, Massachusetts Institute of Technology, Cambridge, USA

15:00 - 15:30 *Regulation of embryonic and adult neural stem cell fate*
Yukiko Gotoh, University of Tokyo, Tokyo, Japan

15:30 - 16:00 *Epigenetic regulation of neuronal development and function*
Vijay Tiwari, Institute of Molecular Biology (IMB), Mainz, Germany

16:00 - 16:30 Short Talks:

Kdm5c prevents spurious transcription, silences germ line genes and modulates transcription of regulatory ncRNAs
Marilyn Scandaglia, Universidad Miguel Hernández-CSIC, Alicante, Spain

DOT1L and H3K79 methylation establish transmittable layer identity in progenitors of the cerebral cortex
Tanja Vogel, Institute for Anatomy and Cell Biology, Department of Molecular Embryology, Albert-Ludwigs-University Freiburg, Germany

16:30 - 17:00 Coffe break (Venue's Science Lounge) and informal discussion

Session 2. Modifications of DNA

Chair: Magdalena Götz

17:30 - 18:00 *Epitranscriptomic mechanism regulating mammalian cortical neurogenesis*
Hongjun Song, University of Pennsylvania, USA

18:00 - 18:30 *A role for endogenous retroviruses in human brain evolution*
Johan Jakobson, Wallenberg Neuroscience Center, Lund University, Lund, Sweden

18:30 - 19:00 *Gene regulation in chromatin*
Dirk Schübeler, FMI, Basel, Switzerland

19:00 - 19:30 *The Chromatin deacetylase SIRT6 drives early cell-fate decisions in embryonic development*
Raul Mostoslawsky, The Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA USA

19:30 - 20:00 *Transposable elements, their polydactyl controllers and the uniqueness of the human brain*
Didier Trono, EPFL, Geneva, Switzerland

20:00 - 20:30 *Epigenetic control of neural stem cells during corticogenesis*
Federico Calegari, CRTD, Dresden, Germany

20:30 - 22:00 Dinner and informal discussions

Day 2 – 8 September 2017

Session 3. Non-coding Genome

Chair: Francois Guillemot

09:00 - 09:30 *Long non-coding RNAs (lncRNAs) in neural development*
Dan Lim, UCSF, San Francisco, USA

09:30 - 10:00 *Molecular basis of synaptic plasticity*
Michael Kiebler, Ludwig-Maximilians-University, Munich, Germany

10:00 - 10:30 *Systematic identification of regulators of an alternative splicing regulatory network underlying nervous system development and autism spectrum disorders*

Benjamin J. Blencowe, University of Toronto, Toronto, Canada

10:30 - 11:00 *Bimodal gene regulation of neural progenitors by Nup153 in cooperation with Sox2*

Rusty Gage, Salk Institute for Biological Studies, La Jolla, USA

11:00 - 11:30 Short Talks:

miRNA-independent functions of Dgcr8 control mouse corticogenesis: implications for neocortex expansion

Davide De Pietri Tonelli, Neurobiology of microRNA lab, Istituto Italiano di Tecnologia, Genoa, Italy

Transcriptional and chromatin accessibility dynamics during status epilepticus

J. Fernàndez-Albert, Instituto de Neurociencias de Alicante (Universidad Miguel Hernández-CSIC), Alicante, Spain

11:30 - 12:00 Coffe break (Venue's Science Lounge) and informal discussion

Non-coding Genome Continues

12:00 - 12:30 *L1 retrotransposition in the mammalian brain*

Geoffrey J. Faulkner, University of Queensland, Brisbane, Australia

12:30 - 13:00 *Quantitative and dynamic analysis of neurogenesis with single cell resolution at the tissue level*

Nancy Papalopulu, The University of Manchester, Manchester, UK

13:00 - 13:30 *Chromatin regulation of cerebellar granule neuron maturation*

Anne West, Duke University, Durham, USA

13:30 - 14:00 *Choroid Plexus releases miR-204 regulating the number of neural stem cells in the subependymal zone*

Jovica Ninkovic, Helmholtz Zentrum München and LMU, Munich, Germany

14:00 - 15:00 Lunch (Venue's Social Lounge) and informal discussion

15:00 - 17:30 **Poster Session I –** Presenting odd numbers

16:30 - 17:30 Coffee in parallel to poster sessions

Session 4. Chromatin remodeling

Chair: Vijay Tiwari

17:30 - 18:00 *Shaping the Neuronal Epigenetic Landscape in development and disease by neuron-specific nBAF Complexes*
Jerry Crabtree, Stanford University, San Francisco, USA

18:00 - 18:30 *Chromatin as a hub integrating environmental signals and fate decisions in adult neural stem cells*
Ana Martin Villalba, DKFZ Heildeberg, Germany

18:30 - 19:00 *Role of the chromatin remodeller CHD7 in neurogenesis and brain diseases*
Haikun Liu, DKFZ Heidelberg, Germany

19:00 - 19:30 *Proliferation control, temporal identity and cell immortalization in Drosophila neural stem cell lineages*
Jurgen Knoblich, IMBA, Vienna, Austria

19:30 - 20:00 Short Talks:

Epigenetic and genetic programming of growth by modulating Zdbf2 dosage in the brain
Juliane Glaser, Institut Curie, PSL Research University, INSERM, CNRS, Paris, France

The POU homeobox gene BRN3A maintains the identity of multiple neuron types throughout the CNS
Esther Serrano-Saiz, Columbia University of New York

20:00 - 22:00 Dinner (Venue's Social Lounge) and informal discussion

Day 3 – 9 September 2017

Session 5. Transcription Factors

Chair: Federico Calegari

09:00 - 09:30 *Post-translational regulation of stem cell quiescence and activity in the adult brain*
Francois Guillemot, The Francis Crick Institute, London, UK

09:30 - 10:00 *What does the FOX say: Gene regulatory networks in the developing neocortex*
Soo-Kyung Lee, Oregon Health & Science University, Portland, USA

10:00 - 10:30 *Transcriptional dynamics in cortical development*
Verdon Taylor, University of Basel, Basel, Switzerland

10:30 - 11:00 *Uncovering spatiotemporal windows in cortical development*
Shubha Tole, Tata Institute of Fundamental Research, India

11:00 - 11:30 Short Talks:

Sox2 is required for global functional chromatin connectivity in brain-derived neural stem cells
Silvia Nicolis, Department of Biotechnology and Biosciences, University Milano-Bicocca, Milano, Italy

Identifying Novel Transcriptional Regulators of Neurogenesis
Angela Garding, Institute of Molecular Biology (IMB), Mainz, Germany

11:30 - 12:00 Coffe break (Venue's Science Lounge) and informal discussion

Transcription Factors continues

12:00 - 12:30 *Decoding the gene regulatory logic of neuronal cell type specification in C. elegans*
Oliver Hobert, Columbia University, New York, USA

12:30 - 13:00 *Dynamic transcriptional control of neural stem cells*
Ryoichiro Kageyama, Kyoto University, Kyoto, Japan

13:00 - 13:30 *Epigenetic regulation of aging neural stem cells*
Anne Brunet, Standford University, San Francisco, USA

13:30 - 14:30 Lunch (Venue's Social Lounge) and informal discussion

14:30 - 17:30 **Poster Session II** – Presenting even numbers

16:30 - 17:30 Coffee in parallel to poster sessions

Session 6. Distal gene regulation and nuclear organization

Chair: Li-Huei Tsai

17:30 - 18:00 *Chromosomal enhancer nuclear syntax: ligand-dependent 3D redistribution of "First Tier" enhancers generate mega-enhancers that associate with subnuclear phase-separated structures to dictate enhancer robustness*
Michael Rosenfeld, University of California, San Francisco, USA

18:00 - 18:30 *Gene regulation in development and evolution of the human cerebral cortex*
Christopher A. Walsh, Boston Children's Hospital, Boston, USA

18:30 - 19:00 *Genome Architecture Mapping: discovering chromatin contacts in rare cell types*
Ana Pombo, MDC, Berlin, Germany

19:00 - 19:30 Short Talks:

Gene network governing the commitment of radial glial cells to ependymal lineage
Stavros Taraviras, Department of Physiology, Medical School, University of Patras, Greece

Mechanisms of epigenetic priming that underpin changes in neuronal identity
Philipp Mews, Icahn School of Medicine at Mount Sinai, New York, USA

20:00 - 22:00 Dinner (Venue's Social Lounge) and informal discussion

Day 4 – 10 September 2017

Session 7. Single cell transcriptome and epigenome

Chair: Jovica Ninkovic

09:00 - 09:30 *Patterning principles in the early Drosophila nervous system development*
Robert Zinzen, MDC, Berlin, Germany

9:30 - 10:00 Short Talks:

Programming cell fates using a comprehensive human transcription factor library
Volker Busskamp, TU Dresden - Center for Regenerative Therapies

Systematic analysis of neural development in vivo
Christopher Esk, IMBA – Institute of Molecular Biotechnology, Vienna, Austria

10:00 - 10:30 *Polycomb proteins as topological facilitators of enhancer function during neural induction*
Alvaro Rada-Iglesias, University of Cologne, Cologne, Germany

10:30 - 11:00 *Modeling human brain development and disease at single cell resolution using brain organoids*
Giorgia Quadrato, Harvard University, Boston, USA

11:00 - 11:30 *Genome-wide analysis of DNA cis-regulatory element activity during neuronal specification of pluripotent stem cells*
Davide Cacchiarelli, TIGEM, Naples, Italy

11:30 - 12:00 Coffe break (Venue's Science Lounge) and informal discussion

Single cell transcriptome and epigenome continues

12:00 - 12:30 *Genomic insights into human cortical development*
Arnold Kriegstein, UCSF, San Francisco, USA

12:30 - 13:00 *Genetic evolution of cortical size determinants*
Victor Borrell, Instituto de Neurociencias, Alicante, Spain

13:00 - 14:30 Lunch (Venue's Social Lounge) and informal discussion

Session 8. Novel mechanisms. Molecular tools and approaches

Chair: Yukiko Gotoh

14:30 - 15:00 *Human-specific genes, neural stem cell amplification, and neocortex expansion in development and human evolution*
Wieland Huttner, MPI, Dresden, Germany

15:00 - 15:30 *Novel molecular mechanisms of neurogenesis*
Magdalena Götz, Helmholtz Zentrum, Munich, Germany

15:30 - 16:00 *Editing of neural epigenomes*
Stefan Stricker, Helmholtz Zentrum, Munich, Germany

16:00 - 16:30 Closing remarks by organizers

Session 1
Histone Modifications

Epigenetic priming contributes to lineage-appropriate binding of transcription factors

GUNNAR SCHOTTA [1,5]

*Cernilogar F.M. [1], Hasenöder S. [2,3], Burtscher I. [2,3], Scheibner K. [2,3], Wang Z. [1], Lickert H. [2,3,4]

[1] Ludwig Maximilians University, Biomedical Center, Munich, Germany.
[2] Helmholtz Zentrum München, Institute of Stem Cell Research, Neuherberg, Germany.
[3] Helmholtz Zentrum München, Institute of Diabetes and Regeneration Research, Business Campus Garching, Germany
[4] German Center for Diabetes Research (DZD), Germany
[5] Munich Center for Integrated Protein Science, Munich, Germany.

Pioneer transcription factors (PTFs) are key to induce cell fate changes during embryonic development and in cell reprogramming. A distinct feature to these transcription factors is that they can bind nucleosomal DNA. This can initiate a cascade of events, often leading to local chromatin opening, recruitment of other factors and, ultimately, enhancer activation. However, in each cell type PTFs bind only a subset of their potential genomic binding sites and only a portion of these binding events result in an active enhancer. This raises two important questions: 1) How are targets sites selected in distinct cell types? 2) What determines if a PTF binding will result in chromatin opening and enhancer activation? We addressed these questions by studying the transition from mouse ES cells to definitive endoderm cells, which strictly depends on the paradigm PTF Foxa2. We found that Foxa2 binding needs a permissive chromatin featured by low levels of active chromatin modifications. Furthermore we found that co-binding of Foxa2 with other transcription factors favors the local chromatin opening, an essential step for enhancer activation. Together, our results suggest that the presence of an appropriate chromatin state at a subset of PTF binding sites defines the developmental competence of progenitor cells.

NOTES:

Mechanisms regulating synapse-specific gene expression by HDAC2 in neurons

LI-HUEI TSAI

Hidekuni Yamakawa, Jemmie Cheng, Jay Penney, Fan Gao, Jun Wang, Satoko Yamakawa, Oleg Kritskiy, Richard Rueda, Elizabeth Gjoneska

Picower Institute for Learning and Memory, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, USA

The histone deacetylase HDAC2, which negatively regulates neuronal plasticity and synaptic gene expression, is upregulated both in Alzheimer's disease (AD) patients and mouse models. We previously showed that targeting HDAC2 using shRNA in the hippocampus of a mouse model of severe neurodegeneration rescued learning and memory deficits and markedly elevated synaptic density. Therapeutics targeting HDAC2 are speculated to be a promising avenue for ameliorating AD related cognitive impairment. Therefore, it is imperative to better understand the mechanism by which HDAC2 is specifically targeted to regulate synapse-specific genes in neurons. Here, we take a novel approach utilizing integrative genomics to identify proteins that mediate HDAC2 recruitment to synaptic plasticity genes. Functional screening of the top candidates revealed that knockdown of certain DNA binding proteins belonging to the HDAC2 complex are able to phenocopy HDAC2 knockdown, and that specific HDAC2 co-factors may facilitate the recruitment of HDAC2 to synaptic genes in neurons. Importantly, like HDAC2, expression of key members of the HDAC2 complex were elevated in AD patients and mouse models, where synaptic dysfunction can be ameliorated via knockdown of specific HDAC2 binding proteins. Furthermore, exogenous expression of an HDAC2 fragment containing the region necessary for binding to these HDAC2 co-factors fully restored synaptic plasticity and memory in a mouse model with severe neurodegeneration. Our findings indicate that targeting a specific HDAC2 complex could enhance synaptic and cognitive function, without affecting HDAC2 function in other processes.

NOTES:

Regulation of embryonic and adult neural stem cell fate

YUKIKO GOTOH

Department of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan

One of the fundamental questions in understanding tissue development is how multipotent progenitors/tissue stem cells give rise to various cell types in a defined order to achieve appropriate tissue organization. Neural stem/progenitor cells (NPCs) attract much attention since these cells give rise to neuronal and glial cell types in a developmental-stage dependent manner with striking precision. We have previously shown that polycomb group (PcG) complex and high mobility group A (HMGA) proteins play pivotal roles in driving fate switches of NSCs during neocortical development. I would like to talk about how these proteins control the fate of NPCs in a developmental stage-dependent manner. I would also like to discuss the mechanisms underlying long-term maintenance of adult neural stem cells and emergence of these cells during development.

This research was supported by AMED-CREST from Japan Agency for Medical Research and Development, AMED and JSPS KAKENHI Grant Numbers JP15H05773, JP16H06481.

NOTES:

Epigenetic regulation of neuronal development and function

VIJAY TIWARI

Institute of Molecular Biology (IMB), Mainz, Germany

Cell-fate specification during mammalian development involves stable resetting of transcriptional programs and the role of chromatin-mediated regulation in this process has been increasingly appreciated. The nervous system is the most complex organ in all mammalian organisms. The last decade has observed extensive research in understanding how this complexity is generated during neuronal development. Despite exciting progress, very little is known about the function of epigenetic mechanisms in neurogenesis and neuronal activity. Using epigenetics, genomics and molecular biology tools in combination with extensive computational biology approaches we are investigating i) the crosstalk of transcription factors with chromatin during specification of neuronal fate, ii) dynamics of chromatin accessibility during neurogenesis and iii) the function of novel epigenetic regulators during neuronal development. In addition, we are also investigating whether neuronal- activity-dependent gene regulation involves epigenetic reprogramming.

NOTES:

SHORT TALK

Kdm5c prevents spurious transcription, silences germ line genes and modulates transcription of regulatory ncRNAs

MARILYN SCANDAGLIA[1]

Jose Pascual Lopez-Atalaya[1], Alejandro Medrano-Fernandez[1], Maria Teresa Lopez-Cascales[1], Beatriz del Blanco[1], Michal Lipinski[1], Eva Benito[1, 4], Roman Olivares[1], Shigeki Iwase[2], Yang Shi[3], Angel Barco[1]

[1] Instituto de Neurociencias de Alicante (Universidad Miguel Hernández-CSIC), Alicante, Spain
[2] University of Michigan, 5815 Medical Science II, Ann Arbor, MI, USA
[3] Boston Children's Hospital and Harvard Medical School, Boston, MA, USA
[4] Present address: Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), c/o European Neuroscience Institute, Göttingen, Germany

Mutations in the lysine demethylase 5C gene (KDM5C) cause a rare X-linked intellectual disability disorder. This enzyme demethylates histone H3 di- or trimethylated at lysine 4 (H3K4me2/3), two histone modifications associated with active transcription. To investigate the differential role of KDM5C in developing and adult brain, we compared the behaviour, transcriptome and epigenomic landscapes of Kdm5c null (KO) and forebrain-restricted inducible knockout (ifKO) mice. KOs showed strong neurological phenotypes mimicking patients' symptoms such as decreased learning and memory and increased emotional responses. In contrast, ifKOs were mildly affected in behavioural performance, suggesting a predominant developmental component in KDM5C-associated intellectual disability. Consistent with the proposed general function for Kdm5c as a transcriptional repressor, both KO and ifKO mice showed excessive hippocampal transcription and histone H3K4 tri-methylation at promoters and enhancers. Interestingly, both strains exhibited events of spurious transcription coinciding with local increases of H3K4me3 in non-canonical and cryptic TSSs suggesting an instructive role for this histone mark on transcriptional activation. Furthermore, conventional KOs also showed (i) neuronal expression of germ line genes that escaped developmental silencing by DNA methylation, and (ii) over-activation of activity-regulated enhancers involved in cognitive processes, such as those regulating the Npas4, Arc and Fos loci. In summary, we suggest that Kdm5c plays a critical role during development both as an epigenetic repressor and as a fine-tuner of enhancers. Although the importance of these functions declines after neuronal maturation, Kdm5c still retains a genome surveillance role preventing the illegitimate activation of non-neuronal and cryptic promoters in adult neurons.

SHORT TALK

DOT1L and H3K79 methylation establish transmittable layer identity in progenitors of the cerebral cortex

TANJA VOGEL[1]

Henriette Franz[1], Alejandro Villarreal[1], Rolf Backofen[2], Thomas Manke[3], et al.

[1] Institute for Anatomy and Cell Biology, Department of Molecular Embryology, Medical Faculty, Albert-Ludwigs-University Freiburg, Germany

[2] Bioinformatics Group, Department of Computer Science, Albert-Ludwigs-University Freiburg, Germany

[3] Max Planck Institute of Immunobiology and Epigenetics, Germany

Cortical development is controlled by transcriptional programs that are orchestrated by activation of key determinants such as transcription factors. Stable inheritance of temporo-spatial activity of factors influencing cell fate and regionalization in different layers is only partly understood. We show that deletion of the Disruptor of telomeric silencing 1 (Dot1l) in the murine telencephalon leads to cortical layering defects. DOT1L activity and chromatin methylation at H3K79 control the cell cycle by preventing premature differentiation through activation of expression of genes regulating asymmetric cell division. In addition, DOT1L and H3K79 methylation transmit cell fate as well as layering information from early to late progenitors. Specifically, DOT1L establishes transcriptional programs characteristic for upper layer neurons already at an early developmental stage, and thereby consolidates restricted cell fate of late cortical progenitors. Further, DOT1L activates transcription of the Sox gene family, suggesting a conserved mechanism, by which H3K79me2 replaces the inactivating H3K9me3 at Sox gene promoters to activate their expression during neural differentiation.

NOTES:

NOTES:

Session 2
Modifications of DNA

Epitranscriptomic mechanism regulating mammalian cortical neurogenesis

HONGJUN SONG

University of Pennsylvania, USA

In the embryonic mouse cortex, radial glia cells (RGCs) function as neural stem cells, sequentially giving rise to neurons residing in different cortical layers and then switching to glial production before birth. Such a precise and predictable developmental schedule requires a highly coordinated genetic program. Previous studies have revealed transcriptional cascades that orchestrate the dynamics of mammalian cortical neurogenesis. Modified nucleotides in mRNAs were initially discovered over 40 years ago, but little was known about the extent, transcript identities, and potential functions of various reversible chemical modifications until very recently. New high-throughput sequencing approaches have revealed a dynamic "epitranscriptome" landscape for many mRNA modifications in various organisms from yeasts to humans, including N6-methyladenosine (m^6A), N1-methyladenosine (m^1A), 5-methylcytosine (m^5C), 5-hydroxymethylcytosine (hm^5C), pseudouridine (**Ψ**), and 2'-O-methylnucleotides. Among these modifications, m^6A is the most abundant internal modification in mRNAs of eukaryotic cells. Recent discoveries of widespread mRNA chemical modifications raise the question of whether this mechanism plays any regulatory role in cortical neurogenesis. Identification of the molecular machinery mediating m^6A mRNA methylation provides an entry point to explore physiological functions of this pathway in vivo. I will present our latest results from investigating the role and underlying mechanism of m^6A during cortical neurogenesis using mouse and human forebrain organoid models.

NOTES:

A role for endogenous retroviruses in human brain evolution

JOHAN JAKOBSSON

Wallenberg Neuroscience Center, Lund University, Lund, Sweden

The complexity of human brain development differs markedly from other mammals and is thought be important for the emergence of higher cognitive functions. However, the precise genetic changes, as well as the existence of human-specific gene regulatory networks underlying the evolution of the human brain remains poorly explored. Most of our knowledge about human brain development is restricted to evolutionary conserved developmental pathways, while much less is known about primate- and human-specific developmental mechanisms. Identification of novel mechanisms that regulate human brain development is important for our understanding of the human brain and may also provide new links to the biology of human brain disorders.

About 8% of the human genome is composed of endogenous retroviruses (ERVs). These sequences are derived from retroviruses that have invaded vertebrate hosts for millions of years leaving traces as inherited ERVs through germ line infection and subsequent transposition. Several studies have found that ERV transcription is tightly controlled at multiple levels in early human development and they have been proposed to participate in the control of gene regulatory networks.

We have found a region- and developmental stage-specific expression pattern of ERVs in the developing human brain, which is linked to a transcriptional network based on ERVs. Several thousand ERVs, many that are primate-specific, act as docking platforms for the epigenetic co-repressor protein TRIM28, which results in the establishment of local heterochromatin around these ERVs. This repressive transcriptional network modulates expression of protein-coding transcripts important for brain development, thereby providing an additional layer of transcriptional regulation. Our findings open up for several exciting future studies on the role of ERVs as potential drivers of human brain evolution, their contribution to individual variation and the implication in human brain disorders.

NOTES:

Gene regulation in chromatin

DIRK SCHÜBELER

Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

How is chromatin and DNA methylation involved in gene regulation? We are using mouse stem cells and their differentiation into neurons as a model to monitor the epigenome and its dynamics in an unbiased way and to identify its dependency on DNA sequence. Our goal is to generate regulatory, which we test in cellular models by genetic perturbation and genome editing approaches.

To create functional genomic binding maps we use a biotin tagging approach that we utilized to map all MBD domain proteins and a set of isoforms (Baubec et al., Cell 2013). We further determined chromosomal binding and site-specific activity of the mammalian de novo DNA methyltransferases DNMT3A and DNMT3B. While binding explains sites of enzymatic activity, DNMT3B furthermore preferentially methylates active genes. This targeting requires SETD2-mediated methylation of lysine 36 on histone H3 revealing reveal how sequence and chromatin cues guide de novo methyltransferase activity to ensure methylome integrity (Baubec et al. Nature 2015).

In order to identify transcription factors that are sensitive to DNA methylation we have mapped DNaseI hypersensitive sites that change upon global loss of DNA methylation. Among others this identified NRF1 as a TF that occupies many additional sites in the unmethylated genome. Importantly, restoring de novo methyltransferases initiates remethylation at these sites and ablates NRF1 binding. This illustrates that binding of DNA methylation-sensitive transcription factors critically depends on other factors to induce local hypomethylation (Domcke, Bardet et al., Nature 2015).

These results highlight the interplay and dynamics between the machineries that set and read chromatin and TF readout.

NOTES:

The Chromatin deacetylase SIRT6 drives early cell-fate decisions in embryonic development

RAUL MOSTOSLAVSKY

The Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA USA

Chromatin factors have emerged as key regulators of early embryonic differentiation, providing unique driving roles to establish cell fate identity at these steps of embryonic development. Recently, we discovered that the mammalian histone deacetylase SIRT6 is a key chromatin factor, with roles in DNA repair, metabolism and cancer (Zhong et al, 2010; Sebastian et al., 2012; Kugel et al., 2016). At the cellular level, SIRT6 directly regulates expression of several key glycolytic and ribosomal genes, co-repressing Hif1a and Myc and acting as a histone H3 lysine9 (H3K9) and lysine 56 (H3K56) deacetylase to inhibit expression of their target genes. In recent studies, we identified SIRT6 as a critical modulator of early embryonic differentiation, acting as a robust co-repressor of pluripotent genes, and modulating stem cell fate in a TET-dependent manner. In the absence of SIRT6, failure to repress pluripotent genes drives ES cells towards the neuroectoderm lineage, as a consequence of increased Tet-dependent 5-OHMeC levels in neuro-development genes Etchegaray et al., 2015). Strikingly, we recently identified the first human syndrome with a SIRT6 mutation, where defective neuro- and cardiac development caused perinatal lethality. Results from these studies will be discussed at the meeting.

Zhong, L., et al., and **Mostoslavsky, R.** (2010). The histone deacetylase SIRT6 regulates glucose homeostasis via Hif1a. ***Cell***, 140, 280-293.

Sebastian, C., et al. and **Mostoslavsky, R.** (2012). The Histone Deacetylase SIRT6 is a novel tumor suppressor that controls cancer metabolism. ***Cell*** 151, 1185-1199.

Etchegaray., et al. and **Mostoslavsky, R.** (2015). The histone deacetylase SIRT6 controls embryonic stem cell fate via TET-mediated production of 5-hydroxymethylcytosine. ***Nature Cell Biol.*** 17, 545-557.

Kugel et al., and **Mostoslavsky, R.** (2016). SIRT6 suppresses pancreatic cancer through control of Lin28b. ***Cell*** 165, 1401-1415.

Transposable elements, their polydactyl controllers and the uniqueness of the human brain

DIDIER TRONO

Ecole Polytechnique Fédérale de Lausanne (EPFL), School of Life Sciences, Lausanne, Switzerland

Transposable elements (TEs), which probably account for at least two-thirds of the human genome, are subjected to epigenetic control through sequence-specific recognition by KRAB-containing poly-zinc finger proteins (KZFPs). KZFPs constitute the largest group of transcription factors encoded by higher vertebrates, and act by recruiting inducers of heterochromatin and, for some, other modulators. Owing to the continuous spread of TEs during evolution, these mobile elements exhibit great degrees of lineage- and species-specificity, and correspondingly their KZFP ligands display very limited inter-species orthology. I will present evidence demonstrating that TEs and KZFPs partner up to establish species-restricted regulatory networks that fundamentally influence a wide range of biological processes and seem to play key roles in the development and physiology of the human brain.

NOTES:

Epigenetic control of neural stem cells during corticogenesis

FEDERICO CALEGARI [1]
Florian Noack [1], Martin Schneider [2], Abhijeet Pataskar [3], Vijay Tiwari [3], Frank Buchholz [2]

[1] DFG-Research Center for Regenerative Therapies, Cluster of Excellence, TU-Dresden, Germany
[2] Medical Systems Biology, UCC, Medical Faculty Carl Gustav Carus, TU Dresden, Germany.
[3] Institute of Molecular Biology, Mainz, Germany
*Correspondence: federico.calegari@crt-dresden.de

During development, the switch of neural stem cells (NSC) from proliferation to differentiation establishes the shape and size of the adult brain. To unravel the role of epigenetic marks in the switch to neurogenesis, we isolated proliferating NSC, neurogenic progenitors and newborn neurons during mouse cortical development and assessed their DNA (hydroxy-)methylation signatures. Interestingly, cell type-specific differential methylation was found enriched in neurogenesis-related genes and bHLH transcription factor binding motifs. We next sought to site-specifically manipulate DNA methylation to assess the role of epigenetic marks in programming and reprogramming of NSC by Cas9-mediated delivery of DNA methyltransferase (DNMT3a) or Tet methylcytosine dioxygenase (Tet1) in developing mouse embryos by in utero electroporation. This approach allowed us to site-specifically manipulate DNA methylation and validate the role of locus-specific epigenetic marks in gene expression and reprogramming of NSC in vivo.

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Session 3
Non-coding Genome

Long non-coding RNAs (lncRNAs) in neural development

DAN LIM

UCSF, San Francisco, USA

The mammalian genome produces many thousands of lncRNAs – transcripts >200 nucleotides long that do not code for protein, and it is now clear that certain lncRNAs have critical biological functions and roles in human neurological disease. Both lncRNA knockdown and more recent conditional genetic knockout methods indicate that one particular lncRNA called Pnky has key roles in the regulation of neurogenesis from neural stem cells in the embryonic and postnatal brain. Single cell sequencing of neural precursors from the developing human brain indicate that many lncRNAs are abundantly expressed in specific cell types including radial glia. In a more recent large scale CRISPR interference (CRISPRi) based screen, we have identified several hundred lncRNAs that have essential roles in cell proliferation including that of glioblastoma multiforme (GBM).

Surprisingly, lncRNAs were found to have exquisitely cell-type specific function. We are continuing to determine the function of many of these lncRNAs and the molecular mechanisms by which they regulate neural development and brain tumors.

NOTES:

The molecular basis of synaptic plasticity

MICHAEL A KIEBLER

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Targeting of mRNAs to synapses and the subsequent regulation of local synaptic translation are essential for hippocampal synaptic plasticity and for learning and memory. A set of RNA-binding proteins (RBPs) critically contributes to these processes, however, the precise machinery and the underlying mechanisms involved in dendritic mRNA localization remain poorly understood.

In my talk, I would like to present several independent lines of investigation that we are currently exploring to elucidate the role of the double-stranded RBP Stau2 at the molecular, the cellular and at the organismic level. On the molecular level, we benefit of having recently identified physiologically relevant target mRNAs for Stau2 (Heraud-Farlow et al., 2013, Cell Reports). Amongst them is a set of transcripts including (1) Rgs4 (regulator of small G protein signaling) whose protein products all cluster in the signaling pathway of G-protein coupled receptors and (2) an intron-containing isoform of Calm3 (calmodulin 3) in its 3'-UTR. To delineate the *in vivo* role of Stau2, we generated – in collaboration with Dusan Bartsch (Mannheim) a transgenic rat model, in which Stau2 expression is conditionally silenced by Cre-inducible expression of a miRNA targeting Stau2 mRNA in adult forebrain neurons. Interestingly, this transgenic rat revealed synaptic strengthening upon stimulation together with deficits in hippocampal spatial working memory, spatial novelty detection and in associative learning for operant rewards. Together, our findings provide new insight into how Stau2 contributes to learning and memory.

NOTES:

Systematic identification of regulators of an alternative splicing regulatory network underlying nervous system development and autism spectrum disorders

BENJAMIN J. BLENCOWE[1,2]
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Alternative splicing generates vast transcriptomic and proteomic complexity and is especially prevalent in the mammalian nervous system. Previously, we discovered nSR100/SRRM4, a neuronal and vertebrate-specific splicing regulator that activates a highly conserved program of 3-27 nt microexons (1,2) as well as longer neuronal exons. Knockout of nSR100 disrupts nervous system development, in part by causing the skipping of a neural-specific exon that normally silences REST, a transcriptional repressor of neurogenesis genes (3,4). Reduced levels of nSR100 and its target microexon program are detected in the brains of approximately one-third of analyzed human subjects with autism spectrum disorders (ASD) (2). Remarkably, mice haploinsufficient for nSR100 recapitulate microexon disruption and display multiple hallmark ASD-like features, including altered social behaviours (5). These observations suggest that pharmaco-modulation of nSR100 may represent a strategy for the treatment of a substantial fraction of autism cases. However, the molecular pathways that converge on nSR100 to regulate its activity have not been systematically explored.

To address this, we have developed genome-wide CRISPR-Cas9 screens to identify genes that impact the splicing levels of nSR100-dependent microexons. Neural cell lines were engineered to stably express microexon splicing reporters. Genome-wide CRISPR knock-out and activation libraries were introduced in these cell lines. Dozens of genes were identified that positively or negatively regulate microexon splicing. The CRISPR screens successfully captured known regulators (e.g. nSR100 and PTBP1) as well as many new and unexpected factors, including RNA-binding, chromatin and post-translational regulators. Affinity purification-mass spectrometry and BioID experiments demonstrated that several of these factors physically interact with – or are proximal to – nSR100 in cells. Moreover, independent validation of microexon splicing by knockdown followed RNA-Seq analysis revealed that some

of the regulators extensively affect microexons and other neural exons, impacting many genes that function in synaptic transmission. Remarkably, genes identified as regulators in our CRISPR-Cas9 screens have also been identified recently in large-scale genome sequencing projects as ASD-risk genes.

In summary, we demonstrate that CRISPR-Cas9 genetic screens coupled to complementary methods facilitate the definition of comprehensive networks of factors that impact alternative splicing events of interest. Our application of these technologies to the analysis of nSR100-dependent microexons has broadened our knowledge of the mechanisms underlying the regulation of a critical neuronal splicing network that is widely disrupted in ASD. These results further provide insight into possible targets for the pharmaco-modulation of nSR100.

1. Calarco et al. Cell, 138: 898-910 (2009)
2. Irimia et al. Cell 159, 1511–1523 (2014)
3. Raj et al. Mol Cell. 43: 843-850 (2011)
4. Quesnel-Vallieres et al. Genes and Dev. 29: 746-759 (2015)
5. Quesnel-Vallieres et al. Mol Cell 64, 1023-1034 (2016)

NOTES:

Bimodal gene regulation of neural progenitors by Nup153 in cooperation with Sox2

RUSTY GAGE

Tomohisa Toda and Fred H. Gage

The Salk Institute for Biological Studies, La Jolla, California, USA

Although a number of key transcription factors for the maintenance of neural progenitor cells (NeuPCs) have been identified, how nuclear structural proteins, which are intimately linked to cell type-specific gene regulation, are involved in the maintenance of NeuPCs remains poorly defined. Here, we report that the nucleoporin Nup153 interacts with the transcription factor Sox2 in adult NeuPCs. Nup153 is enriched in NeuPCs, is indispensable for maintaining NeuPCs and is instrumental for cell fate specification. Genome-wide analyses revealed that Nup153 and Sox2 bind and co-regulate hundreds of downstream genes. Unexpectedly, we found that Nup153 exhibits binding position-dependent gene regulation in which Nup153 differentially regulates gene expression when binding to the promoter region or to the transcription end site. Together, these data reveal a novel layer of gene regulation by the nucleoporin in cooperation with a key transcription factor for the regulation of specific cell fate.

NOTES:

SHORT TALK

miRNA-independent functions of Dgcr8 control mouse corticogenesis: implications for neocortex expansion

DAVIDE DE PIETRI TONELLI

Nadin Hoffmann, Federica Marinaro

Neurobiology of microRNA lab, Istituto Italiano di Tecnologia, Genoa, Italy

The neocortex is the brain region that has undergone greatest enlargement during evolution in primates. The evolutionary expansion of the neocortex is based on differences in Neural Progenitor Cell (NPC) biology. Of note the basal (or intermediate) progenitor (bIP) hypothesis proposes that evolutionary neocortical expansion may be due to an increase in the genesis of bIPs, mostly owing to a substantial change in their mode of division and control in their proliferation-differentiation fate. The molecular mechanisms at the base of the precise coordination of cell proliferation-differentiation process of bIPs in evolution are still largely unknown.

MicroRNAs (miRNAs) are small, single-stranded, regulatory non-coding RNAs that play a key role in post-transcriptional control of gene expression in cortical development. The biogenesis pathways of miRNAs and their core components are well characterized. Recent evidence indicates that some of the components of miRNA biogenesis machinery, especially the Microprocessor moieties DROSHA and DGCR8 (encoded by the Di George Critical Region gene 8), exert also miRNA-independent gene silencing functions (Marinaro et al., EMBO Reports, 2017).

In the present study, we found that sustained expression of dgcr8 in the dorsolateral telencephalon of embryonic day E12.5 mouse embryos, by In utero electroporation, led to a significant expansion of Tbr2+ biP and to a two-fold decrease in the proportion of Tbr1 positive neurons at E14.5. Identification of downstream targets of Dgcr8 is in progress.

Our results open the possibility that Dgcr8-dependent miRNA-independent control of gene expression might have played a role in the evolutionary expansion of the mammalian neocortex.

NOTES:

SHORT TALK

Transcriptional and chromatin accessibility dynamics during status epilepticus

J. FERNÀNDEZ-ALBERT

Michal Lipinski, M.Teresa López-Cascales, A. Barco

Instituto de Neurociencias de Alicante (Universidad Miguel Hernández-CSIC), Alicante, Spain

It is well known that pharmacologically evoked status epilepticus (SE) causes transcriptional bursting in the hippocampus. This robust induction of gene expression correlate with specific histone posttranslational modifications and changes of the nuclear structure of hippocampal neurons. These changes are thought to cause long-lasting transcriptional deregulation and contribute to circuital dysfunction in epilepsy, although it remains unclear how these genomic events participate in the pathoetiology of epilepsy. Here, we combined the genetic tagging of neuronal nuclei and ribosomes with several high-throughput sequencing methods to conduct the first multidimensional analysis of transcriptional, epigenetic, and DNA accessibility changes occurring in hippocampal principal neurons upon SE. Hundreds of transcripts involved in neuroplasticity, including both protein-coding mRNAs and non coding RNAs of different types, were upregulated in hippocampal neurons upon SE. These changes were consistently associated with chromatin opening spilling over the gene limit, pinpointing the participation of structural proteins that topologically delimit locus responsiveness. Chromatin opening was strongly linked to the binding of transcription factors of the AP1, SRF and CREB families whose activation is known to follow neuronal depolarization, and to covalent histone modifications. Overall, our genomic screens provide a novel and essential insight into the genomic impact of epileptic seizures on the neuronal function.

NOTES:

L1 retrotransposition in the mammalian brain

GEOFFREY J.FAULKNER

Mater Research Institute - University of Queensland, TRI Building, Australia.

Queensland Brain Institute, University of Queensland, Brisbane, Australia.

The retrotransposon LINE-1 (L1) is a mobile genetic element that comprises ~17% of the human genome. L1 retrotransposition, a "copy and paste" process, can occur in the germline, generating new L1 insertions and allowing L1 to increase its genomic copy number over evolutionary time. In recent years, L1 retrotransposition has also been demonstrated to occur in tumour cells, the developing embryo, during neurogenesis, and in post-mitotic neurons. In this talk, I will present our experimental data showing that endogenous L1 mobilisation can occur in the pluripotent cells of the developing embryo, leading to germline and somatic mosaicism. As embryogenesis proceeds, L1 activity becomes spatiotemporally restricted, and appears to occur mainly in the neuronal lineage, and to a lesser extent in glia. These data show that L1 mosaicism found in neurons (by single-cell genomics, deep sequencing of "bulk" DNA obtained from brain tissue and engineered L1-EGFP reporter assays) begins in the early embryo, continues during neurogenesis and, it appears, in mature neurons. Although a consensus view has formed that L1 mosaicism occurs in the brain, the key question of neurobiological impact in healthy and disease contexts remains unanswered.

NOTES:

Quantitative and dynamic analysis of neurogenesis with single cell resolution at the tissue level

NANCY PAPALOPULU
Cerys Manning, Veronica Biga, Ximena Soto, Emma Johns, James Boyd

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The University of Manchester, UK

In recent years, our understanding of how cells make cell state transitions has been transformed by the application of single cell molecular and imaging technologies. This is well exemplified by the dynamic, short-period (ultradian), oscillations of gene expression described for the Hes family transcription factors in neural development. Such oscillations are thought to maintain neural progenitor cells in a proliferative state and further evidence suggests that the duration of such oscillations may time the onset of differentiation. Most of these studies have been done in cultured dissociated cells and it is not clear how these single cell dynamics, which are on the whole asynchronous, are integrated at the tissue level.

Here, we take a quantitative and dynamic approach to analyse single-cell heterogeneity in Hes expression at the tissue level. We use two systems, the developing mouse spinal cord in ex-vivo live spinal cord sections and the zebrafish hindbrain in whole live embryos. In the mouse we describe two broad progenitor domains of Hes5 expression, a ventral domain (p0-pMN) that generates motorneurons and ventral interneurons (E9.5-E11.5) and an additional dorsal interneuron domain (dP1-2) appearing at E10.5. Direct protein quantitation using fluorescence correlation spectroscopy (FCS) in single cells in live embryonic spinal cord sections shows that Hes5 expression has a quantifiable 10-fold range within each expression domain. We report that the variability between cells is spatially encoded as the ventral domain has higher cell-to-cell variability than the dorsal domain, resulting in spatially distinct Hes5 expression dynamics in neural progenitor cells along the dorso-ventral axis. Live imaging of Hes5 protein dynamics ex-vivo suggests that the increased cell-to-cell variability in the ventral domain is due to higher fluctuations of Hes5 expression in this domain. Within each domain populations with distinct dynamics are characterised by hierarchical clustering and can be related back to the cell position and division properties. This positional organisation is observed in zebrafish as revealed by Her6 dynamic analysis obtained from live knock-in zebrafish, generated by CRISPR/HR. Although oscillations are on the whole asynchronous, surprisingly, our analysis also reveals a spatial "micro-pattern".

These findings allow insight into the single cell heterogeneity and organisation of neural tissue. We hypothesise that the degree of dynamic and fluctuating Hes/Her gene expression relates to spatial rate of neural differentiation.

Chromatin regulation of cerebellar granule neuron maturation

ANNE WEST

Fang Liu, Christopher L. Frank, Ranjula Wijayatunge, Urann Chan, Gregory E. Crawford

Department of Neurobiology, Duke University Medical Center, Durham, NC USA

Cellular differentiation requires the precise spatial and temporal orchestration of gene expression programs. Temporal control of gene transcription is particularly important in postmitotic neurons of the postnatal brain. These neurons undergo extensive changes in their intrinsic gene expression programs as they mature, and they must coordinate the timing of these intrinsic transcriptional programs to match critical periods of sensory-driven brain development. During early organismal development, epigenetic regulation of genomic DNA and its associated histone proteins, collectively called chromatin, is known to play a role in establishing cell-type specific programs of gene expression. However to what degree chromatin regulatory mechanisms are repurposed for the control of gene expression during the maturation of postmitotic neurons remains to be fully understood. To discover chromatin mechanisms of neuronal differentiation, and to determine how these mechanisms are regulated across multiple stages of neuronal maturation, we have investigated regulation of chromatin accessibility, enhancer activation, and gene expression in differentiating cerebellar granule neurons (CGNs) of the postnatal mouse cerebellum in vivo. We have observed that thousands of regulatory elements show chromatin accessibility changes as CGNs differentiate, we have verified that many of these differentially accessible regions function as developmental stage-specific enhancers of neuronal genes, and we have identified the Zic family of transcription factors as key mediators of enhancer activation in CGNs. Furthermore we have identified a specific subgroup of the genes selectively expressed in mature CGNs of the cerebellum that are repressed by histone H3 lysine 27 trimethylation (H3K27me3) at early postmitotic stages of CGN differentiation yet lose this mark as CGNs mature via a mechanism that involves the histone demethylase Kdm6b. Taken together these studies offer new insight into the chromatin mechanisms that coordinate the temporal regulation of gene expression programs in a single neuronal cell type over the full course of differentiation.

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Choroid Plexus releases miR-204 regulating the number of neural stem cells in the subependymal zone

JOVICA NINKOVIC[1, 4]

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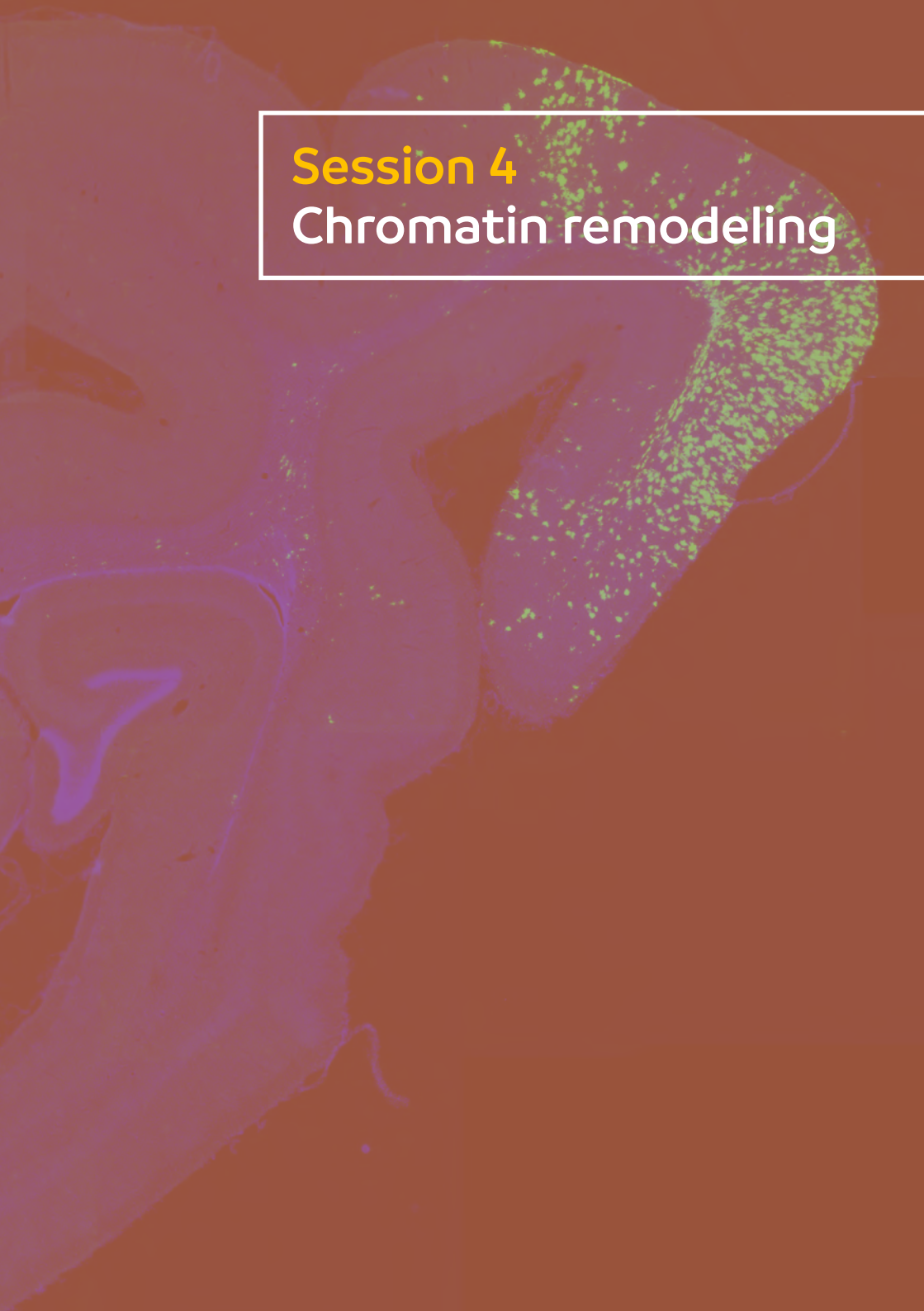
Regulation of adult neural stem cell (NSC) number is particularly important as NSCs deplete with age due to limited self-renewal. We identified here translational control, involving the microRNA miR-204, as a major mechanism regulating the maintenance of quiescent (q)NSCs and therefore life-long neurogenesis. Mir-204 regulates the translation of a spectrum of transcripts involved in cell cycle control, neuronal migration and differentiation in the qNSCs. Most strikingly, we could identify the choroid plexus (ChP) of the lateral ventricle as the major source of miR-204 that is released within exosomes into the cerebrospinal fluid and taken up by NSCs within the subependymal zone (SEZ). Importantly, ChP-specific knockdown of miR-204 reduced the number of qNSCs in the SEZ. Taken together, our results describe a novel mechanism to maintain adult somatic stem cells in their specific niche via secreted microRNAs.

NOTES:



Session 4
Chromatin remodeling

Shaping the Neuronal Epigenetic landscape in development and disease by neuron-specific nBAF Complexes

JERRY CRABTREE

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During the transition from pluripotency to multipotent neural stem cells to post mitotic neurons the BAF chromatin remodeling complex changes subunit composition, proceeding from esBAF to npBAF to nBAF, respectively. The nBAF complex contains three subunits found only in post mitotic neurons (Lessard Neuron 2007; Wu et al Cell 2012). The transition from the npBAF complex to the nBAF complex is driven by REST inhibition of mir9 and 124, which in turn inhibit expression of the subunits of the npBAF complex(Yoo et al Nature 2009). Forcing the formation of the nBAF complex using mir9/124 converts human fibroblasts to a wide variety of neurons (Yoo, Nature 2011, Neuron 2016). Remarkably, BAF250b or Arid1b is the most frequently mutated gene in de novo mental retardation (Nature 2014) and is also mutated in syndromic speech disorders, autism and neurodevelopmental disorders. Several of the other subunits of the nBAF complex are also frequently mutated in human autism and other neurodevelopmental disorders. In mice, the subunits of the nBAF complex are essential for depolarization-induced dendritic outgrowth and a clue to the understanding of these neurodevelopmental disorders comes from the observation that mutation of BAF53b in flies results in perfect dendritic retargeting in the fly olfactory system. Remarkably, the human homologue rescues the phenotype. Hence, these complexes somehow direct neural circuit formation.

Despite the importance of these complexes in human neurodevelopment their mechanism of action is relatively unknown. To understand their mechanism, we have engineered mice (CiAO system) in which it is possible to probe the actions of any chromatin regulator at any site in the genome of a neuron or other cell type (Hathaway et al Cell 2012, Kadoch et al *Nature Genetics* 2017, Braun *Nature Communications* 2017 in press). Using this system we find that BAF complexes rapidly evict polycomb repressive complexes over the genome and human neurodevelopmental disease mutations interfere with this eviction. Indeed BAF complexes rapidly evict polycomb from fate determining genes such as ASCL1. We show that PRC eviction occurs by direct binding and ATP-dependent release.

In addition, I will describe studies of mutations in a neuron-specific nBAF subunit that causes autosomal dominant Mendelian autism. Here we show that these mutations actually cause autism, rather than just being associated with it, making their mechanism particularly informative for understanding autism.

NOTES:

Chromatin as a hub integrating environmental signals and fate decisions in adult neural stem cells

ANA MARTIN VILLALBA

DKFZ Heildeberg, Germany

Regenerative processes in adult tissue require re-activation of developmental programs to enable a proper tissue repair. This is the case in highly regenerative organism such as the Zebrafish or axolotl. However, the adult mammalian brain is an example of very poor regenerative capacity, owing in part to the failure in activating an endogeneous developmenental program following injury. To address the reasons underlying this failure to repair, we examined the reaction to an ischemic injury of endogenous neural stem cells (NSCs) within the natural environment of the adult brain. Combination of in vivo lineage tracing, transcriptome analysis and tagmentation-based whole genome bisulfite sequencing (TWGBS), has uncover changes undertaken by endogenous progenitors following injury. In my talk I will discuss what we have learned about neural stem cell heterogeneity in the adult brain during homeostasis and following ischemic injury. In particular, I will discuss recent finding on how injury activates a transcriptional and epigenetic program in adult neurogenic progenitors, and their possible impact on fate choices.

NOTES:

Role of the chromatin remodeler CHD7 in neurogenesis and brain diseases

HAIKUN LIU

Division of Molecular Neurogenetics, German Cancer Research Center, Heidelberg, Germany

Mutation of the chromatin remodeler CHD7 is the major cause of CHARGE (coloboma, heart defects, atresia choanae, growth retardation, genital abnormalities, and ear abnormalities) syndrome. Most of CHARGE patients also have CNS abnormality during development. In recent years, we generated a set of different mouse models to dissect the role of CHD7 in adult neurogenesis, cerebellum development, and forebrain development. The results demonstrate that CHD7 is essential for governing neural differentiation during brain development. Molecular analysis shows the genomic distribution of CHD7 in active neuronal genes during brain development, and loss of CHD7 leads to loss of active histone marks on essential CHD7 target genes. This study reveals an interesting approach for molecular intervention of CHARGE syndrome, which will also be presented during the meeting.

NOTES:

Proliferation control, temporal identity and cell immortalization in Drosophila neural stem cell lineages

JURGEN KNOBLICH

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Stem cells are of crucial importance for tissue development, maintenance and regeneration in our body. They also play a critical role in tumor formation and growth. We are using Drosophila neuroblasts and the developing mouse cortex as models to understand, how stem cell proliferation is controlled, how stem cells can generate the enormous complexity of the brain and how defects in cell fate control can lead to tumor formation.

Drosophila neuroblasts undergo repeated rounds of asymmetric cell division generating one neuroblast and one more differentiated cell. The asymmetric segregation of the cell fate determinants Numb, Prospero and Brat is responsible for establishing distinct fates in the two daughter cells. When any of those determinants are mutated, neuroblasts over proliferate leading to the formation of lethal transplantable brain tumors. Using the genetic tools available in Drosophila, we have identified several epigenetic regulators that act as tumor suppressors in the Drosophila brain. These have helped us to characterize the molecular mechanisms leading to stem cell immortalization in tumors at a unique level of detail. Our results point to a surprisingly important role for epigenetic modifications and a non-coding RNA in the conversion of normal stem cells to tumor initiating cells.

NOTES:

SHORT TALK

Epigenetic and genetic programming of growth by modulating Zdbf2 dosage in the brain

JULIANE GLASER[1]

Maxim V.C. Greenberg[1], Fatima El Marjou[1], Carles Gaston-Massuet[2], Deborah Bourc'his[1]

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[2] Centre for Endocrinology, William Harvey Research Institute, Queen Mary University of London, London, UK

We recently described that adult phenotypes can be preemptively and indelibly programmed during the first days of development. At the imprinted Zdbf2 locus, an epigenetic switch from polycomb to DNA methylation occupancy occurs on the paternal allele in the pluripotent embryo and is stably maintained throughout life. Strikingly, this early epigenetic event is absolutely required for activating Zdbf2 expression later in the postnatal brain, which in turn is required for optimal growth. Very little is known about the molecular function of the protein product of Zdbf2, other than the presence of a DBF4 zinc finger domain and a predominant expression in the hypothalamo-pituitary axis. To assess the biological impact of Zdbf2 gene dosage in the brain, we generated three complementary mouse models of loss-of-function, gain-of function and parental inversion of Zdbf2 expression. Strikingly, our results show that Zdbf2 is a dose-sensitive but not parent-sensitive positive regulator of growth: 0 dose leads to nanism, 2 doses leads to gigantism, and 1 dose, of maternal or paternal origin, is enough. Importantly, these phenotypes seem to correlate with levels of circulating growth hormone. In conclusion, both genetic and epigenetic regulation of Zdbf2 impacts on the hypothalamo-pituitary determination of growth potential in mice. As the Zdbf2 locus and its imprinted status in conserved from mice to human, these findings have important implications for our understanding of height determination in human populations.

NOTES:

SHORT TALK

The POU homeobox gene BRN3A maintains the identity of multiple neuron types throughout the CNS

ESTHER SERRANO-SAIZ[1, 2]
Eduardo Leyva-Diaz[1, 2], Estanislao De la Cruz[1, 2], Oliver Hobert[1, 2]

[1] Columbia University of New York
[2] Howard Hughes Medical Institute

Many distinct regulatory factors have been identified to initiate neuron-type specific differentiation programs throughout the vertebrate CNS, but much less is known about how the differentiated state of neuronal cell types is actively maintained. While post-translational modifications of chromatin have been proposed to maintain gene expression states, a more parsimonious explanation is that transcription factors that initiate terminal differentiation programs continuously act to maintain the differentiated state throughout the life of a neuron. In the nematode C. elegans terminal selector-type transcription factors have indeed been identified to not only initiate, but also actively maintain the differentiated state of many neuron types. One of these factors, the C. elegans Brn3 ortholog unc-86 is thought to collaborate with distinct factors to initiate and maintain the identity of at least dozen distinct cholinergic, glutamatergic and serotonergic sensory, inter- and motor neurons. The vertebrate unc-86 ortholog Brn3a is known to initiate the differentiated program of different nuclei in the vertebrate nervous system. To assess whether BNR3A also maintains the differentiated state of vertebrate neurons, we first characterized the expression of BRN3A in different nuclei in the adult mouse nervous system and then conditionally removed Brn3a in the adult CNS. We find that BRN3A is required for the maintenance of the neurotransmitter identity of the glutamatergic neurons of several nuclei and interestingly, we found that the loss of terminal identity leads to the cell death of these neurons. We have therefore demonstrated that the differentiated state is actively maintained by a sequence-specific transcriptional regulatory factor.

NOTES:

Session 5
Transcription Factors

Post-translational regulation of stem cell quiescence and activity in the adult brain

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Neural stem cells located in restricted regions of the adult brain produce neurons that have important functions in memory and mood control. While the niche signals that control neural stem cells fates have been extensively investigated, little is known of the intrinsic mechanisms that mediate the activity of these extrinsic signals and implement appropriate fate decisions. Using high throughput genomic analysis and genetic approaches in stem cell cultures and in vivo, we characterise transcription factors that regulate the transitions between neural stem cell quiescence, activation and differentiation in response to niche signals.

We found that expression of the transcription factor Ascl1 by stem cells of the adult hippocampus is essential for their activation, and that regulation of Ascl1 protein levels determines the rates at which stem cells self-renew and new neurons are produced. Reduced levels of Ascl1 slow down stem cell proliferation and neurogenesis while increased levels accelerate proliferation and lead eventually to stem cell exhaustion and inhibition of neurogenesis. Ascl1 protein levels are controlled by different post-translational mechanisms in proliferating stem cells and quiescent stem cells.

NOTES:

What does the FOX say: Gene regulatory networks in the developing neocortex

SOO-KYUNG LEE

Oregon Health & Science University, Portland, USA

The cerebral cortex is responsible for higher cognitive and emotional functions, and it has served as an ideal model to study CNS development and cognitive disorders due to a great level of cellular complexity and unique neuronal organization. The mechanisms underlying the orderly production of the diverse cortical neuronal types are beginning to be understood thanks to the discovery of transcription factors that are expressed with temporal and regional specificity within the neocortex. The forkhead transcription factor FOXG1 is strongly expressed in neural progenitor cells of the forebrain, in which FOXG1 regulates self-renewal and a timing of neurogenesis. FOXG1 is then downregulated during neuronal differentiation of neural progenitor cells, which facilitates the cell cycle exit and cell differentiation. Interestingly, FOXG1 is re-expressed in maturing cortical neurons, in which FOXG1 promotes neuronal entry into the cortical plate. Notably, the mutations in the FOXG1 gene results in the neurodevelopmental disorder FOXG1 syndrome, which was initially described as a congenital variant of Rett syndrome. Prominent clinical features of the FOXG1 syndrome include microcephaly, corpus callosum agenesis, profound intellectual disability with autistic features, and seizures. Duplication of FOXG1 is also associated with epilepsy, ID, and severe speech & social impairment. Together, these results indicate that human brain development is highly sensitive to the dosage of the FOXG1 gene. While the past studies suggest the essential and cell context-dependent roles of FOXG1 in cortex development, the gene regulatory mechanisms by which FOXG1 controls the sequential steps of cortex development and how these mechanisms relate to the FOXG1 syndrome pathology remain unclear. We are investigating these critical issues using an ensemble of cellular, biochemical, genetic, and comprehensive genome-wide approaches.

NOTES:

Transcriptional dynamics in cortical development

VERDON TAYLOR

Embryology and Stem Cell Biology, Department of Biomedicine,
University of Basel, Basel, Switzerland

Although the cerebral cortex of adult mammals contains billions of cells with hundreds of functionally distinct neurons types, it is generated from a thin sheet of neuroepithelial cells which display neural stem cell (NSCs) properties. Coordinated progenitor expansion, neuronal fate commitment and gliogenesis are critical for cortex formation and neuronal subtype formation is linked to the birthdate of the cells. Disruption of the coordinated progenitor differentiation results in aberrant cortical formation and function and in humans leads to sever neurological disorders and cognitive impairments. How the complexity of the cerebral cortex is generated has been a major challenge for developmental neurobiologists. Currently, the most widely accepted model of corticogenesis, the common progenitor model, implies that NSCs are multipotent and become fate restricted with time during brain development. Thus, it implies that NSCs are equivalent at any point in time and undergo a coordinated shift in fate as development progresses. However, this model has been challenged with the suggestion that NSCs may not be equivalent and that some may have distinct and restricted fates. We have addressed the dynamics of transcriptional changes during cortical development over time. By isolating NSCs, basal progenitors and newborn neurons at every day of cortical development in mice, we addressed how gene expression correlates with cell fate. In addition, we looked at human corticogenesis in vitro and examined potential fate restriction during deep to upper layer neuron generation. We will show ongoing work to uncover how cortical development is linked to changes in gene expression and dynamic changes in heterogeneity within the progenitor populations.

NOTES:

Uncovering spatiotemporal windows in cortical development

SHUBHA TOLE

Tata Institute of Fundamental Research, India

The cerebral cortex arises from a simple sheet of neuroepithelial tissue in the embryonic forebrain. How this sheet is patterned to create an array of distinct cortical structures in a reliable and reproducible manner is a question of both evolution and development. We uncovered a fundamental mechanism that controls the formation of the cerebral cortex early in embryogenesis. A single protein, transcription factor Lhx2, serves as a master regulator that specifies cortical identity in neuroepithelial cells (Mangale et al., 2008). In addition to this early role, Lhx2 has distinct functions in the medial versus the lateral cortical primordium at later stages in development. The diversity of developmental phenomena regulated by Lhx2 includes control of the neuron-glia cell fate switch in the developing hippocampus, epigenetic regulation of neuronal subtype in the developing neocortex, and modulating the circuitry of innervation of the somatosensory barrel cortex (Subramanian et al., 2011; Shetty et al., 2013; Muralidharan et al., 2017).

Taking advantage of insights from other systems, we have developed a strategy to understand how different protein complexes may be recruited to generate the diversity of spatially and temporally distinct roles associated with this single transcription factor. Our studies reveal that Lhx2 participates in an evolutionarily conserved "tetrameric model" together with a key protein co-factor to execute some of its functions. Ongoing studies will examine how different combinations of factors form part of a "tool kid" to generate complexity and diversity of function in the nervous system.

References: Mangale et al, Science, 2008; Subramanian et al., PNAS, 2011; Shetty et al., PNAS, 2013; Muralidharan et al., J. Neurosci, 2017.

NOTES:

SHORT TALK

Sox2 is required for global functional chromatin connectivity in brain-derived neural stem cells

SILVIA NICOLIS[1]

Jessica Bertolini [1], Rebecca Favaro [1], Chee-Hong Wong [2], Miriam Pagin [1], Marit Vermunt [3], Menno Creyghton [3], Paola Bovolenta [4], Giulio Pavesi [5], Francois Guillemot [6], Chia-Lin Wei [2,7], et al.

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Lineage-specific transcription factors establish cell type-specific gene expression by binding to promoters and distal enhancers, which are brought into contact via long-range chromatin interactions. The SOX2 transcription factor is critical for neural stem cells (NSC) maintenance and brain development. In humans, SOX2 heterozygous mutation leads to a spectrum of CNS defects (involving eyes, hippocampus, seizures, motor control problems and intellectual disability). We are presently investigating Sox2 targets, and the mechanisms of their regulation by Sox2. Comparing normal and Sox2-ablated NSC, cultured ex-vivo from mouse forebrain, by genomic approaches (ChIA-PET), we found an unexpected role for Sox2 in the maintenance of a global pattern of long-range interactions in chromatin, mediated by RNApolII. Sox2-dependent long-range interaction "anchors" are highly enriched in Sox2 binding, and epigenetic enhancer marks, and predict new enhancers that guide expression of reporter transgenes to the forebrain in vivo in transgenic organisms. Reduction of gene expression observed in mutant cells (by RNAseq) is accounted for by the loss of Sox2-mediated activation via promoter-enhancer interactions. Further, genes controlled by Sox2-dependent interactions identify new mediators of Sox2 function, able to rescue the self-renewal defect of Sox2-ablated NSC. They also include genes involved in Sox2-related human brain disease. Our results highlight the maintenance of long-range enhancer-promoter interactions as a new aspect of Sox2 function as a transcription factor, and give access to thousands of novel distal enhancers of potential relevance for brain development and disease, by the definition of their connectivity to specific target genes.

SHORT TALK

Identifying Novel Transcriptional Regulators of Neurogenesis

ANGELA GARDING[1]

Vilma Raklii [2], Johannes Jung [1], Abhijeet Pataskar [1], Sudhir Thakurela [1], Johan Holmberg [2], Vijay K. Tiwari [1]

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Cell-fate specification during mammalian development involves stable resetting of transcriptional programs and the role of chromatin-mediated regulation in this process has been increasingly appreciated. Despite exciting developments, very little is known about epigenetic and transcriptional regulatory networks during embryonic neurogenesis. By employing a multidisciplinary approach, we attempted to discover novel transcriptional regulators of neurogenesis and identified AG1 to play an important role in gene regulatory program underlying cortical development.

Interestingly, AG1 displays neuronal tissue specific expression pattern in mouse as well as during primate evolution and is transiently induced during embryonic cortical development. Knockdown of this factor in vivo led to a defective neurogenesis during development, suggesting its functional role. Furthermore, transcriptome characterization of cells following such knockdown revealed misregulation of a number of critical genes involved in cell cycle regulation (URGs) and neuronal migration and maturation (DRGs). We are currently comprehensively identifying the genomic targets of AG1 during neuronal differentiation in vivo to reveal genes that are under its direct transcriptional control. Having observed an interaction of AG1 with chromatin repressive complexes including LSD1 and HDACs we are attempting to decipher whether this association occurs on chromatin and if AG1 plays a role in targeting these repressive complexes to its target genes to remodel chromatin and consequently the gene expression program during neurogenesis. In combination with further computational modeling approaches, we aim to be able to generate mechanistic models of how AG1 contributes to the gene regulatory program underlying cortical development.

NOTES:

Decoding the gene regulatory logic of neuronal cell type specification in *C. elegans*

OLIVER HOBERT

Howard Hughes Medical Institute, Columbia University, New York, USA

One of the central questions in developmental neurobiology is how a developing organism can generate a vast array of distinct neuronal cell types. For a terminally differentiating neuron this questions boils down to a gene regulatory question: how is the expression of the distinct batteries of genes that define the terminal, functional properties of distinct neuron type induced and maintained? Through the decoding of cis-regulatory elements and forward genetic screens in the nematode C.elegans, my laboratory has begun to uncover what appear to some simple, phylogenetically conserved principles that underlie the generation of diverse neuronal identities.

NOTES:

Dynamic transcriptional control of neural stem cells

RYOICHIRO KAGEYAMA

Institute for Frontier Life and Medical Sciences; iCeMS, Kyoto University, Kyoto, Japan

During brain development, neural stem cells proliferate intensively while they change their competency over time, giving rise to various types of neurons and glial cells sequentially. It is therefore very important to maintain neural stem cells until the final stage of development to generate a sufficient number of cells and a full diversity of cell types. We previously found that cell fate determination factors such as Hes1, Hes5 and Ascl1/Mash1 are expressed in an oscillatory manner in neural stem cells but exhibits sustained expression during cell fate determination and differentiation, and that Hes1 and Ascl1 oscillations are important for proliferation of neural stem cells. The expression of the Notch ligand Delta-like1 (Dll1), which is controlled by Hes1 and Ascl1, is also oscillatory in neural stem cells, but when Dll1 oscillations were dampened, Hes1 oscillations were also dampened. Under this condition, proliferation of neural stem cells was impaired, which causes microcephaly. We next generated transgenic mice that steadily express Hes5 in neural stem cells at high levels and found that switching from deep- to superficial-layer neurogenesis and from neurogenesis to astrogenesis is accelerated in these mice. Conversely, in the absence of Hes5, the switching is delayed. We also found similar phenotypes in Hes1-mutant mice. These results suggest that Hes1/Hes5 oscillations may be important for both proliferation and competency switching of active neural stem cells in the embryonic brain. By contrast, in quiescent neural stem cells, Hes1 expression is sustained, and Ascl1 expression is repressed. Furthermore, sustained Hes1 expression is sufficient to inhibit neurogenesis and maintain quiescent neural stem cells in the adult brain. These results suggest that oscillatory versus sustained Hes1/Hes5 expression may be important for active versus quiescent neural stem cells.

NOTES:

Epigenetic regulation of aging neural stem cells

ANNE BRUNET

Department of Genetics, Stanford University, San Francisco, USA

Aging is accompanied by a decline in tissue regeneration in mammals. In the nervous system, neural stem cells are thought to be critical for learning and memory. During aging, both the pool of neural stem cells and their ability to give rise to new neurons decline. Thus, neural stem cell decline may underlie age-dependent cognitive deterioration. However, the mechanisms that promote a youthful neural stem cell pool are largely unknown. Epigenetic changes in chromatin states may be particularly important in aging neural stem cells. We have previously shown that conserved chromatin modifiers of the COMPASS family, which is responsible for trimethylation of lysine 4 on histone H3 (H3K4me3), regulate longevity in the worm *C. elegans*. We are currently characterizing epigenetic changes, specifically changes in H3K4me3, in neural stem cells in aging mouse cohorts. We used next-generation sequencing to identify the genome-wide distribution of H3K4me3 in young and old adult neural stem cells. By directly purifying young and old neural stem cells, we have also examined coding and non-coding RNAs in aging neural stem cells. This global analysis has provided key insights into how this chromatin mark may promote youthful neural stem cell function. The knowledge of the epigenetic network controlling adult neural stem cell homeostasis might help counter brain aging in long-lived species, including humans.

NOTES:

Session 6
Distal gene regulation and nuclear organization

Chromosomal enhancer nuclear syntax: ligand-dependent 3D redistribution of “First Tier” enhancers generate mega-enhancers that associate with subnuclear phase-separated structures to dictate enhancer robustness

MICHAEL ROSENFELD

Sreejith J. Nair, Amir Gamliel, Lu N. Yang, Dimple Notani, Dario Meluzzi, Soohwan Oh, Qi Ma, Tom B. Suter, Wenbo Li, Amir Gamliel, Feng Yang, Ranveer Jayani

Howard Hughes Medical Institute, School of Medicine, University of California, San Diego, La Jolla, CA, USA

A basic, unresolved question in neurons and all cell types is the mechanism by which enhancers might interact at vast distances in the 3D space of the nuclus. Enhancers have been considered to be transferable elements that regulate proximal coding gene transcription based on their intrinsic properties. However, an important but minimally explored, question is whether the strongest regulated enhancers, although separated by vast linear distances on a chromosome, might be induced to functionally cooperate to yield higher levels of transcriptional robustness. We will present evidence that estradiol-17ß (E2) rapidly induces eRNA-dependent 3D physical proximity of a cohort of the most highly-induced MegaTrans- and ERα-bound enhancers, imposing cooperative enhancement of activation on the interacting enhancers and their regulated coding gene targets. Our data reveal previously unappreciated “spatially-redistributed mega-enhancers,” with robust enhancer transcription apparently requiring both Condensin-dependent, E2-induced enhancer proximity, the presence of eRNAs and association within an interchromosomal granule (ICG). These events, possibly augmenting liquid-liquid phase separation (LLPS) in the confined volume of this sub-nuclear architectural structure, would favor-increased concentrations of critical cofactor machinery to impart robustness to the first tier enhancers. In parallel, we will provide evidence that the altered strength of a subset of chromosomal boundaries underlies the altered transcriptional program in Rett Syndrome.

Gene regulation in development and evolution of the human cerebral cortex

CHRISTOPHER A. WALSH

Division of Genetics and Genomics and Howard Hughes Medical Institute,
Boston Children's Hospital and Harvard Medical School, Boston, USA

Although the noncoding genome includes vast numbers of sequences that appear to be essential regulators of gene activity, it remains a challenge to identify noncoding mutations that are associated with human disease. A remarkable noncoding mutation in GPR56, which encodes a G-protein coupled receptor that is essential for normal development of the cortex and cerebellum, disrupts a noncoding promoter-enhancer sequence regulating one of 17 or more alternative splice forms of the GPR56 locus. The noncoding mutation disrupts gene expression preferentially in the lateral cerebral cortex, and causes a malformation of the lateral, perisylvian regions of the human cortex, including Broca's language area and the symmetrical region of the right hemisphere. This noncoding region shows evolutionary divergence in humans compared to other species, also suggesting that evolutionary changes in this noncoding mutation may relate to the anatomical diversity of the perisylvian region.

Human Accelerate Regions (HARs) are genomic sequences, most of them noncoding, that are highly conserved in nonhumans, but show accelerated divergence between humans and nonhumans, suggesting that changes in these sequences may relate to human evolution. We tested HARs for rare homozygous mutations in patients from consanguineous families with autism and/or intellectual disability, and found an excess of rare HAR mutations in cases compared to controls, suggesting that some HARs are essential for cognitive and social behavior. Analysis of existing or custom 4C-sequencing identified target genes of the HARs that carried mutations. Further study of rare HAR mutations may help identify which among the ≈4000 HARs in the genome have essential roles in human brain evolution.

Supported by the NIMH, NINDS, the Allen Frontiers Program, and the HHMI.

NOTES:

Genome Architecture Mapping: discovering chromatin contacts in rare cell types

ANA POMBO

Berlin Institute for Medical Systems Biology, Max Delbrueck Center for Molecular Medicine, Berlin, Germany

The folding of chromosomes and the structural organization of the genome impacts human health and disease. Gene expression is controlled by long-range chromatin contacts between non-coding regulatory regions and their target genes. Recent development in novel methodologies to map chromatin contacts have shown that disruption of chromatin contacts due to disease-associated structural changes in the linear genome can result in altered patterns of gene expression. However, the analyses of specific cells types, such as neurons in specific areas of the brain remain a challenge.

To study the relationship between 3D genome folding and gene expression in specific cell types, we have developed Genome Architecture Mapping (GAM), a novel ligation-free technique, which overcomes limitations of current 3C-based approaches. GAM extracts spatial information by sequencing DNA from a large collection of thin nuclear sections, before quantifying the frequency of locus co-segregation across the collection of sections. By applying GAM to mouse embryonic stem cells, we have identified specific chromatin contacts enriched for interactions between active genes and enhancers spanning large genomic distances. We currently apply GAM in neuronal subtypes directly microdissected from mouse brain. Our work shows that genome architecture is highly dependent on cell-type specific gene expression patterns at both short and long genomic distances.

NOTES:

SHORT TALK

Gene network governing the commitment of radial glial cells to ependymal lineage

STAVROS TARAVIRAS[1]

Marialena Lalioti[1], Konstantina Kaplani[1], Chrisitna Kyrousi[1], Marina Arbi[2], Georgia Lokka[1], Eleni Damianidou[1], Zoi Lygerou[2]

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Multiciliated ependymal cells are key components of the adult subventricular zone niche. They carry multiple motile cilia on their apical surface, thus controlling the circulation of the cerebrospinal fluid (CSF) within the adult brain. Moreover, they provide multiple regulatory cues for the adult neural stem cells self-renewal and production of new neurons. Ependymal cells are generated from radial glial cells (RGs) shortly after birth, though their specification has been established earlier during embryogenesis, however the molecular pathways governing these early fate decisions of RGs are poorly understood.

Our findings reveal that GemC1/Lynkeas, a member of the Geminin family, is the earliest known marker RGs committed to the ependymal cell lineage in the developing mouse brain. We have shown that RGs upon GemC1/Lynkeas overexpression lose their neural stem cells characteristics and prematurely differentiate into multiciliated ependymal cells. Moreover, GemC1/Lynkeas is a key upstream transcriptional regulator which collaborates with E2F5 to directly activate the expression of key transcription factors of multiciliogenesis, including Mcidas and Foxj1. Moreover, we have shown that Geminin and Notch act antagonistically to GemC1 maintaining progenitors in undifferentiated state. GemC1/Lynkeas deficient mice develop hydrocephalus and exhibit complete lack of early committed and mature multiciliated ependymal cells in the SVZ. Moreover, cellular components of the SVZ niche are severely affected by the absence of Lynkeas leading to impaired neurogenesis.

Our results show that GemC1/Lynkeas is a master regulator of radial glial cells fate initiation to the ependymal lineage and the establishment of the SVZ niche.

NOTES:

SHORT TALK

Mechanisms of epigenetic priming that underpin changes in neuronal identity

PHILIPP MEWS

Hope Kronman, Erin S. Calipari, Eric J. Nestler

Icahn School of Medicine at Mount Sinai, New York, USA

At the heart of cellular identity lies the capacity to store information over long periods of time. In the adult brain, epigenetic signatures that govern gene expression preserve cellular memory. These epigenetic processes can integrate environmental information to permanently adapt neuronal gene transcription and identity. Currently, the precise mechanisms underlying such plasticity remain opaque. An important context of permanently altered neuronal gene regulation relevant to human health is drug addiction. Drugs of abuse, in addition to their acute effects, cause persistent aberrations in neuronal activity and gene expression. In the nucleus accumbens - the key brain region of reward learning - we found that cocaine permanently alters the inducibility of key neuronal genes to future stimuli, referred to as gene priming and desensitization. We hypothesize that drug-induced changes in the epigenetic landscape underlie such latent transcriptional dysregulation. A critical challenge is to determine the neuronal subtypes that are responsible: the nucleus accumbens is principally composed of two functionally exclusive types of medium spiny neurons (MSNs), the D1 and D2 dopamine receptor-expressing subtypes. First, we defined the differential transcriptional priming caused by cocaine in the distinct MSN subtypes. We then identified the long-lasting aberrations in chromatin structure that underlie epigenetic priming and altered MSN activity. Using ATAC-seq, we surveyed chromatin accessibility genome-wide and differentiate acute from permanent changes caused by cocaine in D1 vs D2 MSNs. An advanced understanding of the epigenetic processes that regulate priming and neural identity will ultimately identify novel targets for epigenetic therapies of psychiatric diseases.

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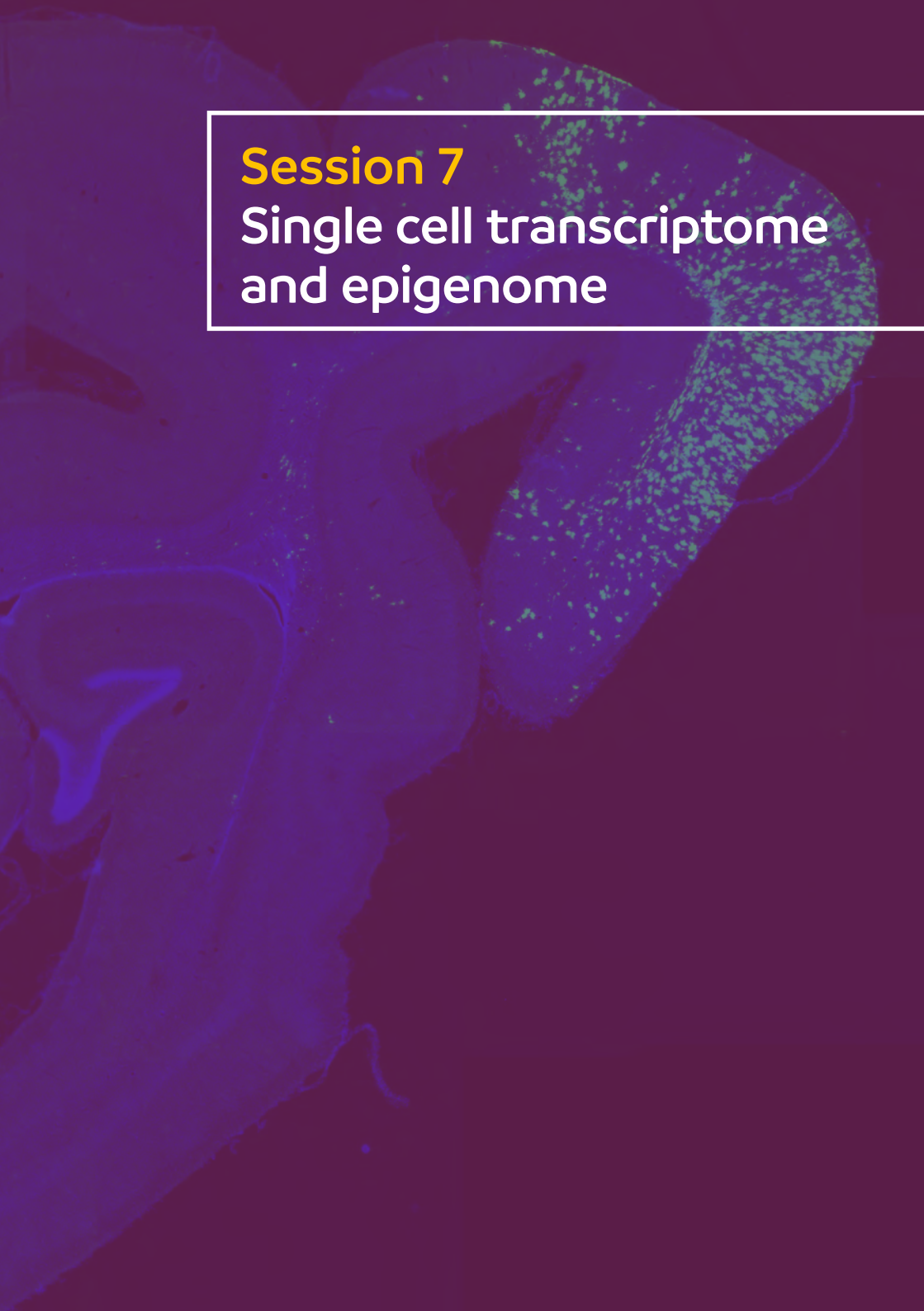

Session 7
Single cell transcriptome and epigenome

Patterning principles in the early Drosophila nervous system development

ROBERT ZINZEN

MDC, Berlin, Germany

NOTES:

SHORT TALK

Programming cell fates using a comprenhensive human transcription factor library

VOLKER BUSSKAMP [2]

Alex Ng [1], Parastoo Khoshakhlagh [1], Seth Shipman [1], Anka Swiersy [2], Didier Trono [3], Jussi Taipale [4], David Hill [5], Marc Vidal [5], George Church [1]

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The ability to produce any human cell type in a robust and facile manner would advance the development of realistic microtissues and organoids. To expand the breadth and access of in vitro cell types, we conducted large-scale cell fate engineering approach to generate cell types from human induced pluripotent stem cells (hiPSCs). First, we created a comprehensive human transcription factor (TF) expression library (the "human TFome") to systematically screen TFs that differentiate human stem cells. Second, we mapped transcription factor-cell type relationships by pooled TFome over-expression and RNA sequencing. From these approaches, we identified 204single TFs that are potent to program stem cells into differentiated cell types of multiple tissues. We achieved potent (>90%) cellular programming without the addition of growth factors, mechanical processes or purifications by maximizing TF expression and selecting potent TF isoforms. Engineered cell types include electrically active neurons, lumen-forming endothelial cells and stromal cells. Large-scale engineering of human cell identity could pave the way towards the production of many human cell types in vitro.

NOTES:

SHORT TALK

Systematic analysis of neural development in vivo

CHRISTOPHER ESK[1]
Johannes Zuber [2], Juergen A. Knoblich [1]

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Mammalian neurogenesis in the cerebral cortex generates millions of neurons while maintaining an active population of undifferentiated progenitors. This requires a tight balance of stem cell division modes and solid establishment of self-renewing and differentiating fates in neural progenitors and prospective neurons respectively. So far, a systematic analysis of factors involved has been missing. Here, we describe an in vivo loss-of-function screening approach that allows for systematic testing of a large number of genes for their involvement in mammalian neurogenesis. We have developed a method for efficient viral delivery into the murine neocortex at E10.5 so that we can target both cycling neural stem cells and their differentiating progeny. Viral constructs are highly competent in transducing stem cells, exhibit sustained expression in differentiated daughter cells and downregulate target genes when expressing shRNAs. By separating progenitors and neurons at E17.5 and testing viral composition of these populations through deep-sequencing of PCR-amplified viral inserts, we have characterized shRNA effects on both proliferation and differentiation. We demonstrate that hundreds of genes can be tested simultaneously for their role in fate induction allowing for identification of functional modules in mammalian neurogenesis. Initial candidate shRNAs target transcriptional and epigenetic factors as these are key to understanding the complex pathways underlying proliferation and fate induction. Screen results confirm known factors and identify chromatin remodeling complexes such as polycomb repressive complex 1 as having pivotal roles in controlling neurogenesis. In summary, we present a sophisticated in vivo loss-of-function screening approach for systematic analysis of mammalian neurogenesis.

NOTES:

Polycomb proteins as topological facilitators of enhancer function during neural induction

ALVARO RADA-IGLESIAS

University of Cologne, Cologne, Germany

Poised enhancers marked by H3K27me3 in pluripotent cells were previously proposed to facilitate the establishment of somatic expression programs upon embryonic stem cell (ESC) differentiation.

However, the functional relevance and mechanism of action of poised enhancers remain unknown. Here, we identify and functionally annotate poised enhancers in mouse ESC, uncovering a previously unappreciated link with the establishment of anterior neural identity. Most importantly, we use genetic deletions to demonstrate that poised enhancers are necessary for the induction of their target genes, which includes major anterior neural regulators. Interestingly, poised enhancers already establish physical interactions with their target genes in ESC in a Polycomb repressive complex 2 (PRC2) dependent manner. Loss of PRC2 led to neither the activation of poised enhancers nor the induction of their putative target genes in undifferentiated ESC. In contrast, loss of PRC2 severely and specifically compromised the induction of major anterior neural genes representing poised enhancer targets upon mESC differentiation. Finally, we show that poised enhancers have an intrinsic sequence composition that enables

PRC2 recruitment and H3K27me3 deposition at these regulatory elements.

Hence, both poised enhancer and their target genes have sequence features that, through the recruitment of polycomb proteins, can mediate their physical communication. Overall, our work illuminates a novel function for polycomb proteins, which we propose facilitate neural induction by providing major anterior neural loci with a permissive regulatory topology. Last but not least, based on the preferential association of poised enhancers with major anterior neural genes and the essential role during their activation, we hypothesize that poised enhancers could represent a novel and important component of the classical “default” model of neural induction, whose genetic and/or epigenetic basis remains largely unknown.

Modeling human brain development and disease at single cell resolution using brain organoids

GIORGIA QUADRATO

Harvard University, Boston, USA

In vitro models of the developing human brain such as 3D brain organoids offer an unprecedented opportunity to study aspects of human brain development and neurodevelopmental disorders in a format amenable to large-scale production and genetic engineering. However, it remains undefined what cell populations are generated within organoids and to what extent brain organoids recapitulate the regional complexity, cellular diversity, and circuit functionality of the human brain.

Here, we analyzed gene expression in over 80,000 individual cells isolated from 31 human whole-brain organoids that has developed for 3-6 months. We find that organoids can generate a broad diversity of cells, which we show are related to known endogenous classes, including subpopulations of neurons and progenitors of the cerebral cortex and of the retina. Organoids could be developed over extended periods (>1 year) enabling unprecedented levels of maturity including the formation of dendritic spines and of spontaneously-active networks. Neuronal activity within organoids could be controlled using light-stimulation of photosensitive cells, which may offer a way to probe the functionality of human neuronal circuits using physiological sensory stimuli. All together, these data provide insight into the range of cellular diversity that results when generating brain organoids and further validate their use to model human brain development and cell-type specific changes in physiological and pathological conditions.

NOTES:

Genome-wide analysis of DNA cis-regulatory element activity during neuronal specification of pluripotent stem cells

DAVID CACCHIARELLI

Telethon Institute of Genetics and Medicine (TIGEM) - Naples (Italy)

Exit from the pluripotent state represent one of the first cell fate decisions towards cell specialization. The rapid development of next-generation sequencing technologies has dramatically increased our capacity to investigate the architecture of such transitions, spanning from the annotation of genic regions to epigenomic and transcriptional states. By chromatin immunoprecipitation we defined dynamic regulatory transitions at cis-regulatory regions occurring during the early neuronal commitments of differentiating Pluripotent Stem Cells (PSC).

To move from a descriptive study to a functional analysis of the mapped cis-regulatory elements we performed a genome-wide functional activity assay to quantitatively estimate the differential contribution of each single promoter or enhancer region to PSC specification. I will present how this novel approach allowed us to identify bona fide regulatory elements alongside with an accurate estimation and validation of their corresponding transcription factors that, either alone or in combination, define the regulatory network controlling the transcriptional modulation of early neurogenesis.

NOTES:

Genomic insights into human cortical development

ARNOLD KRIEGSTEIN

UCSF, San Francisco, USA

Radial glia, the neural stem cells of the neocortex, are located in two niches: the ventricular zone and outer subventricular zone. Although outer subventricular zone radial glia (oRG) appear to generate the majority of human cortical neurons, their molecular features remain elusive. We have begun to sequence mRNA from single human progenitor cells for unbiased classification of cell identity and for detection of activated signaling pathways. By analyzing gene expression across single cells, we find that oRG cells preferentially express genes related to extracellular matrix formation, migration, and stemness, and we relate these genes to the position, morphology, and behaviors previously used to classify these cells. Many of these genes are involved in growth factor signaling and self-renewal pathways, suggesting that outer radial glia cells establish a self-sustaining proliferative niche in the OSVZ. Using single cell clonal lineage analysis, we find that oRG cells can generate hundreds of daughter neurons of deep and upper layer identity, establishing the extensive proliferative and neurogenic capacity of this cell type. Finally, by using novel markers that reveal the morphology of oRG or ventricular radial glia cells selectively, we find that oRG cells form the primary scaffold for migration of neurons to the cortical plate during mid- and late-phases of cortical neurogenesis. More generally, we have expanded this approach to identify the genes and pathways distinguishing diverse cell types during cortical development.

These molecular insights have already informed a novel model of primate corticogenesis, suggested a relationship between oRG cells and brain tumors, provided insights into the specific cell types affected by genetic forms of lissencephaly, and have helped identify the mechanism of Zika virus microcephaly.

NOTES:

Genetic evolution of cortical size determinants

VICTOR BORRELL

Institute of Neuroscience, CSIC-UMH, San Juan de Alicante, Spain

We study developmental mechanisms relevant for the expansion of the cerebral cortex through evolution. Cortex size in reptiles, birds and mammals differs dramatically owing to developmental differences in neuron production and final abundance of neurons, but the genetic mechanisms behind this evolution of neurogenesis are unknown. I will present unpublished work showing that in evolutionarily old brain structures, direct neurogenesis from Radial Glia Cells is abundant, driving fast but limited neuron production, whereas in evolutionarily more recent structures most neurogenesis is indirect via basal progenitors, thus slow but abundant. We have identified a conserved signaling pathway behind these changes during evolution. Gain- and loss-of-function manipulations in mouse, chick and snake embryos, and also human organoids, demonstrate that activation of this pathway is necessary and sufficient to drive direct neurogenesis. This pathway is prevalent in the cerebral cortex of developing chick and snake embryos but dampened in mammals, thus favoring indirect neurogenesis, which suggests that attenuation of this signaling pathway contributed to increase neuron production and cortical expansion during vertebrate evolution.

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Session 8
Novel mechanisms. Molecular tools and approaches

Human-specific genes, neural stem cell amplification, and neocortex expansion in development and human evolution

WIELAND B. HUTTNER

Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Our group studies neural stem and progenitor cells in the context of the expansion of the neocortex in development and evolution. Two major classes of cortical stem and progenitor cells (CSPCs) can be distinguished. First, CSPCs that reside in the ventricular zone (VZ), i.e. neuroepithelial cells, apical radial glia (aRG) and apical intermediate progenitors, collectively referred to as apical progenitors (APs). Second, CSPCs that reside in the subventricular zone (SVZ), i.e. basal radial glia (bRG) and basal intermediate progenitors, collectively referred to as basal progenitors (BPs). Neocortex expansion is thought to be linked to an increased abundance and proliferative capacity of BPs.

To gain insight into the genomic changes that underlie neocortex expansion, notably in humans, we have analyzed the transcriptomes of human vs. mouse VZ and SVZ, and of human vs. mouse aRG and bRG. This led to the identification of the human-specific gene *ARHGAP11B* as a major player. Specifically, ARHGAP11B promotes the generation of BPs from aRG and the subsequent BP proliferation, thereby increasing BP abundance. Moreover, ARHGAP11B is able to induce folding of the embryonic mouse neocortex, which normally is smooth. The ability of ARHGAP11B to amplify BPs is based on a single C-to-G base substitution which creates a novel splice donor site; this leads to the removal of 55 nucleotides upon mRNA splicing, resulting in a reading frame shift and generating a human-specific 47-amino acid sequence that is thought to be key for BP amplification.

BP generation involves their delamination from the ventricular surface and apical adherens junction belt. We recently identified the adherens junction belt-specific protein Plekha7 as a key player and found that the transcriptional repressor Insm1, previously shown to promote BP generation, down-regulates Plekha7 expression, and that disruption of Plekha7 expression causes CSPC delamination.

Given that the mouse is secondarily lissencephalic, we searched for relicts of features of the gyrencephalic ancestror and found these in the mouse E18.5 medial neocortex. Specifically, the mouse medial neocortex exhibits and outer SVZ and a high abundance of bRG which express the human bRG-enriched marker Hopx. Disruption of Hopx expression in mouse medial neocortex reduces bRG abundance. Conversely, forced expression of Hopx in mouse lateral neocortex increases bRG abundance. Thus Hopx

is necessary and sufficient to achieve a gyrencephalic-like bRG abundance.

To compare neural stem cell division between human and great ape developing neocortex, we have performed live imaging using iPSC-derived 3D cerebral organoids. This revealed a specific lengthening of metaphase during AP mitosis in human as compared to chimpanzee and orangutan.

Finally, we have found that specific components of the extracellular matrix (ECM) are capable of inducing the folding of human neocortex in organotypic slice and hemisphere culture. The implications of these findings will be discussed.

NOTES:

Novel molecular mechanisms of neurogenesis

MAGDALENA GÖTZ

Institute for Stem Cell Research, Helmholtz Center Munich and Biomedical Center, University of Munich, Germany

We study the mechanisms of neurogenesis in order to implement them for neuronal repair. I will present unpublished work about the role of a novel centrosomal protein which is crucial for formation of the subventricular zone and retention of cells therein, a key event in cerebral cortex evolution. I will further present new data on the molecular function of Trnp1, a novel nuclear protein, with key roles in cerebral cortex folding and seeding of the basal radial glial cells into the SVZ in ferret (Stahl et al., Cell 2013; Martinez-Martinez et al., Nature Comm. 2016). I will comment on its interactome and novel molecular function. Both these proteins, as well as the function of Uhrf1 in cerebral cortex development (Ramesh, Bayam et al., 2016) highlight that cell biological functions that were considered as rather general in all cells are instead highly cell type specific and their modulation even in subtypes of neural stem cells exerts key roles during neurogenesis and expansion of the cerebral cortex. I will close by linking these mechanisms also to the processes of direct neuronal reprogramming.

NOTES:

Editing of neural epigenomes

STEFAN STRICKER

Helmholtz Zentrum, Munich, Germany

The bacterial CRISPR system is fast becoming the most popular genetic and epigenetic engineering tool due to its universal applicability and adaptability. The desire to deploy CRISPR-based methods in a large variety of species and contexts has created an urgent need for the development of easy, time- and cost-effective methods enabling large-scale screening approaches. Here we describe method for the generation of comprehensive gRNA libraries for CRISPR-based screens and show that gRNA libraries can be derived from any source of DNA without the need of complex oligonucleotide synthesis. Furthermore, we will introduce an efficient strategy for multiplexing and customizing gRNA expression and show how CRISPR tools can be used to influence cell identities.

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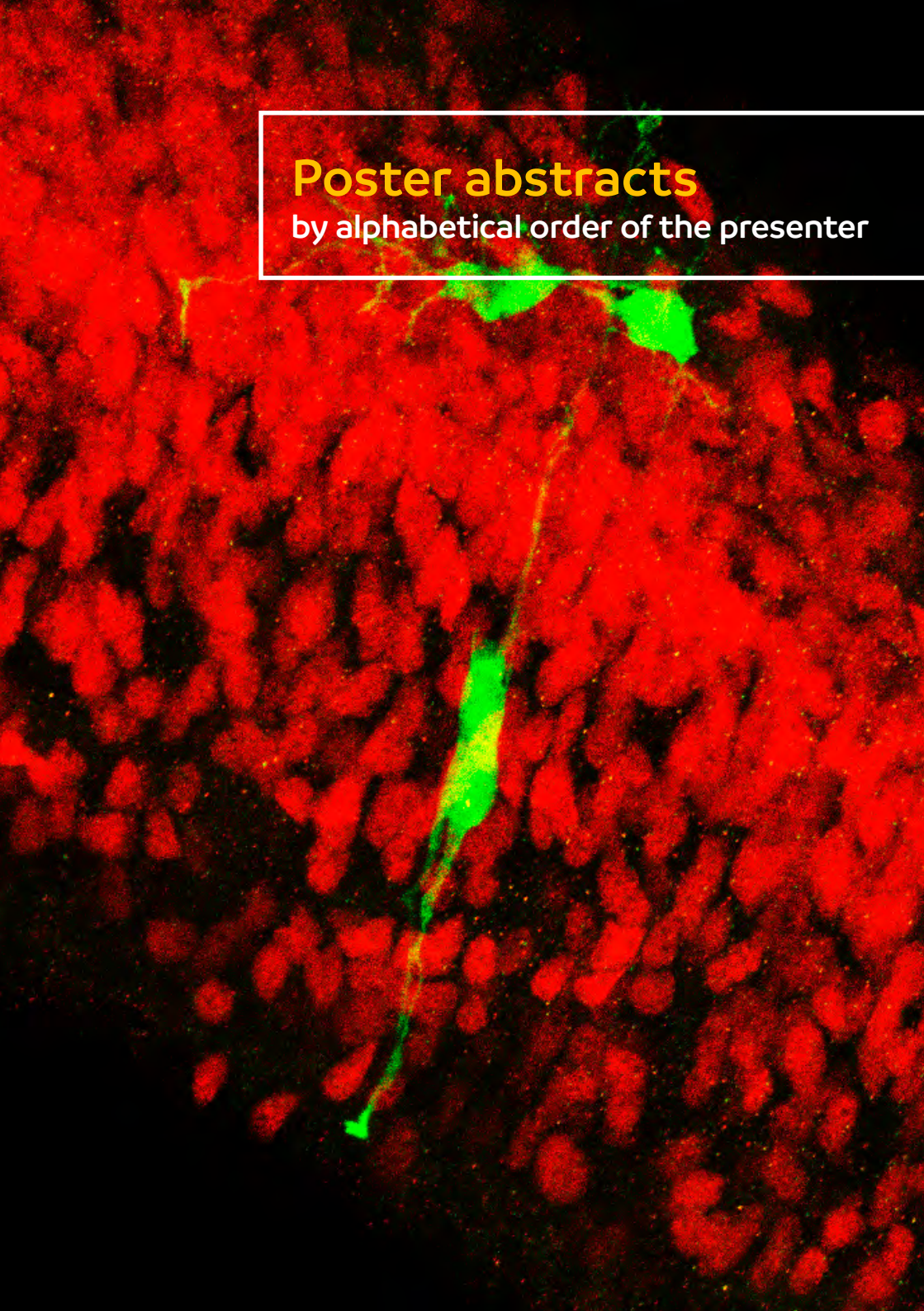
Poster abstracts

by alphabetical order of the presenter



1 | From single-cell transcriptomics to forebrain evolution

Kaia Achim, Detlev Arendt

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GABAergic and glutamatergic neurons are the principal types of neurons in the mammalian brains. Given the vast diversity in their localization, projection patterns, morphology and physiology, it is perhaps not surprising that these neurons represent two fundamentally distinct cell types in the brain.

We have earlier proposed a model of GABAergic neuron development whereby brain is divided in three broad regions where, regardless of the distinct patterning across the neuromeres, the GABAergic fate determination converges on distinct molecular toolkits: telencephalon–anterior diencephalon (DLX type), posterior diencephalon–midbrain (GATA2 type) and hindbrain–spinal cord (PTF1A and TAL1 types) (Achim et al 2014). Recent single-cell sequencing studies confirm both the fundamental split between the GABAergic and glutamatergic fates, and reveal the core determinants of forebrain GABAergic neurons. Furthermore, these studies provide insights into generation and manifestation of the diversity of GABAergic neurons in cortex, subcortical areas as well as hypothalamus. Based on these studies, we now delineate the evolutionary descent of different GABAergic neuron types in the vertebrate forebrain.

Achim K, Salminen M, Partanen J. 2014. Mechanisms regulating GABAergic neuron development. Cell Mol Life Sci 71: 1395-415

2 | Epigenome profiling and editing of neocortical progenitor cells during development

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The generation of neocortical neurons from neural progenitor cells (NPCs) is primarily controlled by transcription factors binding to DNA in the context of chromatin. To understand the complex layer of regulation that orchestrates different NPC types from the same DNA sequence, epigenome maps with cell type resolution are required. Here we present genome-wide histone methylation maps for distinct neural cell populations in the developing mouse neocortex. Using different chromatin features, we identify potential novel regulators of cortical NPCs available for future exploration. Moreover, we identify extensive H3K27me3 changes between NPC subtypes coinciding with major developmental and cell biological transitions. Interestingly, we detect dynamic H3K27me3 changes on promoters of several crucial transcription factors, including the basal progenitor regulator Eomes. We used catalytically inactive Cas9 fused with the histone methyltransferase Ezh2 to edit H3K27me3 at the Eomes locus in vivo, which results in reduced Tbr2 expression and lower basal progenitor abundance, underscoring the relevance

of dynamic H3K27me3 changes during neocortex development. Taken together, we provide a rich resource of neocortical histone methylation data and outline an approach to investigate its contribution to the regulation of selected genes during neocortical development.

3 | Zika virus-associated microcephaly is caused by ER stress up-regulation in cortical progenitors

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[8] Equal contribution to the work

The recent outbreak of the Zika virus (ZIKV) infection in Brazil has raised the interest of the international scientific community due to an increased incidence of congenital microcephaly in infected foetuses. Several hypotheses have been formulated to explain how ZIKV infection induces microcephaly, but no convincing mechanisms have been described so far. By combining analyses on mouse and human samples we show that ZIKV induces microcephaly during embryonic development by triggering ER stress and unfolded protein response (UPR) in neocortical neuron progenitors, as well as in their progeny, during development. In the first phases of cortical neurogenesis, neurons are directly produced from apical progenitors, which are the earliest committed progenitor population (direct neurogenesis). At later stages, apical progenitors generate intermediate progenitors that amplify the production of neurons (indirect neurogenesis). Previous studies have demonstrated that the gradual down-regulation of UPR in apical progenitors promotes the shift from direct to indirect neurogenesis. By injecting ZIKV in mouse foetal brains we observed a long-lasting up-regulation of several UPR mediators together with a strong decrease of intermediate progenitor number and a severe microcephaly. This phenotype was reverted by injecting chemical inhibitors of the UPR pathway in infected embryonic brains. Our results shed new light on the etiology of ZIKV-induced microcephaly and portend potential therapeutic interventions towards counteracting, and possibly preventing ZIKV infection.

4 | Characterizing of Robo downstream signalling to promote direct neurogenesis

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Understanding the genetic and molecular mechanisms that control the development and size of the cerebral cortex across phylogeny is needed. The process of neurogenesis and the balance between progenitor self-renewal and consumption is under the influence of a quite complex regulation. We previously found that in the Neocortex very low levels of the surface receptors Robo1 and 2 are essential in RGCs to control the balance between IPC production and RGC self-renewal, in part by driving Hes1 transcription, a component of the Notch signalling pathway. Recently, our lab found that high levels of Robo receptor signalling, concomitant with low Dll1 levels (Delta like-1, a ligand of Notch) in RGCs promote direct neurogenesis in the cortex and olfactory bulb whereas inversion of these levels lead to indirect neurogenesis via IPCs .This suggests that attenuation of Robo signalling is responsible for the increased neurogenesis and cortical expansion during vertebrate evolution. Based on these findings, we are characterizing the downstream molecular signalling that leads to direct neurogenesis upon activation of Robo receptors and low Dll1 levels, we are using immunopreciptaion of the Robo receptor followed by mass spectrometry to identify the interactome of Robo and also RNA sequencing to identify the transciptome that leads to increased direct neurogenesis.

Keywords: direct neurogenesis, signalling, Robo, Cortex

5 | Identification of a novel transcription regulator NeuroEpi1 in neurogenesis

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The role of chromatin-mediated regulation in cell-fate specification during mammalian development has been increasingly appreciated. However, very little is known about epigenetic regulatory networks during embryonic neurogenesis. In search for novel epigenetic regulators of neuronal development, we find that NeuroEpi1 (NE1) is specifically expressed in the cortex during embryonic development. Interestingly, knockdown of NE1 during cortical development leads to a defective neurogenesis. Immunoprecipitation assay combined with mass-spectrometry re-

vealed that NE1 associates with the repressive chromatin machinery including histone deacetylases, suggesting that it likely functions in gene repression. Further biochemical analysis revealed that NE1 has higher affinity for H3K4me1 compared to other modifications states of histone H3 at Lysine 4 (K4). Moreover, our recent data suggests that NE1 is targeted to regulatory regions in the genome. Future work will aim to generate mechanistic models of how NE1 contributes to the epigenetic reprogramming underlying cell-fate changes during neurogenesis.

6 | Regulation of adult NSC quiescence by inflammatory signals

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Neurogenesis persists in specific niches of the adult mammalian brain and is supported by long-lived stem cells present in these locations. The subependymal zone (SEZ) of the adult murine brain is a very active neurogenic niche in which a relatively quiescent population of radial glia/astrocyte-like GFAP+ neural stem cells (NSC) continually produces new neurons and oligodendrocytes, via a population of rapidly-diving transit-amplifying progenitor cells. NSC co-exist in equilibrium of different cycling phases (quiescent and active), and the regulation of this transition plays an essential role in stem cell maintenance during tissue homeostasis. Although activation and cell division of NSC has been extensively studied, little is known about the maintenance and regulation of the quiescent state.

Apart from producing new differentiated progeny during the adult life, neural stem cells can also respond to a plethora of stimuli and pathological situations. Among them, neuroinflammation seems to generate a complex scenario where both positive and negative effects have been observed.

In the present work, we have studied the regulation of NSC quiescence/activation in different in vivo scenarios where SEZ homeostasis is compromised such as acute inflammation or chemotherapy. We identify TNFα and Progranulin as potential key regulators in both processes. Additionally, signaling through their common receptor TNFR2 seems to be an important element in the conservative activation of the quiescent NSC pool in order to restore homeostasis.

7 | Reconstructing cortical gene expression dynamics of Hes5 in vitro

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The complexity of processes that concertedly lead to the development of the CNS in the embryo obscures our understanding of how gene expression is regulated at the single cell level and in turn how dynamics give rise to distinct biological function. Thus a strategy to deconstruct and reconstruct gene expression in vitro is needed to understand internal cell regulatory mechanisms and distinguish them from non-genetic heterogeneity introduced by the cell environment.

We used a combination of advanced Fluorescence Correlation Spectroscopy (FCS) and computational techniques to recapitulate gene expression dynamics of the cerebral cortex in the dish. We focused on the dynamics of Notch effector Hes5 which prevents neural stem (NS) cells from premature differentiation by repressing pro-neural genes (Kageyama et al. 2007). Using transgenic mice containing a Hes5 reporter (Imayoshi et al. 2013), we found that NS cells in cortical tissue slices expressed on average ~21,000 molecules of Hes5 per nucleus and that this level was preserved between E12.5-E14.5.

However, Hes5 expression was reset upon dissociation and spontaneously regained over 2-3 days in adherent (2D) cultures of cortical NS cells. Upregulation was enhanced by cell density, however, steady-state levels were limited to ~3-4,000 molecules per nucleus indicating a bottleneck. We hypothesised that this is caused by disruption of Notch activity due to reduced cell:cell contact compared to the tissue. Indeed, NS cells grown as neurospheres did express Hes5 at levels comparable to tissue. Our findings demonstrate that the culture environment quantitatively impacts gene expression dynamics.

8 | Uncovering a central role for Id4 in the regulation of adult hippocampal neural stem cell quiescence

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The transition of adult hippocampal neural stem cells (AHNSCs) between the quiescent and active state is tightly controlled by a combination of signals derived from their niche, a regulation essential for their long-term maintenance. However, the precise mechanisms regulating the quiescence/activation switch are not well understood.

In order to study this, we have developed an in vitro system of BMP4-induced AHNSC quiescence. Genome-wide transcriptional profiling of BMP4-induced AHNSCs shows a clear 'quiescence' signature and similarities to published data sets of ex vivo quiescent NSCs[1,2].

Amongst the genes identified to be enriched in BMP4-induced AHNSCs, Inhibitor of differentiation-4 (Id4) was highly and specifically expressed in the quiescent state. Moreover, Id4 protein is highly expressed in more than 95% of stem cells in the adult SGZ in vivo. Id proteins are known negative regulators of bHLH proteins, therefore we are investigating a mechanism whereby Id4 induces and maintains NSC quiescence by negatively regulating the bHLH transcription factor Ascl1, a crucial factor for NSC activation[3]. In support of this hypothesis, we have found Id4 and Ascl1 protein expression to be anti-correlated in hippocampal stem cells in vitro and in vivo. Moreover, conditional deletion of Id4 in AHNSCs in vivo results in increased Ascl1 protein immunoreactivity and increased activation of the stem cells. We are further testing this mechanism using in vitro overexpression of Id4.

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2. Codega, P. et al., (2014) Neuron. doi: 10.1016/j.neuron.2014.02.039.
3. Andersen, J. et al., (2014) Neuron. doi: 10.1016/j.neuron.2014.08.004.

9 | Dynamic, multi-scale 3D genome rewiring during mouse neural differentiation

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Chromatin-conformation capture technologies have revealed important insights on genome folding. Yet, how spatial genome architecture is related to gene expression and ultimately cell fate remains unclear.

We mapped comprehensively 3D chromatin organization during mouse neural differentiation, both in-vitro and in-vivo, generating the highest resolution Hi-C maps available to date. At the global scale, we found reduced interactions between active domains and increased long-range contacts between inactive domains during differentiation. We uncover that in addition to CTCF, chromatin insulation is tightly linked to gene expression. Active promoters can insulate adjacent regions even when not present at domain boundaries and interact to form strong chromatin loops. Furthermore, using CRISPR-dCas9 we examine in details the causal relationship between transcription and insulation.

In addition to changes in domain architecture, we find dynamic rearrangement of chromatin loops at various scales. We discover that an extensive Polycomb-linked contact network in stem cells is disrupted independently of H3K27me3 during differentiation, while novel interactions are established in neural progenitors and cortical neurons driven by the transcription-factors Pax6, NeuroD2 and Tbr1. Finally, we show that the majority of the enhancer-promoter interactions are cell-type specific and are established concomitantly with gene expression.

Collectively, this work shows that chromatin architecture is more dynamic than previously anticipated and provides a framework to study its influence on gene expression and cell fate.

10 | Chronic in vivo 2-photon imaging of remyelination processes in the adult mouse brain

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Oligodendrocytes form myelin sheaths around axons allowing for the rapid transduction of electrical signals. A number of neurological diseases, among others multiple sclerosis (MS) and epilepsy, are associated with oligodendrocyte loss and subsequent demyelination leading to progressive axonal degeneration and neurological decline. The adult brain retains the potential for remyelination through injury-induced activation of parenchymal and neural stem cell (NSC)-derived oligodendrocyte progenitor cells (OPCs) that upon demyelination start to proliferate and differentiate into remyelinating oligodendrocytes. However, the cellular mechanisms underlying remyelination, the migratory behavior of OPCs towards the injury site, and the timing of remyelination remain largely unknown on a single cell level. We here used chronic in vivo 2-photon imaging combined with sparse genetic labeling strategies to follow parenychmal and NSC-derived OPCs in response to a demyelinating injury in the murine corpus callosum (CC). Aim of our study is to delineate the cellular principles of remyelination in the adult mouse brain.

11 | In Vitro modeling of human basal ganglia development using human induced pluripotent stem cells

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Tridimensional (3D) cortical organoids derived from human induced pluripotent stem cells (hiPSCs) are emerging as an accurate and versatile in vitro model with the capability to mimic early aspects of human brain development. Current organoid protocols give rise to neuronal cells mainly comprised of cortical excitatory lineages, resulting from a dorsal telencephalic commitment. Treatment of 3D cortical organoids with ventral morphogens such as fibroblast growth factors (FGFs) can

enhance their ventral telencephalic identity. The subsequent addition of differentiationLpromoting factors will enable organoid cultures to mature into specific types of neurons that populate the ventral forebrain. Careful optimization of the type, concentration and timing of these agents is needed in order to favor ventral forebrain development over hindbrain. Organoid analyses via immunocytochemistry, real time PCR, in situ hybridization, and RNA sequencing will enable comparison of organoids with human developmental datasets to confirm the achievement of a human embryonic basal telencephalic identity and whether it will be possible to obtain separate medial and lateral ganglionic regions, which originate GABAergic neurons for the cerebral cortex and the striatum, respectively. This model will foster empirical exploration of both human brain development and complex neurodevelopmental disorders affecting basal ganglia function, such as Tourette Syndrome.

12 | The role of increased neurogenesis in olfaction

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The subventricular zone is the main source of neural stem cells (NSC) in the mammalian brain and, although the physiological role of adult neurogenesis remains controversial, current strategies aim to manipulate this process as a promising approach towards therapy.

Despite several efforts, controlling the proliferation versus differentiation of NSC remains a challenge. Our group has shown that the expansion of endogenous NSC can be controlled during embryonic development and adulthood by shortening the G1 phase of their cell cycle upon overexpression of Cdk4/cyclin D1 (4D) (Artegiani et al., 2011; Nonaka-Kinoshita et al., 2013).

We generated a transgenic mouse line that allows the temporal and reversible control of 4D overexpression. We found that switching on 4D increases the population of NSC while switching it off afterwards allows their physiological differentiation. As a consequence of the transient expansion of NSC, the final neuronal output is increased. The 4D-derived neurons survive for more than two months, integrate into the local circuit and are electrophysiologically active.

Interestingly, mice with more neurons display a similar olfaction proficiency as compared to controls, but seem to be faster when discriminating between very similar odors and when challenging their olfactory memory.

13 | The epigenetic impact of transposable elements in human neural progenitor cells and mammalian brain development

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Endogenous retroviruses (ERVs) are increasingly being recognized as important drivers of evolution by re-wiring of the gene regulatory networks. We find a region- and developmental stage-specific expression pattern of ERVs in the developing human brain. We demonstrate that almost ten thousand, primarily primate-specific ERVs, act as docking platforms for the epigenetic co-repressor protein TRIM28 in human neural progenitor cells (hNPCs), which results in the establishment of local heterochromatin. Thereby, TRIM28 represses ERVs and consequently regulates the expression of neighboring genes. These results uncover a gene regulatory network based on ERVs that participates in control of gene expression of protein-coding transcripts important for brain development.

14 | Time-resolved, gene-specific assessment of neural human organoids for High-Throughput Approaches

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Over the last few years, 3D cell culture has found a rapid following in the form of self-organizing organoids. As organoids more closely mimic cells' tissue-specific microenvironments, organoid approaches may help bridge the gap between in vitro and in vivo experiments. The ultimate goal would be to utilize tissue-specific organoids in High-Throughput Screening (HTS) approaches; however, rigorous standardized gene-specific quantification of organoids as needed for screening has so far been elusive.

HTS-compatible screening readouts need to be fast, automatable, and cost-effective to render them scalable to thousands of samples. Organoids are inherently heterogeneous, as their protocols must allow for self-organization, which – to a degree – is random. Thus, no organoid resembles another organoid, even in the same batch. This makes screening strategies more challenging, requiring a pre- and post-treatment measurement for each particular organoid within a screen. Neural organoids tend to be large (1-3 mm diameter) and optically dense, making them unsuitable for HTS-compatible light or fluorescent microscopy. Biochemical strategies (ELISAs, PCRs) as endpoint measurements destroy their samples. This, coupled with the heterogeneous nature of organoids, prevents a pre- and post-treatment assessment, necessitating novel analysis strategies.

Here we have developed a secretable dual-luciferase-based readout that can quantify and normalize gene-specific activity pre- and post-treatment on a per-organoid basis. We demonstrate this by measuring Sox2 and TH activity in human neural organoids during several weeks of differentiation. Our findings enable the use of quantitative HTS-based approaches in the field of organoid biology and may lay the foundation for next generation 3D HTS.

15 | Chromatin accessibility and transcriptional landscapes during neurodevelopment

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Development of multicellular organisms from a pluripotent state is a complex process, which requires the repression of pluripotency and the activation of a particular lineage-specific differentiation program. During this process cells undergo dramatic changes at the level of chromatin organisation, interactions between transcriptional factors, gene expression and protein production. Many in vitro differentiation systems are extensively used for genetic manipulations and as disease models. Mouse embryonic stem cells (ESCs) induced with retinoic acid to differentiate into post-mitotic neurons, represent an attractive system to delineate neuronal development, as it generates a homogenous population of neurons that form functional synapses. Our differentiation protocol robustly produces a uniform population of cortical neurons containing mostly excitatory glutamatergic neurons with a small amount of inhibitory GABAergic neurons and no detectable non-neuronal cells. Here we integrate chromatin accessibility (ATAC-seq) and gene expression (RNA-seq) data acquired at several time points of neuronal differentiation to better understand chromatin dynamics from ESCs to post-mitotic cortical neurons. Using a custom-developed pipeline, we are able to find transcription factor binding sites in genomic regions with differentially accessible chromatin across neurodevelopment and to infer the potential consequences on gene expression. In addition, we integrate publicly available epigenome modifications and genome interaction data to correlate gene expression changes with altered chromatin interactions. This study provides new insights into the chromatin changes that underlie normal neurodevelopment.

16 | Maternal thyroid hormones function as a cell fate determinant during neurodevelopment in zebrafish

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Maternal thyroid hormones are amongst signals regulating neural development in vertebrates. However the role for these hormones as been largely neglected and only now evidences are arising that it plays a key role in the development of the nervous system. MTHs are involved in cell fate decision during zebrafish neurodevelopment. Depletion of MTHs during zebrafish embryogenesis influences the balances between differentiation of different classes of neurones and glial cells. In parallel neural stem cell pools are depleted in the absence of MTHs signalling due to precocious exit from cell cycle. Our data suggest that MTHs act via NOTCH signalling and depletion of the hormones leads to increased post-mitotic neurones at the expense of decreasing neural stem cells. As well our data points that MTHs are also involved in the correct balance between different glial cell types in the developing nervous system of zebrafish. Collectively, MTHs seem to function as key factor in the establishment of correct nervous system architecture. We are currently investigating the detailed molecular and cellular mechanisms by which this regulation is achieved. This body of data withholds an unexpected importance of MTHs in zebrafish neurodevelopment and interactions with key developmental pathways up until now unknown.

17 | From cohorts to neuronal fate circuitry: dose-response patterns of endocrone disruption in human neurodevelopmental models

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Endocrine disrupting chemicals (EDC) are a class of compounds interfering with the human hormonal system. A growing body of evidences correlates early life EDC exposure with neurodevelopmental disorders but the molecular events triggered by EDC remain to be elucidated.

For the first time we interrogate the impact of EDC mixtures while previous studies were focusing on single compounds. We break new ground by integrating a uniquely large mother-child pregnancy cohort, where a specific EDC mixture was associated to adverse outcomes, with in vitro exploration of the altered gene networks in human cellular systems.

We used three complementary models that transcriptionally resemble the early stages of brain development: cortical neural precursor, differentiated from human pluripotent stem cells with the ngn2 overexpression and through the generation of 3-dimensional cortical brain organoids, as well as human fetal neural progenitors.

Several mixture dilutions, including the environmental concentration, were exposed for 48 hours on the three systems.

The transcriptome analysis across EDC concentrations highlighted differentially expressed genes (DEGs) that, through clustering and regression models, followed non-linear and non-monotonic dose-response patterns reflecting the complexity of mixture effects.

DEGs were significantly enriched for autism spectrum disorders and intellectual disability-causative genes as well as gene ontology categories related to chromatin modulation and regulation of gene expression, confirming that EDC have a significant impact on cell fate acquisition during early brain development.

In conclusion, we show the first elucidation of the transcriptional impact of real life and hence policy-relevant EDC exposure, on gene networks linked to neurodevelopmental disorders.

18 | Attenuation of Robo signaling promoted cerebral cortex expansion during evolution

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Brain size in reptiles, birds and mammals differs dramatically owing to developmental differences in speed of production and final abundance of neurons, but the genetic mechanisms behind this evolution of neurogenesis are largely unknown. Here we analyzed neurogenesis in the mouse olfactory bulb and neocortex, two structures with different size and growth rate. In the olfactory bulb, direct neurogenesis from Radial Glia Cells is abundant, driving fast but limited neuron production, whereas in the larger neocortex most neurogenesis is indirect via basal progenitors. Gain- and loss-of-function manipulations in mouse, chick and snake demonstrate that high Slit/Robo signaling, combined with Robo-dependent low Dll1, are necessary and sufficient to drive direct neurogenesis. This mechanism is prevalent in the cerebral

cortex of chick and snake embryos but dampened in mouse, allowing indirect neurogenesis. Our findings suggest that attenuation of Robo signaling contributed to increase neuron production and cortical expansion during mammalian evolution.

19 | Divergent trajectories of adult neurogenesis in Kabuki syndrome

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Kabuki syndrome (KS) is an intellectual disability (ID) disorder caused by loss of function alleles encoding the histone-modifying enzymes KMT2D (in KS1) or KDM6A (in KS2). These enzymes modulate histone methylation at H3K4 and H3K27, respectively, and clinical overlap is thought to result from commonality of mechanism, namely reduced chromatin accessibility at critical genomic loci. Prior work in a KS1 mouse model demonstrated genome-wide deficiency of H3K4me3, impaired adult neurogenesis in the dentate gyrus (DG), and hippocampal memory defects, all of which were rescued by histone deacetylase inhibition. Now, quantitative imaging of the adult neurogenic cascade in two KS mouse models reveals alterations at neural stem cell (NSC) and neural progenitor cell (NPC) stages, chiefly in the visuospatial-linked septal DG region, compared to wild-type littermates. The radial glia-like NSC niche shows similar deficiency in both KS1 and KS2, but surprisingly, upon transition to proliferative NPC and neuroblast stages, KS1 and KS2 show diametrically opposing phenotypes; these cells show a paucity in KS1, while in KS2 they show a surplus. Despite quantitative differences, neuroblasts of both KS1 and KS2 showed aberrant migration in the DG. How divergent neurogenic trends are reconciled to produce indistinguishable clinical ID phenotypes in KS patients remains unclear, but single-cell expression profiling of adult-born NPCs reveals bifurcating maturation trajectories that mirror the observed proliferation divergence. Collectively, our results suggest that the pathophysiological basis of ID in KS involves aberrations in NSC cell cycle, as well as proliferation, maturation, and integration defects of NPCs in the adult hippocampus.

20 | Investigating the molecular mechanisms of direct neuronal reprogramming by forced expression of proneural genes

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Direct reprogramming is a powerful tool to generate specific cell types difficult to obtain, such as neurons. Ascl1 is as a key reprogramming factor used in different cellular contexts: interestingly, it converts astrocytes into GABAergic neurons (Heinrich et al., 2010), and mouse embryonic fibroblasts into glutamatergic neurons (Chanda et al. 2014) or motor neurons (Gascón et al, unpublished), highlighting the influence of the cell of origin for the outcome of the direct neuronal reprogramming.

To explore the molecular mechanisms governing direct neuronal reprogramming and to investigate the impact of the cellular context in the reprogramming outcome, we took advantage of the OHT-inducible form of Ascl1 (Ascl1ERT2) (Masserdotti et al. 2015) and examined the initial phases of direct neuronal conversion of mouse embryonic fibroblasts (MEFs) and murine postnatal astrocytes following Ascl1ERT2 activation. First, we have verified that Ascl1ERT2 could convert MEFs into neurons as efficiently as Ascl1. Subsequently, we have sorted transduced MEFs (24 and 48 hours) and astrocytes (4, 24 and 48 hours) expressing only DsRed or Ascl1ERT2 activated or not by OHT at different time points and analysed the transcriptome by RNA-seq.

This approach revealed that MEF-to-neuron conversion is delayed compared to the astroglia-to-neuron conversion and Ascl1ERT2 regulates different genes and pathways (GO biological processes) in these different cell types to achieve direct neuronal reprogramming. Taken together, these data suggest that the cell of origin has a major impact on the function of Ascl1, inducing apparently different pathways to generate neurons when starting from different cell types.

21 | Nuclear envelope limited chromatin sheets are a unique nuclear envelope structure conserved in neural stem cells of different species

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Neural stem cells (NSCs) of the adult ventricular-subventricular-zone (V-SVZ) originate in the embryo and show astrocyte features. In many vertebrates, neurogenesis continues postnatally and into adulthood in this region. NSCs are reactivated postnatally to function as primary progenitors for neurons destined for the olfactory bulb. In our recent work we describe that a subpopulation of quiescence B cells has a unique nuclear envelope invagination specialization identified as nuclear envelope-limited chromatin sheets (ELCS). This nuclear structure represents an specific nuclear compartment. We have found that B cells with ELCS dramatically decrease with age in the V-SVZ of rodents. Here we describe that nuclear ELCS are conserved in astroglial cells located in the V-SVZ of different vertebrates such as teleosts, lizards and non-human primates.

22 | Regulation of cerebral cortex development by miR3607

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The presence of an extensive OSVZ in the developing primate cerebral cortex is thought to be highly responsible for gyrification. This germinal layer houses a special type of progenitor cell, basal Radial Glia Cell (bRGC), with high amplification potential and directly related to folding. Cortical areas with greater OSVZ proliferation give rise to folds, and areas with lower OSVZ proliferation give rise to fissures. The molecular mechanisms regulating this regional difference in germinal zones are unknown. A microarray analysis was carried out in our lab to identify genes differentially expressed in germinal layers of prospective gyrus and sulcus, revealing some miRNAs along with several of their target genes. Based on this analysis and in silico predictions, we hypothesize that miR3607 might act as an important regulator of expansion and gyrification of the cerebral cortex. Experiments of gain of function for miR3607 in mouse embryos indicate its involvement in the regulation of proge-

nitor cell lineage and neuronal migration. Overexpression of miR3607 altered distribution of cells across the cortex compared to miRscrambled control with a loss of cells from germinal layers and an increase in neuronal layers. These differences were accompanied by changes in the proportion of Pax6 and Tbr2 positive cells. These findings suggest that miR3607 levels regulate the balance between progenitor cell self-renewal and neurogenesis. Ongoing work is aimed at investigating the role of miR3607 in gyrification of the cerebral cortex using ferret as a model system.

23 | Regulation of region-specific interneuron distribution

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The mammalian cerebral cortex is a remarkably complex organ responsible for the perception of sensory stimuli, the execution of motor actions, learning, cognition, and consciousness. From medial to lateral, the mammalian cerebral cortex consists of three major regions: archicortex, neocortex and paleocortex; each cortical region performs specific functions, has specific cytoarchitecture and neuronal connectivity. The two major neuronal types in the cortex are the excitatory projection neurons and the inhibitory interneurons (INs). Although these two types of neurons are generated separately - the projection neurons are generated in the dorsal telencephalon (dTel) and migrate to their destination by radial migration, while the GABAergic interneurons are primarily generated in the medial and caudal ganglionic eminences (MGE and CGE) of the ventral telencephalon (vTel) and enter the cortex by tangential migration - it is critical for brain function to establish a balance between excitation and inhibition. Here, with a panel of IN subtype markers, we demonstrate that the IN density is consistent in different cortical regions, but the IN distribution patterns are different in the six-layered neocortex and the three-layered piriform cortex (PC). To study how IN distribution is regulated, we studied the IN distribution in the Lhx2 conditional knockout (cKO) mice, in which Lhx2 is deleted by Emx1-Cre. In the Lhx2 cKO cortices, the lateral neocortex is re-fated to generate an ectopic piriform cortex.

24 | Characterization of the spatio-temporal transcriptional and epigenetic landscape of cortical folding

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Brain development relies on sophisticated gene expression programs, which in turn are potentially guided by a timely remodeling of the epigenetic landscape at distinct genomic regions. Compared to species with a smooth cortex like mouse, species with a folded cortex like human and ferret have more complex germinal layers. Despite advances in understanding their differences at the morphological level, the underlying gene regulatory networks are not known. Here, by using ferret as a model system, we have compared the transcriptomes of germinal layers between sulci and gyri, of different cortical areas, and across three developmental stages, to achieve a comprehensive understanding of the spatio-temporal dynamics of gene expression of cortical progenitors. This analysis allows us to understand: 1. Genes important for the gyrus vs. sulcus formation regardless of developmental stages; 2. Genes regulated by developmental gradients regardless of sulcation; 3. Fold-changes in gene expression proportional to depth of folding. Furthermore, we have also characterized the epigenetic landscape of germinal layers at E30 and E34, a critical period for their development, to correlate changes in chromatin at promoter and enhancer regions with the observed changes in gene expression. By performing motif analysis of differentially activated regulatory elements of sulci and gyri and integrating differentially expressed genes (DEGs), we reveal transcription factors which might have a critical role in determining cortical folding. The genes targeted by these factors belong to important pathways implicated in proliferation and neurogenesis. Overall, findings from our comprehensive analysis provide deep biological insights into the complex transcriptional and epigenetic landscape that underlies gyrencephalic brain development and folding.

25 | Loss of CBP interferes with SRF-dependent neuronal growth and synaptic maturation

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Alterations in dendrite branching and morphology are present in many neurodevelomental disorders, which underscores the importance of understanding the transcriptional and epigenetic mechanisms that regulate neuronal outgrowth. The CREB binding protein (CBP) is a large protein with intrinsic lysine acetyltransferase (KAT) activity that functions as transcriptional co-activator for numerous transcription factors. Furthermore, CBP hemi-deficiency causes severe intellectual disability in humans and cognitive impairments in mice. We investigated here the consequences of selectively eliminating CBP in recently born neurons through the characterization of nestin-cre::Crebbpf/f and nestin-creERT2::Crebbpf/f mice. Nestin-cre::Crebbpf/f pups suffer perinatal death as a consequence of diaphragm innervation defects. In accor-

dance with these results, ex vivo and in vivo morphological analyzes demonstrate that the absence of CBP in hippocampal neurons interfered with dendrite outgrowth, spine maturation and chemically induced long-term potentiation (cLTP). RNA-seq and Chip-seq data linked these phenotypes with defective activation of transcriptional programs involved in dendritogenesis, synaptogenesis and synaptic activity. Some important activity-regulated genes affected were Bdnf, Npas4, Fos and Nptx2, which are known to be downstream of the serum response factor (SRF), an activity-regulated transcription factor that interacts with CBP. To specifically examine the contribution of impaired formation of CBP-SRF complexes to the growth and transcriptional defects, we assessed the effect of the viral transduction of a constitutively active SRF protein. Notably, SRF-VP16 ameliorated the growth and transcriptional defects, indicating that CBP loss in newborn neurons specifically interferes with SRF-dependent transcription, dendritic growth and synaptic maturation.

26 | Implication of the RNA-binding protein MEX3A in adult neurogenesis

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The subependymal zone (SEZ) in the lateral ventricles is the most active neurogenic niche in the mammalian adult brain. Subependymal neural stem cells (NSCs) continually generate neuroblasts and oligodendroblasts that migrate to the olfactory bulb (OB) and corpus callosum, respectively. NSCs in this niche are highly regulated by both extrinsic and intrinsic factors. Recently, RNA-binding proteins have emerged as interesting key regulators of many cellular processes such as cell division and differentiation, as they can quickly switch gene expression by stabilizing or promoting degradation of mRNAs. Here, we show that RNA-binding protein MEX3A is highly expressed in neuroblasts in vivo. Interestingly, Mex3a expression is also observed in NSCs and progenitors, although these populations show lower levels of expression. This suggests that MEX3A might be playing different roles in each population within the niche. Preliminary results show that Mex3a deficiency results in increased proliferation of PSA-NCAM+ neuroblasts and neurogenesis to the OB, but also in a premature exhaustion of the NSC pool.

27 | Integration of intercellular signaling and mechanobiology in the control of adult neural stem cell pools homeostasis

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The majority of neural stem cells in the adult brain are quiescent but can be activated i.e. re-enter the cell cycle when recruited. The molecular pathways controlling adult Neural Stem Cells (aNSCs) quiescence are beginning to be unraveled, but nothing is known on how aNSC quiescence/activation is coordinated in time and space at the tissue level.

My project aim to explore whether and how intercellular signaling and the physical cellular microenvironment are integrated to regulate aNSC activation.

The dorsal telencephalon (pallium) of the adult zebrafish harbors a constitutively active neural germinal zone located superficially and which overlaps areas homologous to the two neurogenic niches of adult rodents. As a first step, we have developed a non-invasive method to image populations of over 1.000 aNSCs in their endogenous environment in the zebrafish pallium, at single cell resolution and over weeks. Using this method, we have analyzed the spatiotemporal distribution of quiescent and activated aNSCs at the population scale. We are now working on cross-correlating this pattern of aNSC activation with the spatiotemporal dynamics of Notch3 signaling, a known gatekeeper of NSC activation, and with the mechanical microenvironment of the germinal zone (collab. with Dr Bellaiche).

28 | Intrastriatal treatment with microtubule-depolymerizing agent disrupts the non-cell-autonomous long-range sonic hedgehog signaling in the mesostriatal circuit of mice

Terence Duarte, et al.

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The secreted Sonic Hedgehog (Shh) is a signal that specifies midbrain dopaminergic (DA) neurons during development (Hynes et al., 1995). Opposed this short-range in-

duction, evidence implicates that Shh long range signaling from DA neurons to striatum is crucial for the maintenance of mature mesostriatal circuit. Gonzalez-Reyes et al. (2012) showed that ablation of Shh from DA neurons results in cellular and functional corruption of this structure. Related to this mesostriatal circuit, there are evidences showing that the DA axons are more vulnerable to dysfunction of microtubules. Intrastriatal injections of colchicine, a drug that suppress axoplasmatic transport, induce an autotoxic response to certain CNS. The present study was designed to investigate if the Shh trophic loop within the nigro-striatal circuit was affected by this colchicine-induced toxicity. Intrastriatal injection of colchicine (0.05 mg/kg) induced a decrease of TH-positive fibers from injected side on day 14 post treatment, when the striatum underwent atrophy. In contrast, the number of TH-positive neurons in SNpc did not show significant loss. Stereological quantitation of Ach neurons revealed a reduction in the number of these neurons in the dorsal striatum. A significant increase in astrocytic reactivity in striatum from day 3 through 14 post colchicine where observed. In our in vitro experiments, cultured astrocytes did not release Shh 3 days after application of different doses of colchicine. In our in vivo experiments, Western Blot showed that there is a decrease of Shh expression in the forebrain from day 3 through 14 after injection. We also found an upregulation of Shh expression in the ventral midbrain ipsilateral to the toxin injection.

29 | The AhR pathway regulates restorative neurogenesis

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The stem cells of zebrafish brain, ependymoglial cells, react to the injury and generate new neurons, which migrate to the lesion site and contribute to the tissue restoration. (Barbosa et al., 2015; Baumgart et al., 2012; Kroehne et al., 2011).

In order to understand molecular mechanisms underlying this regenerative neurogenesis we analysed the heterogeneity of ependymoglial response in Tg(gfap:GFP) mi2001 zebrafish line using the live in vivo imaging (Barbosa et al., 2015) and found that a particular population of ependymoglia expressing low levels of the GFP transgene directly converts to the post-mitotic neurons in response to the injury.

Changes in the transcriptome of ependymoglia cells identified Aryl hydrocarbon receptor (AhR) to be involved in regulation of ependymoglia differentiation towards post-mitotic neurons. High AhR signalling promotes direct conversion of ``GFP low``

ependymoglia into neurons, whereas decrease in AhR signalling promotes ependymoglial proliferation. We also observed inactivation of the AhR signalling shortly after the injury and return to the basal levels 7 days post injury suggesting the role of AhR in proper timing of regenerative neurogenesis, ensuring the successful repair. Finally, pharmacological activation of AhR signalling in mammalian cerebral cortex after traumatic brain injury decreases reactive astrocytes proliferation and directs them towards acquisition of the neural stem cell properties.

Overall, we identified important role of AhR signalling in regenerative neurogenesis in zebrafish brain as well as functional conservation of the aryl signalling pathway controlling the differentiation state of glial cells in the Vertebrate brain and therefore our data open a potentially new avenue for therapeutic interventions after the traumatic brain injury.

30 | Deciphering the molecular function of the novel nuclear protein Trnp1 in brain development

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Evolution of the mammalian brain encompassed a remarkable increase in size of the cerebral cortex(CC) including tangential and radial expansion. We have identified Trnp1 as a regulator of CC expansion in both of these dimensions; however the molecular mechanisms to promote both expansions are still unknown.

Predictions of Trnp1 structure revealed N-and C-terminus intrinsically disordered regions (IDRs) and a central alpha-helix domain with the capacity to form coiled-coil (CC) structures. Using different mutant forms of Trnp1 we showed that the first 16 conserved aminoacids are important to promote proliferation, the CC is important for its retention in the nucleus most likely by interacting with chromatin factors, and the IDRs are important for Trnp1 oligomerization.

IDRs containing proteins (IDPs) have a central role in the regulation of crucial cellular processes like regulation of transcription, splicing, translation and cell cycle. IDPs also play a central part in the ordered assembly of macromolecular machines such as the ribosome, in the organization of chromatin, in the assembly/disassembly of microfilaments/microtubules and in the functioning of chaperones among others. MS analysis

of the Trnp1 interactome revealed that Trnp1 interacts with proteins involved in most of these processes. Most excitingly, the mutant Trnp1 proteins that no longer increase proliferation also fail to localize nucleolar proteins. Thus, Trnp1 contributes to the nucleoli biogenesis which affects cell proliferation. The functional relevance of other core interactors including those affecting splicing will be presented.

Taken together, Trnp1 is a multifunctional protein using different domains for protein interactions affecting nucleolar, chromatin or splicing function.

31 | Molecular control of lineage diversification in the ventral telencephalon

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In the developing central nervous system (CNS) area-specific programs initiated by patterning processes in the early embryo result in the regionalization of the CNS. Different brain regions exhibit variable degrees of lineage expansion from the primary stem cells to the final progeny resulting in different number of neurons generated from a single neural stem cell (NSC). But also within a given region of the developing brain different progeny and distinct lineages are established. The molecular programs driving these diversification processes are poorly understood. The lateral ganglionic eminence (LGE) in the ventral telencephalon harbours a higher diversity of progenitor subtypes and shows larger lineage amplification as compared to most other CNS regions. Moreover, the LGE generates most adult NSCs in the SEZ , all together making the LGE an ideal system to study diversification processes in the developing CNS. Here we show that the cleavage plane orientation in embryonic NSCs regulates the number of NSCs entering quiescence during embryonic development and hence the seeding of future adult NSCs in a time-dependent

manner. Thus, the bifurcation of the lineage of future aNSCs from the lineage of embryonic NSCs is controlled by the cleavage plane orientation. Searching for further molecular determinants we performed transcriptome analysis at the population and single cell level. This led to the identification of Esrrg regulatign the balance between proliferation and differentiation of embryonic NSCs in the LGE but not in the cerebral cortex, showing a brain area-specific control of Esrrg on the expansion of the progenitor pool.

32 | Pax6 regulates cell proliferation and differentiation in mouse cerebral organoids

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The cerebral cortex is the seat of much higher cognitive function and the structure contains highly diverse populations of neurons that form complex neuronal networks. Studies using genetically modified mice have shown that normal corticogenesis requires the transcription factor Pax6, which regulates proliferation and differentiation of radial glia cells. Cerebral organoids, a 3D culture system in which cerebral cortex-like tissue can be grown in vitro from pluripotent stem cells present an attractive tool to decipher the underlying mechanism of Pax6. Since the system is recently developed, our aim is to determine the extent to which cerebral organoids accurately recapitulate early cortical development by comparing Pax6-/- mutant cerebral organoids to that of Pax6-/- embryos. We used a previously established Pax6-/- mouse embryonic stem cell line to generate cerebral organoids and analysed them at day 8 and 9 by immunohistochemistry. We found that both mutant (Pax6-/-) and control (Pax6+/+) organoids formed neuroepithelium with apical-basal polarity and spatial separation of progenitor and neuronal layers. Basal progenitor cells (Tbr2+) and deep layer neurons (Tbr1+) were also present in the correct arrangement, in both mutant and control organoids, indicating cortical identity. However, Pax6-/- mutant organoids had a thicker neuronal layer with a lower proportion of Tbr2+ cells suggesting abnormal differentiation. Furthermore, quantitative analysis showed a higher proportion of abventricular mitosis and a significant difference in the proportion of cells in S-phase, suggesting proliferation dysfunction. Taken together, these defects are characteristic of the small eye (Pax6-/-) mutant mouse, suggesting comparable genetic pathways between these two systems.

33 | Exploring RNA Polymerase II role in circular RNA biogenesis in neurons

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Rpb1 is the largest subunit of RNA polymerase II (RNAPII) and it contains a long C-terminal domain which is targeted by dynamic post-translational modifications at different stages of the transcription cycle. These modifications are important for proper processing of nascent transcripts, but have not been explored in terminally differentiated neurons. CircRNAs have been recently described as a novel class of RNAs that is most abundant in neuronal cells and tissues, and whose function and biogenesis remains ill understood. To understand whether regulation of RNAPII pausing and post-transcriptional modifications of its CTD regulate circRNA formation, we have mapped the chromatin occupancy of modified forms of RNAPII by ChIP-seq and identified circRNAs by RNA-seq at different stages of in vitro differentiated dopaminergic and spinal motor neurons. CircRNAs are produced at all differentiation time-points peaking at differentiated cells. Our current work explores the mechanisms by which RNAPII post-translational modifications and processivity may modulate the production circRNAs in neurons. Understanding how RNAPII regulation affects circRNA production will help clarifying how terminal neuronal cell fate is acquired and maintained.

34 | Role of miRNAS in brain early development

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During early telencephalic development a small domain of progenitors in the rostral pallium initiates a developmental program different from the nearby pallium causing a marked tissue growth and evagination, leading to the eventual formation of the olfactory bulb (OB). The mechanisms regulating this specialization of rostral pallial progenitors into generating the OB remain unknown. Here we tested the potential role of miRNAs in controlling the behavior of OB progenitor cells using a Dicerflox/

flox;Rx-Cre mouse, deficient in Dicer-dependent miRNAs since the onset of telencephalic development. We find that the absence of miRNAs from early stages of brain development causes deficits in OB formation, which is smaller than in WT littermates. Rx-Dicer mutants display a high frequency of cell death from early stages, affecting progenitor cells. At later stages this deficit is compensated by hyperproliferation and the formation of rosette-like structures in the basal telencephalon. RNA sequencing analysis of the primordium of the OB (pOB) from WT and Dicer KO embryos shows a loss of let-7 family miRNas together with an upregulation of genes involved in apoptosis (p53 pathway) and proliferation (like IRS-2) in Dicer mutants. Finally we show how deleting functional p53 in Dicer KO animals, there is no rosette formation and furthermore, performing GOF experiments of IRS-2 by IUE we can generate rosette-like structures in the rostral telencephalon. Our results demonstrate a crucial role of let-7 miRNAs during rostral telencephalon development, controlling oncogenic cascades that includes both apoptosis and cell proliferation.

35 | Differential routing of Mindbomb1 via centriolar satellites regulates asymmetric divisions of vertebrate neural progenitors

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Unequal maturation of centrosomes has been associated with differential fate choices in several models of asymmetric cell division. In asymmetrically dividing Radial Glial cells (RGCs) of the mouse cortex, the "old" and "young" centrosomes are preferentially inherited by the self-renewing RGC and the differentiating cell, respectively. Nevertheless, centrosomal fate determinants have yet to be identified. In addition, how centrosome asymmetry is compatible with symmetric divisions that are predominant at early stages remains elusive. To address these two questions, we took advantage of live imaging techniques to monitor the behavior of Mindbomb1 (Mib1), a regulator of the Notch pathway, in chick neural progenitors. Mib1 is a mono-ubiquitin ligase involved in the trafficking of Notch ligands and promoting their activity.

Here, we show that the Notch pathway regulator Mindbomb1 co-localizes asymmetrically with centriolar satellite proteins PCM1 and AZI1 at the daughter centriole in interphase. Remarkably, while PCM1 and AZI1 remain asymmetric during mitosis, Mindbomb1 is associated with either one or both spindle poles. Asymmetric Mindbomb1 correlates with neurogenic divisions and Mindbomb1 is inherited by the prospective neuron. By contrast, in proliferative divisions, a supplementary pool of Mindbomb1 associated with the Golgi apparatus in interphase is released during mitosis and compensates for Mindbomb1 centrosomal asymmetry. Finally, we show that preventing Mindbomb1 centrosomal association induces reciprocal Notch activation between sister cells and promotes symmetric divisions. Thus, we uncover a link between differential centrosome maturation and Notch signaling and reveal an

unexpected compensatory mechanism involving the Golgi apparatus in restoring symmetry in proliferative divisions

36 | Conserved transcriptional regulatory rules define the serotonergic neuron identity.

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Cell differentiation is controlled by individual transcription factors (TFs) that together activate a selection of enhancers in specific cell types. How these combinations of TFs identify and activate their target sequences remains unknown. Here, we identify the cis-regulatory transcriptional code that controls the differentiation of serotonergic (5HT) HSN neurons in C. elegans. Activation of the 5HT transcriptome is orchestrated by a collective of six TFs. Binding site clusters for this TF collective form a regulatory signature that is sufficient for de novo identification of HSN neuron functional enhancers. Mouse orthologs of this TF collective also regulate 5HT differentiation and can functionally substitute for their worm counterparts. Finally, among C. elegans neurons, the HSN transcriptome most closely resembles that of mouse 5HT neurons, which reveals deep homology. Our results identify rules governing the transcriptional regulatory code of a critically important neuronal type in two species separated by over 700 million years.

37 | Unveiling the impact of developmental origin on oligodendrocyte fate and heterogeneity by single-cell transcriptomics

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Oligodendrocytes (OLs) are the myelinating cells of the central nervous system. We have recently shown that the whole OL lineage is formed of twelve subpopulations/

states (Marques et al., Science 2016). OLs differentiate from Oligodendrocyte Precursor Cells (OPCs). During development, OPCs originate in three waves and domains in the forebrain: embryonic day (E)12.5, 15.5 and postnatal day (P)0 from ventral and dorsal zones, respectively. We have also recently shown that dorsal OPCs are more reactive in case of demyelination compared to ventral OPCs (Crawford et al., Cell Reports 2016).

This evidence suggests that the developmental origin of OPCs might influence their differentiation potential into specific OL subpopulations/stages with distinct functions. In order to investigate this question, we used mice bearing the dual-reporter Emx1::Cre-Sox10-loxP-eGFP-STOP-loxP-TdTomato, in order to distinguish dorsal derived (TdTomato+) from ventral derived (eGFP+) OLs.

We analysed these populations by Single-Cell Drop-Seq transcriptomics. Clustering analysis shows that the environment where OPCs migrate and differentiate has a major influence on their fate. In the corpus callosum, the myelinating MOL2 population is more abundant than in the cortex. We also crossed the dual-reporter line with the Sox10-loxP-eGFP-STOP-loxP-DTA mouse line, to ablate Emx1 derived OPCs and performed single cell Drop-Seq. We observed that ventral OPCs repopulate the region and differentiate in the twelve OL subpopulations/states with apparent partial depletion of the progenitor pool. We are continuing to investigate the influence of the OPC developmental origin, intrinsic and extrinsic factors on the heterogeneity of the lineage and their potential effects on their functions.

38 | The regulation of progenitor position during assembly of the cortex

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Cortical development is a finely regulated process that depends on different progenitor types that have expanded during evolution. Perturbations of progenitors can lead to severe neurological disorders in human. Elucidating the underlying patho-mechanisms sheds light on progenitor regulation and function. We are investigating genetic mechanisms related to severe atypical subcortical heterotopia, a cortical malformation associated with epilepsy and intellectual disability. We have previously studied heterotopic cortex (HeCo) mice which show Eml1 loss of function, and shown EML1 mutations in human patients (Kielar et al, 2014). Eml1 codes for a microtubule-binding protein playing roles in different phases of the cell cycle.

HeCo mouse mutants have misplaced ectopic Pax6+ radial glial cell (RG)-like progenitors during cortical development. We question how a proportion of Pax6+ cell somata leave their normal apical localization to continue dividing in more superficial regions. Studying the ventricular zone, we now show at E13.5 that HeCo metaphase RGs present perturbed spindle lengths and spindle orientation, as well as altered

shapes. Focusing also on interphase RGs, which have apical processes attached to the ventricular lining, en face examination at E13.5 shows an altered density of apical elements, as well as their changed size. These are novel findings which contribute to our understanding of mutant cell characteristics in the neuroepithelium.

Exome sequencing of patients with similar phenotypes also reveals new genes involved in these disorders, which also have an impact on RG mechanisms. Using these approaches, we are piecing together molecular determinants and revealing new biochemical pathways regulating RG function.

39 | Regulation of Cux2 expression in neural progenitors: towards uncovering mechanisms of fate specification

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During neocortical development, neurons are produced by a diverse pool of neural progenitors. A subset of these progenitors express the Cux2 gene and predominantly produce late-born excitatory neurons, but the upstream pathways that specify this subset of progenitors remain unknown. Elucidating how Cux2 expression is activated in forebrain progenitors could provide insights into mechanisms of fate specification. To uncover the transcriptional network that regulates Cux2 expression, we are characterizing an ultraconserved Cux2 enhancer that we find recapitulates endogenous Cux2 expression in the developing forebrain. Using bioinformatic analysis of this enhancer to identify putative transcription factor binding sites, we found several potential binding sites for well-known forebrain patterning genes, including Emx1/2, Lhx2/6/8, and Dlx1/2. We are currently testing the role of these candidate transcription factors in regulating expression from the Cux2 enhancer using a combination of in vitro cell culture and in vivo studies. Additionally, as many putative Cux2 regulators identified in our analysis are directly downstream of BMP signaling, we reasoned that BMPs could regulate Cux2 expression in the developing forebrain, as has been reported in other embryonic tissues. We demonstrate that BMP4 can upregulate Cux2 expression from both the Cux2 enhancer and the endogenous Cux2 locus in cell culture and in neocortical explants, respectively. While BMP signaling is known to be important for patterning forebrain progenitor domains, our results suggest that BMPs may also be an important contributing factor for activating the transcriptional network that directs Cux2+ progenitor fate.

40 | TGFß pathway triggers neuronal differentiation transcriptional program by coordinating local events in the context of enhancer-promoter interaction

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Corticogenesis is a coordinated process in which signaling pathways cooperate with transcription factors and histone modifying enzymes to generate different gene programs that will ultimately lead to the required phenotypical traits. At the megabase level, topological associated domains constrain the transcription factor accessibility and the potential enhancer-promoter interactions occurring in a specific time and space. At the same time, at the local level, DNA sequences present at enhancers exhibit binding sites for a plethora of transcription factors that are necessary in specific cell decisions. How dynamic signaling pathways coordinate transcription factor recruitment in the context of enhancer-promoter interactions and to which extent chromatin modifiers cooperating with the cascades impact genomic loops is still poorly understood.

Our work, demonstrates that in mouse neural stem cells obtained from E12.5 embryo cortices, TGFß signaling pathway is orchestrating the neuronal developmental gene program by activating a specific set of enhancers. Using genome-wide data, we point to the proneural factor ASCL1 as the pioneer factor required for SMAD3 recruitment to cis-regulatory regions and we indicate that the known interactor of SMAD3, JMJD3 histone demethylase, is as a new partner of ASCL1 in enhancer activation. Furthermore, using CRISPR-Cas9 experiments, we decipher the involvement of the classic NEUROG2 enhancer in the neuronal differentiation process and we establish a hierarchical order of recruitment of its activating proteins within the three-dimensional chromatin structure.

41 | Induction of outer radial glia by the random spindle orientation causes severe microcephaly in the Aspm mutant mice

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ASPM/MCPH5 is one of the major responsible genes for microcephaly in human, and plays an important role in the spindle formation during neural progenitor mitoses. However, it is known that in mouse model system, mutations in the Aspm gene lead to only a modest reduction in brain size, implying that the spindle abnormalities caused by the Aspm mutations may have different impacts on the brain development in rodents and primates.

One of the prominent features of the complex brain development is the emergence of non-apical neural progenitors, outer radial glia, residing in the outer subventricular zone (OSVZ) during latter stages of the development. To mimic this situation in mice, we used LGN mutant mice, in which the random spindle orientations frequently transform apical radial glia into ectopic outer radial glia without affecting neural differentiation or cell survival.

In the Aspm mutant mice, we found that neural progenitors exhibited p53-dependent apoptosis in a low frequency. Surprisingly, the rate of apoptosis in the Aspm mutant was dramatically enhanced by inducing outer radial glia by the LGN mutation. The Aspm LGN double mutant showed a severe reduction in brain size with a decrease in the number of neurons. These results imply that the developmental scheme with the OSVZ and the outer radial glia is vulnerable to spindle malformations in neural progenitors. These findings will help us understand the mechanism underlying a serious microcephaly in the gyrencephalic brain due to the spindle abnormalities.

42 | Epigenetic mechanisms underpinning neurogenesis in the cnidarian Nematostella vectensis

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Cnidarians represent the sister group to bilaterian animals and as such are a pivotal group for understanding the evolution of developmental programs. In particular, it is clear that bilaterian and cnidarian nervous systems share a common origin and so a detailed understanding of cnidarian neurogenesis should provide insight into the biology of the ancestral nervous system. Nematostella vectensis has become the foremost cnidarian model with regard to neurogenesis due partly to major technological developments in recent years. We, and others, have identified the neural progenitor cells in Nematostella and begun to unravel the gene regulatory network underpinning Nematostella neurogenesis. We have carried out a micro-array screen to identify genes expressed in neural cells in Nematostella and identified several epigenetic regulators including members of the CoREST complex and Polycomb genes. Research on epigenetics and chromatin in cnidarians is currently in its infancy although it appears that the major principles of bilaterian chromatin organization are conserved in cnidarians. We aim to study the function of these genes in Nematostella neurogenesis in order to uncover the mechanisms shaping the epigenome

during neural differentiation. In addition we will use FACS sorting of transgenic lines labelling different neural populations in combination with RNA sequencing and various chromatin mapping techniques to characterize changes in the transcriptome and epigenome underlying cell fate transitions in the nervous system. This data will help to shed light on the core principles of transcriptional regulation underpinning neurogenesis in cnidarians and identify ancestral features critical for neurogenesis across animal phyla.

43 | CB1 receptor signaling in embryonic stem cell differentiation into cortical neurons

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Pluripotent embryonic stem (ES) cell cultures constitute a powerful tool to investigate key aspects of nervous system development and the regulatory signaling mechanisms involved in neuronal generation and differentiation. The endocannabinoid system exerts a regulatory role of neurodevelopment that influences neural progenitor proliferation, identity and neuronal differentiation. In particular, CB1 receptor signaling controls the differentiation of corticofugal deep layer neurons owing to its ability to regulate the transcription factor switch Ctip2/Satb2. We have developed an ES default neuronal differentiation paradigm aimed to the generation of cortical projection neurons. ES neuronal differentiation generates mainly excitatory glutamatergic neurons (Glu, vGlut1,...) that express distinctive markers characteristic of upper and deep layer cortical neurons (Tbr1, Ctip2, Satb2,...). In addition, ES generated glial cells are although at a lesser extent. Proliferating murine ES cells and their differentiated neuronal progeny express CB1 receptors and therefore constitute a robust tool to investigate the role of CB1 signaling in the transition of pluripotent ES to multipotent neural stem cells, and secondly in their neuronal differentiation program. Pharmacological regulation of CB1 receptor signaling during ES-neuronal differentiation reveals a pro-neurogenic action, with increased expression of deep-layer, and reduced upper-layer, cortical neuronal projection markers. Ongoing characterization of the neuronal differentiation changes upon CB1 receptor knockdown in ES cells will be shown. In summary, these findings support that CB1 receptor signaling exerts a cell-autonomous action in ES-derived neuronal differentiation favouring deep-layer specification.

44 | Determining the role of mammalian H3.3 modifications in developing and mature neurons by mutagenesis of histone tail residues

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Numerous mutations in histone H3-modifying enzymes in neurodevelopmental disorders suggest that a critical level of histone N-terminal modifications is required for cell lineage specification. In support of this, considerable research tightly links many histone modifications with a distinct transcription state during development. In this study we want to identify the functional roles of H3 residues and their post-translational modifications in developing and mature neurons through lysine-to-alanine mutations. Using CRISPR-Cas9 mediated gene editing, we introduce point-mutations into histone variant H3.3 in mouse embryonic stem cells (mESCs). H3.3 variant accumulates in the brain throughout development and into adulthood, where it becomes the predominant H3 protein in neuronal nucleosomes. We profiled the genomic and phenotypic outcomes of lysine 4 and 36 mutations by measuring changes in transcription and histone modifications during neuronal differentiation in vitro. We find that lysine 4 of histone H3.3 is crucial for histone stability and mutations of this residue result in degradation within chromatin and widespread transcription changes even in the ESC state indicating a gain-of-function impact. In contrast, mutation of lysine 36 of H3.3 does not influence histone stability, but significantly reduces tri-methylation (H3K36me3) levels. Comparison of changes in H3K36me3 with transcription levels suggest that the modification states of lysine 36 control specific subsets of genes that are crucial for neurogenesis and functions in post-mitotic neurons. We envision that H3.3 tail mutations can serve as an experimental platform to study the effect of histone modifications on cell lineage specification and transcription.

45 | The acetyltransferase CBP is dispensable for proliferation of neural progenitors but essential for neural differentiation

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Rubinstein-Taybi syndrome (RSTS) is a genetic neurodevelopmental disorder characterized by mental impairment and a wide spectrum of congenital abnormalities, that is caused by hemizygous mutations in the genes encoding the KAT3 family of transcriptional co-activator CREB binding protein (CBP) and the E1A binding protein P300 (p300). Both factors have intrinsic lysine acetyltransferase (KAT) activity and are critically involved in the transcriptional and epigenetic regulation of gene expression. Consistent with this central role, CBP and p300 knockout (KO) mice exhi-

bit early embryonic death and neuronal tube closure defects. However, the precise function of these proteins during the development of the central nervous system has not been clearly stated. Here we assess whether KAT3 proteins are necessary for cell proliferation and neuronal differentiation, since this would explain some of the neurological alterations associated with RSTS. Conditional removal of CBP specifically from retinal progenitors (using Rx-Cre line) or from differentiated retinal neurons (using Brn3b-Cre line) demonstrate that the loss of CBP in differentiated neurons does not affect neuronal viability nor identity, while its deletion in progenitors have a negative impact in the number of retinal neurons. In turn, experiments on neurospheres lacking CBP demonstrate that cell proliferation is normal in the absence of CBP, but both glial and neuronal differentiation are seriously compromised. Ongoing experiments designed to remove either p300 alone or in combination with CBP, both in vitro and in vivo, should further clarify the role of KAT3 proteins in neural differentiation and reveal the redundancy between the two paralog transcriptional coactivators.

46 | The c-Abl kinase modulates synaptic gene expression and maturation

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Introduction: At the earliest onset of Alzheimer's disease (AD) the Aß-oligomers (AßOs) induce a decrease on dendritic spine density in hippocampus and brain cortex, leading to cognitive impairment and memory loss. Our group has shown that the AßOs activate the c-Abl tyrosine kinase downstream activation of the ephrin receptor EphA4, causing synaptic loss, and synaptic gene repression by stabilization of HDAC2. Imatinib, a specific inhibitor of c-Abl, prevents neuronal damage and the synaptic loss induced by AßOs, via inhibition of the c-Abl signalling in primary culture hippocampal neurons. However, other neuronal Imatinib targets, such as ARG, c-kit and PDGFR could be contributing to the synaptic protective effects of c-Abl. This is why we used null neurons for c-Abl to evaluate its role on dendritic spine density and morphology changes induced by AßOs.

Materials and methods: We prepared hippocampal neurons from c-Ablflox/c-Ablflox/Nestin-Cre embryos. At 15 DIV we transfected the neurons with a GFP-expression plasmid to visualize spine morphology. At 19 DIV we treated neurons with AßOs for 5 hours to evaluate the number of dendritic spines and morphology. Additionally, we used immunofluorescence techniques for pre-or post-synaptic markers.

Results: We found that the absence of c-Abl protects against synaptic loss induced by Aß-oligomers. Interestingly, we have also observed dendritic spine enrichment in c-Abl null neurons without treatment, and an increase in immature spines in spite of positive regulation of synaptic genes, like AMPAR: Gria2, Gria3, and NMDAR: Grin2a,

Grin2b. Our results support that c-Abl could be participating in the synaptic elimination in physiological context.

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47 | Alternative splicing regulates neurogenic commitment in the mammalian brain

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During embryonic development cells with an identical genetic information acquire different identities due to regulation of gene expression. Many research have focused on the genes that are activated or repressed during cell fate determination. However this approach does not take into account transcripts diversity originated from alternative splicing, therefore ignoring that a gene can give rise to multiple transcripts with different coding potential, hence function. Our aim to understand how alternative splicing regulates the switch from proliferative to differentiative division of neural stem cells, i.e the neurogenic commitment, in the mammalian developing brain in physiological condition. For this purpose we took advantage of a double transgenic mouse developed by our group that allows the isolation of proliferating progenitors (PP), differentiating progenitors (DP) and neurons, coexisting in space and time in the embryonic mouse cortex. Bioinformatic analysis on the transcriptome obtained from these cell populations allowed me to identify genes differentially spliced in the PP to DP transition. Most of the splicing events detected have an impact on the protein output of these genes. In vivo overexpression by in utero electroporation in the lateral cortex of isoforms with different coding potential derived from a same gene showed isoform-specific effect on neurogenic commitment. These data show that alternative splicing plays a critical role in neurogenic commitment in mammalian brain.

48 | Functions of the p57 imprinted allele in mouse neocortical development

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Mammalian cells have two homologous copies of each chromosome, one from father and one from mother, allowing cells to have a backup. On the other hand, a particular subset of genes have its one allele silenced by an epigenetic mechanism called genomic imprinting. Imprinted alleles are in general believed to have no function. Recent studies, however, showed that the imprinted allele of some genes un-

dergo loss-of-imprinting in the brain and that a few of these derepressed imprinted genes contribute to the maintenance of neural stem cells (Ferrón et al., 2011; 2015). We thus hypothesized that other imprinted genes may also have functions in the brain. In this study, we focused on the cell cycle-dependent kinase inhibitor p57kip2, whose paternal allele becomes silenced during the post-implantation stage. The maternal allele of p57 kip2 is essential for brain development, but the function of its paternal allele remained unknown. We found that the p57 kip2 paternal allele shows a low but significant level of mRNA expression in the developing brain. Importantly, a central nervous system specific deletion of the p57 kip2 paternal allele (p57 kip2 paternal cKO) resulted in malformation of the brain. In the neocortex, the number of upper layer neurons was decreased by p57 kip2 paternal cKO. Consistent with this observation, we found that the number of neural progenitor cells that give rise to upper layer neurons was also decreased by p57 kip2 paternal cKO. These results reveal that the paternal allele of p57 kip2 plays a role in neocortical development.

49 | Decoding Malfunction of Developmental Enhancers in Brain Disorders

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It is essential to understand the etiology of neurological diseases to implement early prevention and intervention. Despite progress in identifying the links between defects during neural development and neurological diseases of childhood and adulthood, underlying molecular mechanisms remain largely unknown. Genome-wide association studies (GWAS) have revealed a number of Single-nucleotide polymorphisms (SNPs) associated with neurological diseases, but the biological relevance of non-coding SNPs, which account for more than 90% of all disease SNPs, remains unclear. In this study, we aim to characterize the influence of non-coding SNPs on enhancer function and transcriptional regulation. For this purpose, we computationally prioritize enhancer SNPs and perform luciferase assays to establish their enhancer activity. To reveal target genes under the control of these distal regulatory elements, we identify the expression quantitative trait loci (eQTL) genes and the nearest genes for enhancer SNPs and experimentally evaluate the effect of distal SNPs on gene expression. To further shed light on the function of enhancer SNPs, a 3C experiment will be performed in a human neuronal system. In addition, we will investigate developmental phenotypes altered by enhancer SNPs using a human brain organoid. To find transcription factors (TFs) whose binding is influenced by enhancer SNPs, we will employ affinity columns with wild type or mutated enhancers as bait sequences and, following the addition of neuronal protein extracts, perform mass-spectrometry. Altogether, this study will identify non-coding SNPs associated with brain disorders and reveal how they affect the distal regulatory landscape, TF binding and gene expression during neuronal development.

50 | The impact of DNA methylation in human neural progenitor cells

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DNA methylation is an epigenetic modification that plays a crucial role in gene regulation throughout development and in adulthood. DNA methylation is traditionally thought upon as a repressor of gene expression. However, with recent literature it is becoming increasingly clear that the view of DNA methylation and gene regulation is far more complex than previously believed.

In this project we are investigating the role of DNA methylation in human neural progenitor cells (hNPCs). By using CRISPR-Cas9 we have successfully deleted DNA methyltransferase 1 (DNMT1), which is the main enzyme responsible for maintaining DNA methylation during replication. Upon the DNMT1 deletion, the hNPCs lost DNA methylation within a few days, as revealed by immunocytochemistry and bisulphite-PCR. In spite of this, the cells were still viable and continued to proliferate, which is in contrast to proliferative somatic cells in mice. The DNMT1 deleted hNPCs were however unable to survive 30days of differentiation towards neurons. In order to further investigate the cells, they were harvested 10days post the DNMT1-deletion and we are currently investigating changes in both the transcriptome, using RNA-sequencing, as well as the proteome, using mass spectometry. In addition, the changes in DNA methylation will be studied using both bisulphite- and oxidative bisulphite sequencing. Together these approaches will allow us to do correlative analyses between methylation (both 5-methylcytosine and 5-hydroxymethylcytosine) and gene- and protein expression. The results will add to the understanding of the complex nature of DNA methylation in human neural development.

51 | Developmental stage-dependent change of SMAD target genes defines the direction of neural stem cell differentiation induced by bone morphogenetic proteins

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During brain development, tight regulation of neurogenesis to astrogenesis switching of neural stem cells (NSCs) is critical to generate a balanced number of each neural cell type for proper brain functions. Accumulating evidence has indicated that a complex array of epigenetic modifications and availability of extracellular factors control the timing of neuronal and astrocytic differentiation of NSCs, however, complete understanding of NSC fate regulation is still far from clear.

Although bone morphogenetic proteins (BMPs) are one group of well-characterized factors to induce astrocytic differentiation of NSCs obtained from mouse forebrain at relatively late-gestational stages (e.g., embryonic day 14 (E14)), we have recently found that BMPs induce neuronal differentiation of NSCs at mid-gestational stages (e.g., E11). To elucidate molecular mechanism underlying this developmental stage specific responsiveness of NSCs to BMPs, we have performed ChIP-seq and RNA-seq analyses of E11 and E14 NSCs after BMP2 stimulation. Genome-wide mapping for the phosphorylated-SMAD1/5 (P-SMADs) binding sites revealed that during this short developmental time period, SMAD target genes were altered dramatically, for example, P-SMADs specifically bound to the promoters of proneural genes, Neurog1 and 2 in E11 but not in E14 NSCs. Conversely, P-SMADs binding on a typical astrocyte marker glial fibrillary acidic protein promoter was observed only in E14 NSCs. Moreover, by analyzing stage-specific P-SMADs binding sites, we identified several novel epigenetically regulated regions, which may contribute to the developmental stage-dependent alteration in the preference of NSC's fate choice.

52 | A mechanism of region-specific neocortical overgrowth relevant to autism spectrum disorder

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The neocortex regulates complex cognitive tasks such as sensory perception and higher cognition. Defects in cortical development are believed to cause neurological disorders including autism spectrum disorder (ASD). ASD children often have an overgrowth of the frontal cortex, but the underlying molecular mechanisms responsible for this region-specific overgrowth as well as its pathological relevance remain elusive. Recently, ASD has genetically been linked to members of fibroblast growth factor morphogens and their signaling pathways. Here we show that manipulation of this signaling pathway in mice from early embryonic stage (around E9.0) results in autism-like region-specific cortical overgrowth at early postnatal stages. Interestingly, manipulation of the same signaling pathway from later embryonic stage (around E11.0) did not cause overgrowth phenotype, suggesting that early embryonic stage before E11.0 is critical for the regional specification of neocortex at postnatal stages. Furthermore, the mutant mice defective in fibroblast growth factor signaling also display autism-like behavioral defects, including increased repetitive behavior and social deficits. Our data together suggest that fibroblast growth factor signaling might have a causal role in brain morphological and behavioral alternations relevant to ASD.

53 | mir-30 family miRNAs: Critical mediators of chronic stress associated changes in the hippocampal neurogenesis

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Depression, anxiety and related mood disorders are major psychiatric illnesses worldwide, where chronic stress appears to be one of the foremost underlying causes. Research in the past decade has shown diverse epigenetic mechanisms involved in mediating the negative effects of chronic stressful events on neural circuits, including the cognitive circuit, where adult neurogenesis gets affected. However, non-coding RNAs, another layer of epigenetic regulation, are relatively lesser studied in this context. Here, using the chronic social defeat stress-induced depression model, we aimed to uncover the dysregulation in miRNA-mRNA networks in the neurogenic dentate gyrus region (DG) of the mouse hippocampus. Analysis of the miRNA array data led us to identify numerous dysregulated miRNAs and the most striking finding was attenuated expression of miRNAs of an entire miR-30 family in defeated mice. To profile miRNAs in the neural stem/progenitor cells (NSCs/NPCs) only, we used the in vitro neurosphere culture system, where adult mouse DG-derived proliferating NSCs/NPCs were subjected to differentiation, and observed differential expression of several miRNAs. Surprisingly, miR-30 miRNAs also appeared in the list of dysregulated miRNAs and showed an upregulation in the differentiated cells. To search for the gene targets of these miRNAs, we performed gene arrays followed by bioinformatic analysis, miRNA manipulation experiments and luciferase assay. Our results suggest that mir-30 miRNAs mediate chronic stress-induced depression, and alter hippocampal neurogenesis and neuroplasticity through regulating few critical epigenetic/transcription and cell signaling regulators implicated in neural differentiation and neurodevelopmental functions.

54 | Pax6 α-enhancer is a battleground of neuronal fate determination in the post-natal retina

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Visual acuity of the animal is sensitive to the number and distribution of retinal neurons that comprise the inner retinal circuitry. Paired-homeobox 6 (PAX6) is a key transcription factor that specifies amacrine cells in the retina. PAX6 expression is regulated by multiple regulatory elements, including the intronic α-enhancer, which is active specifically in GABAergic amacrine cell subsets. We found hydrogen peroxide-induced clone 5 (Hic5)/tumor growth factor ß1-induced 1 (Tgfb1i1) links the

LIM domain transcription factors to form a complex that inhibits the α-enhancer in the post-natal mouse retina. The Hic5-/- mice show elevated α-enhancer activity to overproduce the GABAergic amacrine cell subsets, whereas the mice carry an auto-stimulation-defective Pax6ΔPBS/ΔPBS mutation lose the cells to gain OFF-type bipolar cells. Consequently, the Hic5-/- mouse retinas show sustained light response that becomes more transient in the Pax6ΔPBS/ΔPBS mice. Together, our results suggest the antagonistic regulation of the α-enhancer by Pax6 and a LIM protein complex is necessary for the establishment of a neural circuitry that controls visual adaptation of the retina.

55 | Activity – dependent chromatin regulation during barrelette map formation

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Little is known about how neuronal activity regulates chromatin dynamics in neurons during the assembly of topographic circuits. Here, we investigated the development of brainstem barrelette neurons, relaying whisker-specific somatosensation, as a model system. Using mouse ad hoc genetic tools, we compared epigenetic and transcriptional profiles of control and activity-silenced barrelette neurons at perinatal and early postnatal stages. The analysis includes transcriptome, chromatin accessibility, histone marks and transcription factor binding profiles. By this approach, we identified activity dependent chromatin epigenetic signatures during barrelette map formation.

56 | Functions for miR-128 in cortical neuron maturation and connectivity

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miRNAs have been shown to regulate cellular processes such as migration, proliferation, differentiation and connectivity that are fundamental for the establishment of a functional cortex. Our group showed previously that the brain-enriched miRNA miR-128 plays a decisive role in the developing cortex by regulating migration, dendritic morphology and excitability of upper layer neurons. However the role of this miRNA in deep-layer neurons has not been addressed so far.

We used target site prediction and functional enrichment algorithms to identify the neurotrophin signaling pathways as a probable regulatory node downstream of miR-128. We validated predicted targets in this pathway with known or suspected functions in neurogenesis, connectivity or synaptic transmission using reporter assays. By manipulating miR-128 levels in different developmental stages in the mouse and in neuronal cultures, we are assessing the involvement of these new candidates and their regulation by miR-128 on activity-dependent signaling and cortical development. To achieve a stable and substantial knock-down of miR-128 in vivo, we used the CRISPR Cas9 nickase approach. Combining this technique with in utero electroporation allows specific knock-down of the miRNA in vivo in cell populations of choice in a wildtype background. Successful and specific editing was validated by physical and loss-of-function assays in cultured cells and in vivo. We will report our progress on the functional analysis of miR-128 and its effects on neurotrophin signaling pathways for upper and deep layer neurons using this potent new tool.

57 | Mechanism of neural stem cell transformation by C11orf95-RELA translocations

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Ependymomas are tumors of the central nervous system, arising within the ependymal lining at the ventricle-parenchyma interface. Molecular profiling studies suggests ependymomas in different anatomical compartments are distinct and disparate diseases, with unique cells of origin and genetic drivers. We have recently described a highly recurrent 11q structural variant, producing a fusion translocation between the C11orf95 gene of unknown function and RELA, the principal effector of NF-kB signaling. C11orf95-RELA Fusion proteins, when introduced into neural stem cells, rapidly transform to form ependymoma. Furthermore, recent studies analyzing the genomes and transcriptomes of 500 primary ependymomas have reinforced these findings, showing that C11orf95-RELA fusion proteins are found within ~70% of forebrain (supratentorial) ependymomas and correlated with negative overall survival. However, the molecular events preceding and following Fusion transformation remain largely unknown. In this study we will present our recent efforts integrating transcriptome, proteome, and genome wide mapping of Fusion proteins (as well as their individual components) to understand the mechanisms by which neural stem cells transform to form ependymomas.

58 | A systems level view on miR-124 function during neuronal differentiation from human iPS cells

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The human brain comprises various neuronal cell types, whose underlying developmental programs are still widely unclear. miRNAs, such as miR-124, have been identified as key players of neurogenesis. However, we lack a coherent understanding of miR-124 functions at the systems level.

To reveal which pathways are targeted by miR-124, we have established a complete miR-124 knockout in an easy-to-manipulate cellular system. The model is based on human induced pluripotent stem cells and enables the generation of homogenous neurons within four days. For an extensive analysis we characterized the phenotype, sequenced the RNA over the time course of differentiation and performed RNA immunoprecipitation (RIP-Seq).

Phenotypically, miR-124 knockout cells still differentiate into neurons and express neuronal markers. However, neurite outgrowth capacity is reduced and the differentiation is altered as the cells exit pluripotency faster. In long-term cultures the neurons are more vulnerable suggesting impaired maturation or survival.

Using systems biology and an unbiased consideration of all transcripts under miR-124 control in the RISC complex (via RIP-Seq) we could identify key gene regulatory pathways and targets of miR-124 present in human neurons and monitor their behavior during differentiation.

The data suggests that miR-124 has a modulating role during human neurogenesis. Other factors such as transcription factors still have a greater power. Nevertheless, miR-124 is extremely important for maturation and optimal development of the cells. A systems level view on modulators of neurogenesis and the resulting knowledge about tuners of differentiation can facilitate subtype specification and maturation of neuronal cells from human stem cells.

59 | Insm1 promotes neurogenesis by repressing the neural progenitor program and Notch signaling

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In neural stem cells, the proneural factor Ascl1 controls changes in gene expression to promote the generation of neurons (Castro et al., 2011). In progenitor cells of the ventricular-subventricular zone, Ascl1 induces the expression of Dll1 ligand, which activates transmembrane receptors of the Notch signaling pathway in neighboring cells. This results in upregulation of the repressors Hes1 and Hes5, leading to the inhibition of neuronal differentiation and the maintenance of progenitor cells (Castro & Guillemot, 2011; Imayoshi & Kageyama, 2008).

In neuronal progenitors, Ascl1 induces the expression of the zinc-finger factor Insm1 (Insulinoma-associated 1) that is necessary for neuronal differentiation (Wildner et al., 2008; Farkas et al., 2008; Masserdotti et al., 2015).

Here we show that Insm1 directly represses the expression of distinct Notch family members (e.g. Notch1, Dll1 and Hes1). Genome-wide, Insm1 occupies genomic regions located within the loci of Notch signaling components that are upregulated in the absence of Insm1. Re-introduction of Insm1 in Insm1 deficient differentiating neurons restored the correct expression of the Notch pathway components and rescued the impairment of neuronal differentiation. We propose that Insm1 represses the progenitor program downstream of Ascl1 in neurogenesis.

60 | The role of HAT in the regulation of adult neurogenesis

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Histone acetyltransferases (HAT) and histone deacetylases (HDACs) are important mechanisms to regulate development and cell differentiation. Hypo-acetylation has been linked with a decline of the adult neurogenesis and neurodegenerative diseases. HDAC inhibitors can increase the production of adult neurons and improve memory formation. Moreover, HDACs have been used as therapeutic tools for various neuropathies, including Alzheimer disease, Parkinson's disease or schizophrenia, among others.

TRRAP is a common cofactor of the GNAT and MYST families of HATs. To investigate the mechanisms by which HAT regulates adult neurogenesis, we specifically deleted Trrap in the adult neural stem cells (aNSCs) in the subventricular zone and the subgranular zone within the dentate gyrus. We found that Trrap deletion leads to a gradual reduction and eventually halt of the newborn neuron production at 7 mon-

ths of age, in contrast to 18 months of wild-type controls, showing an accelerating aging process. Moreover, Trrap-null aNSCs formed fewer and smaller neurospheres in cultures compared to wild type aNSCs, indicating a compromised self-renewal of aNSCs. Although Trrap-deleted neurospheres are viable, they underwent cell death when disassociated in culture, suggesting that the cell-cell interaction is essential for Trrap-null aNSCs. Intriguingly, the proliferation defects and lethality of Trrap mutant aNSCs could be rescued by an aggregation with its wild-type counterpart in vitro, suggesting a non-cell autonomous effect of the Trrap deletion in aNSCs. Currently we are investigating the molecular pathways by which TRRAP-HAT controls stemness and fitness of aNSCs in aging processes.

61 | The chick dorsal pallium: a model to study the bases of cortical neurogenesis and progenitor heterogeneity.

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The cerebral cortex, a singularity of the mammalian brain originating from the dorsal telencephalon, is of great interest given its particular importance in higher cognitive functions and the array of brain disorders caused by its dysfunctions. Because its cytoarchitecture is unique to mammals among the animal kingdom, it has long been assumed that the molecular mechanisms and cell types governing its formation were also specific to mammals and emerged de novo during evolution. Yet, recent studies have brought to light that the development of the dorsal pallium in non-mammalian amniotes of the sauropsida clade (birds and reptiles) actually shows unexpected similarities to what is described for mammalian corticogenesis. The conservation of the temporal neurogenetic program and a striking medial-to-lateral gradient of neurogenesis combined with the ease of experimental manipulations, highlight the interest of using these non-mammalian animal models to study the basic molecular mechanisms governing cortical neurogenesis and to tackle the founding principles of cortical cell heterogeneity.

With this idea in mind, we undertook a meticulous characterization of the neurogenic process within the dorsal pallium of the chick embryo. The results obtained so far demonstrate that the principal progenitor and neuronal subtypes and the function of key signaling pathways are conserved between chick and mouse cortical neurogenesis. Moreover, by comparing the dynamics of neurogenesis within the dorso-medial and dorso-lateral pallial subregions we established the existence of striking loco-regional differences in the behavior of cortical progenitors, which might facilitate the identification of mechanisms at the origin of cortical radial growth.

62 | The role of chromatin associated protein Hmgb2 in setting up permissive chromatin states for direct glia to neuron conversion

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The adult mammalian brain lacks the capacity to replace the loss of neurons upon the traumatic brain injury. Direct lineage reprogramming of non-neuronal cell types resident within the central nervous system to generate new neurons is a promising approach to repair damaged brain. Despite very good conversion rate of isolated postnatal astrocytes in vitro and recent advances to produce induced neuronal cells in vivo, the molecular understanding of the reprogramming process remain largely unknown. Toward this end, we developed in vitro model with adjusted growth factor composition lacking EGF that rendered the astroglia into the cell types resistant to the reprogramming. By comparing the cultures prone and resistant to reprogramming we aimed to identify molecular features required for the efficient conversion. The proteome analysis of two cultures suggests that the changes in the chromatin states could maintain the astrocytes in the glial lineage even after neurogenic factor overexpression. To test this hypothesis, we overexpressed the most regulated chromatin related protein, Hmgb2, together with Ngn2 and analyzed reprogramming efficiency. Indeed, the overexpression of Hmgb2 and Ngn2 in the astrocytes resistant to reprogramming significantly increased direct reprogramming suggesting the role of chromatin associated proteins in bypassing the lineage roadblocks. To understand the role of global chromatin rearrangement we performed ATAC-sequencing and searched for Hmgb2 overexpression related changes in the chromatin compaction. In order to access the transcriptional changes induced by Hmgb2 overexpression, we performed RNA-sequencing. Taken together, our data identify chromatin states in the glial cells permissive for lineage conversion and possible role of Hmgb2 in regulation of these states.

63 | MicroRNA filters hox temporal transcription noise to confer the robustness of boundary formation in the spinal cord

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During organism developments, cell lineages are specified by several factors. In the rostrocaudal (RC) patterning of spinal cord development, extrinsic signals act on early neural progenitors within the nascent neural tube. The initial RC patterning lead to differential expression of Hox genes in postmitotic motor neurons (MNs) and specification of MN subtype identity and connectivity. Although several 3' Hox genes are expressed in progenitors in a noisy manner, these Hox proteins are not expressed in the progenitors and only become detectable in postmitotic MNs.

We found microRNA biogenesis impairment leads to precocious expression and propagates the noise of Hoxa5 at the protein level, resulting in an imprecise Hoxa5-Hoxc8 boundary. Using in silico simulation, we uncovered two feed-forward Hox-miRNA loops accounting for the precocious and noisy Hoxa5 expression, as well as the ill-defined boundary phenotype of Dicer mutants. Finally, we identified mir-27 as a major regulator coordinating the temporal delay and spatial boundary of Hox protein expression. Our results describe a novel trans Hox-miRNA circuit that filters transcription noise and precisely controls the timing of proteins expression to confer robust individual motor neuron identity.

64 | Optimisation of interneuron function by direct coupling of cell migration and axonal targeting programs

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Neural circuit assembly relies on the precise synchronization of developmental processes such as cell migration and axon targeting, but the cell autonomous mechanisms coordinating these events remain largely unknown. Here we found that different classes of Somatostatin-expressing interneurons use distinct routes of migration to reach the embryonic cerebral cortex. Martinotti cells, one of the most distinctive classes of cortical interneurons, migrate through the marginal zone, a behavior that is linked to the development of the unique axonal arborization that these cells possess in layer 1. Alteration of the normal migratory route of Martinotti cells by conditional deletion of Mafb – a gene that is preferentially expressed by these cells – cell-autonomously disrupts axonal development and impairs the function of Martinotti cells in vivo. Our results suggest that cell migration and axon targeting programs are mechanistically coupled to functionally optimize the assembly of inhibitory circuits in the cerebral cortex.

65 | KAT3 proteins are crucial for the maintenance of neuronal identity

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Studies in different organisms have demonstrated that neuronal identity needs to be actively maintained throughout life by the combined action of transcription factors and chromatin modifiers. The paralogue lysine acetyltransferases 3 (KAT3) CBP/KAT3A and p300/KAT3B play an important role in the regulation of transcription and chromatin three-dimensional structure. Although both proteins are essential for the normal development of the nervous system, their specific functions in post-mitotic neurons remain unclear. In order to investigate this matter, we produced inducible forebrain-specific knockout (ifKO) mice for either one or for both KAT3 proteins. Interestingly, we observe that only when both proteins are knocked out simultaneously,

mice show severe neurological phenotypes and an increased mortality. These deficits are accompanied by a rapid and progressive reduction in the dendritic complexity of forebrain neurons and loss of normal electrophysiological properties. Remarkably, these dramatic changes correlate with a strong and specific downregulation of neuron-specific gene expression whereas housekeeping genes were mostly unaffected. We also observed that the lack of KAT3 caused a robust decrease in histone acetylation at specific residues, such as H2B panacetylation and H3K27ac. Overall, our experiments demonstrate that KAT3 proteins are necessary for maintaining neuronal identity and function during adulthood, most probably through the regulation of acetylation levels at neuronal enhancers and gene bodies.

66 | Understanding intellectual disability through parallel derivation of 2D and 3D models of human neurodevelopment.

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Cell fate reprogramming has propelled a shift of paradigm in the way human physiopathology can be modeled. Our research uses reprogramming and differentiation to dissect the epigenetic mechanisms underlying intellectual disability and autism. We are harnessing the largest cohort of human induced pluripotent stem cells (iPSC) characterized thus far for two disorders caused by genetic CNVs of the 7q11.23 locus (hemideletion Williams-Beuren Syndrome - WBS, and hemiduplication - 7dupASD), which lead to symmetrically opposite socio-cognitive features with shared intellectual disability. Our analysis of patient-derived iPSC using mass spectrometry, RNAseq and ChIPseq, uncovered a pivotal role of the GTF2I-LSD1 complex in the disease-relevant transcriptional dysregulation during pluripotency. We have developed an innovative approach of parallel differentiation of human neocortical organoids and single-step induction of mature neurons by a seamless transposase system, in which we profile cell lineages and transcriptomes by cell line microarrays and a combination of bulk and single cell RNAseq. Differential expression analysis in cortical organoids from patients at two developmental stages (days18 and 50) in a cohort that includes four controls, four WBS, four 7dupASD and three unconventional WBS with autism, revealed a wave of transcriptional dysregulation that dramatically amplifies upon differentiation (from 49 to 1225 genes) and includes several downstream targets of GTF2I. Phenotypic analysis of mature neurons suggests that this early genetic imbalances result in synaptic aberrations associated to the behavioral outcome. Our results convey transcriptional and functional defects related to the 7q11.23 CNV during brain development as key causative mechanisms of WBS and 7dupASD.

67 | CBP is required for experience-driven EPI-editing of neuronal activity-regulated gene programs

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Growing evidence supports a key role for the lysine acetyltransferase and transcriptional coactivator CBP in cognitive function. However, the precise molecular mechanisms underlying CBP action and specificity remain largely unknown. We report here the first genome-wide mapping of CBP bound sites in the adult mouse hippocampus and evaluate the consequences in target histone posttranslational modifications (hPTMs) and transcriptional activity of the selective ablation of CBP in forebrain neurons (ifCBPKO mice). Dampened transcription of constitutively active gene networks underlying synaptic plasticity and other key neuronal functions in CBP-KO neurons resulted from concomitant reduction of RNAPII occupancy and CBP-targeted hPTMs, namely H2Bpan-ac and H3K27ac levels, at enhancers and gene bodies. These data suggest that CBP is responsible for refreshing gene acetylation of neuronal gene expression programs in order to maintain active gene status over time. Furthermore, we found that behavioural, epigenetic and transcriptional responses to pentylenetetrazol-induced kindling, kainate-induced seizures, and environmental enrichment were strongly reduced in ifCBPKO mice. This set of experiments unveils a critical role for CBP enabling transcriptional competence of neuronal cells. Thus, the consequences of CBP absence becomes more prominent in paradigms that involve chronic or recurrent changes in gene expression driven by experience or environmental changes, underscoring CBP's importance for the epigenetic encoding of novel experiences.

68 | The imprinted genes Igf2 and Cdkn1c interact to induce terminal differentiation of adult neural stem cells

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Maintenance and differentiation of neural stem cells (NSCs) might be modulated by epigenetic mechanisms such as genomic imprinting. This process causes genes to be expressed depending on their parental origin. Igf2 is an imprinted gene expressed from the paternal allele that is implicated in embryonic growth but also regulates NSCs in the SVZ. Another imprinted gene regulating NSCs is Cdkn1c. These two genes have been showed to be linked and their alteration has implications for pathologies as tumours or the Beckwith-Wiedemann syndrome. However, it is not known about the possible link between these two factors in the neurogenesis

process. We show that treatment of NSCs derived from the adult subventricular zone (SVZ) with recombinant IGF2 induces their differentiation into the three neural lineages. Moreover, reactivation of NSCs after this differentiation is impaired in the presence of IGF2 supporting the hypothesis of IGF2 promoting NSCs fate commitment. Importantly, IGF2 increases the expression of the imprinted gene Cdkn1c, cell cycle inhibitor that mediates effects on NSCs. Our previous work suggests that regulation of the imprinting state in some genes is essential for the normal function of the NSCs. Thus, we propose that Igf2 and Cdkn1c act in a common pathway to maintain the NSCs pool in the adult SVZ.

69 | ALS genetic background modulates a global gene regulatory mechanism in early human motor neurogenesis

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Intron retention (IR) is emerging as a rich contributor to transcriptome diversity, however it still remains the least studied mode of splicing. Thus its role in gene regulation during neurogenesis and disease remains unknown.

We sought to resolve the gene regulatory events underlying distinct stages of lineage restriction of human motor neurons derived from healthy controls and patients with VCP-mutation related ALS. We integrated ontogeny-recapitulating directed differentiation induced pluripotent stem cells (iPSCs) into spinal motor neurons with RNA sequencing.

Unexpectedly IR was the predominant splicing change that affects early stages of lineage restriction. A dynamic increase in IR events targeted key genes involved in splicing machinery and was observed at a comparatively earlier stage in samples with an ALS genetic background compared to healthy controls. Many of the upregulated introns also exhibited widespread retention in motor neuron samples of other ALS gene backgrounds including SOD1. Overall exon inclusion increased upon terminal differentiation, involving in neuron differentiation and axonogenesis, without being affected by ALS genetic background.

This study reveals a robust IR program underlying the early stage of lineage restriction of human motor neurons. Furthermore, we find these IR events occur prematurely in ALS cell lines, potentially affecting the expression of many important neural developmental genes. This suggests that different modes of alternative splicing regulate distinct stages of lineage restriction from iPSCs to MNs, and that ALS genetic background modulates a global gene regulatory mechanism in iPSC-derived motor neurogenesis, which may have important functional consequences on overall motor neuronal integrity.

70 | Epigenomic profiling of aged mouse neural stem/progenitor cells identifies Dbx2 as a candidate regulator of age-associated neurogenic decline

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Adult neurogenesis declines with ageing, but the underlying molecular mechanisms are poorly understood. To investigate the intrinsic molecular changes that occur upon neural stem/progenitor cell (NSPC) ageing, we compared the transcriptional, histone methylation and DNA methylation signatures of NSPCs derived from the subventricular zone of young adult (3 months old) and aged (18 months old) mice. Surprisingly, the genome-wide transcriptional and epigenetic profiles of SVZ-derived NSPCs were largely unchanged in aged cells. Despite the global similarities, we identified robust age-dependent changes at several genes and their regulatory elements. Among them, the homeobox gene Dbx2 was upregulated, and its promoter region was DNA hypomethylated, in aged NSPCs. Using functional in vitro assays, we show that elevated Dbx2 expression in adult NSPCs promotes age-related phenotypes, including the reduced growth of NSPC-derived neurospheres and the altered expression levels of genes implicated in NSPC self-renewal and differentiation. Taken together, these results provide new insights into the molecular programmes that are altered on NSPC ageing, and uncover a new functional role for Dbx2 in promoting age-associated neurogenic decline.

71 | Non-conventional roles of cell cycle regulators during neurogenesis

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Increasing evidence in the last years suggest that control of the cell cycle is tightly linked to cell-face decisions during neurogenesis. Aurora A and Polo-like kinase 1 (Plk1) are two serine/threonine kinases involved in centrosome and spindle dy-

namics, among other cell-cycle-related functions. These kinases are functionally linked since Aurora A is able to activate Plk1 by direct phosphorylation and, in addition, they participate in similar cellular processes by phosphorylating related proteins. In Drosophila, mutations in Aurora A and Polo lead to a disruption of the asymmetric localization of Numb, thus resulting in increased symmetric divisions and self-renewal in neuroblasts at the expense of differentiating neurons. In the last years, we have generated mouse models with specific gain- or loss-of-function mutations in either Aurora A or Plk1. Our preliminary data suggest a defect in the asymmetric cell division of neural progenitors in mice hypomorphic for Plk1, a defect accompanied by impaired centrosome regulation and abnormalities in brain size. Plk1 is known to control the proper localization of the microcephaly protein ASPM in neural progenitors, and it also regulates the centrosomal recruitment of CDK5RAP2, an additional protein mutated in autosomal recessive primary microcephaly. On the other hand, two additional centrosomal proteins encoded by genes mutated in microcephaly, WDR62 and CENPJ/CPAP, are directly regulated by Aurora A. Altogether, these data suggest a possible role for these kinases in controlling centrosomal proteins in neural progenitors. The implications of our work in brain development, microcephaly and syndromes with cranial dymorphism and intellectual disability in children will be discussed.

72 | Generation of vascularized and functional iPSC-derived human brain organoids

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Stem cells have the remarkable ability to self-organize in three-dimensional (3D) space into organ-like structures termed Organoids. By harnessing this property, researchers have been able to create such organoids for several tissues that better recapitulate the complexity and physiological properties of tissues and organs. Despite many reports describing the generation of human neural organoids, the generation of vascularized and functional neural organoid graft is not described yet. Here we describe the generation of vascularized, and electrophysiologically active, human cerebral-organoids by transplantation of organoids grown in vitro to an adult mouse brain. Engrafted mice were viable, and exhibit long and high survival rates. Moreover, histological and immunostaining analysis revealed intact grafts with mature neurons, and extensive axonal trajectories from the implant to multiple regions of the host mouse brain. Importantly, live imaging on of the implanted organoids using two-photon microscopy revealed neuronal activity, and intensive vascular network with active blood flow within the organoid. To our knowledge, this is the first report demonstrating the generation of transplanted, vascularized, and functional neural organoids. Moreover, our method creates opportunities for noninvasive recording of neuronal

activity with high spatial and temporal resolution deep within organoid-brain chimera. This powerful combination of in vitro 3D human neural structures, and an in vivo rich environment in the animal brain provides a promising novel approach with broad applications for degenerative and regenerative medicine.

73 | The insulator binding protein SuHw instructs neural cell fate

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Insulators are responsible for the three-dimensional organization of chromatin in the nucleus to regulate gene expression. They organize different parts of chromatin to each other and affect the localization of chromatin in relation to the nuclear envelope. Insulators are thought to loop chromatin, and to interrupt or facilitate interactions of cis-acting regulatory proteins with promoters. Another proposed mechanism is to regulate chromatin through a generally inhibitory interaction with the nuclear envelope. In particular, the insulator binding protein Suppressor of Hairy wing (SuHw) has been shown to localize to the nuclear envelope.

We have identified the insulator binding protein SuHw as a regulator of the expression of neuronal specific genes in Drosophila. Analysis of our cell-type-specific gene expression data from the nervous system, we identified SuHw as a factor that ensures silencing of neuronal genes in non-neuronal cell types. We find that neuronal genes are enriched for SuHw binding sites. These genes are embedded in a repressive chromatin environment. We observe that SuHw is not expressed in neuronal cells, but is expressed in all other cell types, including glia. This suggests that SuHw ensures the silencing and repressed chromatin structure of neuronal genes in non-neuronal cell types. To test this hypothesis, we have altered SuHw expression in neurons and in glia. Our results indicate that SuHw plays an important role in the regulation of neuronal cell fate by mediating the silencing of neuronal genes in non-neuronal cell types and by regulating the interactions of chromatin with the nuclear envelope.

74 | Single Cell Analysis of direct astroglia-to-neuron reprogramming

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Direct neuronal reprogramming is a promising approach to convert non- neuronal cells into neurons, thus allowing to investigate fundamental questions in development from a different perspective, such as cell fate specification, differentiation as well as cell fate maintenance. Our group was first showing that Ascl1 and Neurog2 are sufficient to convert primary astrocytes (Heinrich et al., 2010), obtained from grey matter of postnatal mice, into specific neuronal subtypes, GABAergic and Glutamatergic neurons.

Recently, we have identified a subset of genes downstream of Ascl1 and Neurog2 that are required and sufficient to instruct astroglia-to-neuron reprogramming (Masserdotti et al., 2015). However, it is still not known why only a subset of astrocytes can successfully complete the reprogramming process. To address this question, we decided to study the induction of the neurogenic program at the single cell level at different time points. This allows revealing not only the heterogeneity of the responsiveness to the activation of the transcription factor, but also testing whether all the previously identified downstream targets need to be expressed in a single cell at a given time for successful conversion, or combinations of such factors may be expressed in different cells but nevertheless trigger neuronal reprogramming. Interestingly, our data show a rather homogenous initial response in Ascl1 transduced astrocytes including neuronal gene activation, suggesting that they embark rather homogenously onto the road of fate conversion. We will present data also from later stages and discuss the differences and similarities to reprogramming of other cell types.

75 | Unravelling the global transcriptional and post-transcriptional program underlying adult hippocampal neural stem cell quiescence

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Neurogenesis persists in specific areas of the adult mammalian brain due to the life-long maintenance of neural stem cell (NSC) reservoirs. The vast majority of these NSCs are held away from the cell cycle, in a reversible resting state called quiescence. A tight balance between NSC quiescence and activation is instrumental to meet the neurogenic demands of the adult brain at a given time. Through a combination of genetic and pharmacologic approaches, we previously demonstrated that canonical BMP signalling through the BMPR1A receptor is required for the maintenance of adult hippocampal NSC quiescence. Employing genome-wide gene expression analysis combined with polysomal profiling, here we elucidate the transcriptional and post-transcriptional program that characterizes the adult NSC quiescent state downstream of BMP signalling. In line with recent single-cell omics data, our study reveals molecular signatures of adult quiescent NSCs characterized by both a global reduction in protein translation and an enhanced niche signalling integration capacity. These signatures are further supported by functional assays. The translational silencing is mediated by the coordinated transcriptional repression of several components of the protein synthesis machinery and the phosphorylation of translation initiation factor eIF2a at serine 51 (P-eIF2a). Our functional assays also connect the quiescence signature to an increased sensitivity of the stem cells to extracellular neurogenic cues such as WNT ligands. Thus, our results support the notion that quiescent NSCs are endowed with low translation rates and are primed for rapidly reacting to the neurogenic demands of the brain.

76 | EZH2 is a potential therapeutic target for H3K27M-mutant pediatric gliomas

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Diffuse intrinsic pontine glioma (DIPG) is an aggressive brain tumor that is located in the pons and primarily affects children. Nearly 80% of DIPGs harbor mutations in histone H3 genes, wherein lysine 27 is substituted with methionine (H3K27M). H3K27M has been shown to inhibit polycomb repressive complex 2 (PRC2), a multiprotein complex responsible for the methylation of H3 at lysine 27 (H3K27me), by binding to its catalytic subunit EZH2. Although DIPGs with the H3K27M mutation show global loss of H3K27me3, several genes retain H3K27me3. Here we describe a

mouse model of DIPG in which H3K27M potentiates tumorigenesis. Using this model and primary patient-derived DIPG cell lines, we show that H3K27M-expressing tumors require PRC2 for proliferation. Furthermore, we demonstrate that small-molecule EZH2 inhibitors abolish tumor cell growth through a mechanism that is dependent on the induction of the tumor-suppressor protein p16INK4A. Genome-wide enrichment analyses show that the genes that retain H3K27me3 in H3K27M cells are strong polycomb targets. Furthermore, we find a highly significant overlap between genes that retain H3K27me3 in the DIPG mouse model and in human primary DIPGs expressing H3K27M. Taken together, these results show that residual PRC2 activity is required for the proliferation of H3K27M-expressing DIPGs, and that inhibition of EZH2 is a potential therapeutic strategy for the treatment of these tumors.

77 | TET3 prevents terminal differentiation of neural stem cells in the adult SVZ

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Neural stem cells (NSCs) are able of unlimited self-renew while maintaining their capacity to differentiate. Sustained neurogenesis throughout life occurs in the walls of the lateral ventricles (SVZ). There is evidence suggesting that epigenetic mechanisms like DNA methylation, histone modification and imprinting can interact with transcriptional and environmental factors. In turn, this interaction modulates NSCs maintenance and differentiation. The recently described ten-eleven-translocation (Tet) proteins, named as Tet1, Tet2 and Tet3, are enzymes responsible for the conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) that directly affects epigenetic regulation. We show that Tet3 is the most expressed isoform in the nervous system, especially in NSCs, indicating its possible role in stem cell regulation. Importantly, we also show that TET3 exerts its function on the stem cell pool of the SVZ, regulating their undifferentiated state and preventing the differentiation of B cells to non-neurogenic astrocytes.

78 | SoxD genes in the control of adult neurogenesis

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During the development of the nervous system, the generation of hundreds of subtypes of neurons and glial cells relies upon the relatively fast production, amplification, specification and differentiation of a pool of neural progenitors and stem cells (NSCs). Surprisingly, this strategy is retained to some extent in niches in the adult nervous system throughout lifetime. Although the molecular mechanisms involved in both embryonic and adult neurogenesis are conserved, it is not clear how the differences in the cell production rates and in the temporal extent of neurogenesis can be attained.

Genes of the Sox family of transcription factors are essential during neurogenesis. In the developing spinal cord, Sox5 controls cell cycle exit of neural progenitors and the specification of subtypes of dorsal interneurons, counteracting the Wnt signalling pathway (Martínez-Morales et al., 2010; Quiroga et al., 2015). Moreover, both Sox5 and Sox6 are expressed in the majority of NSCs and in early intemediate progenitor cells (IPCs) in the subgranular zone (SGZ) of the adult mouse dentate gyrus.

Using Sox5fl/fl and Sox6fl/fl mice crossed to a transgenic Sox2-cre-ERT2 line inducible by tamoxifen, we have determined that in the absence of SoxD genes neurogenesis in the SGZ is diminished, with a clear reduction in the number of IPCs and inmature neurons. This is in part due to the fact that Sox5 and Sox6 are required for the self-renewal and survival of NSCs. Together, these results suggest that Sox5 and Sox6 control the activation, proliferation and/or survival of NSC/IPCs during adult hippocampal neurogenesis.

79 | Hippo signaling in mammalian forebrain development

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The cerebral cortex of mammals is composed of billions of neurons (in humans109) organized into functionally distinct layers. The different types of neurons originate from what has been proposed to be a homogeneous pool of neural stem cells

(NSCs). We have undertaken a systems biology approach to understand development of the mammalian cerebral cortex, to elucidate the mechanisms controlling neuronal fate and differentiation, and to examine the homogeneity of the telencephalic NSC population (NeuroStemX, SystemsX.ch). Through collaborative wet biology and computational modeling approaches we are deciphering the signaling and transcriptional networks that regulate the formation of cerebral cortical neurons. The control of these networks modulates the regimental differentiation and characterization of the NSCs, to pattern the complex six-layered structure of the cerebral cortex. The integration of these intrinsic and extrinsic signals controls neuron production and fate. A comprehensive understanding of transcriptional regulation and its interplay with an ensemble of upstream factors will pave the way for regeneration of cortical neurons and structures following disease and could have implications for cellular therapy and drug screening. We have isolated NSC, progenitor, and newborn neuron populations, generated RNA for sequencing and are experimentally analyzing the predicted regulatory nodes controlling neuronal cell fate. From the global transcriptomes, we have initially focused on one, as yet less explored signaling pathways in the brain, Hippo Pathway and its downstream effectors. Hippo effectors change dynamically during forebrain development. Further experimentation indicates their active control in forebrain development.

80 | MEIS2 facilitates PARP1/ARTD1-mediated chromatin opening of neuronal genes

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Binding of the linker histone H1 to chromatin facilitates higher-order chromatin folding, physically limits nucleosome mobility and prevents access of transcription factors or epigenetic modulators to DNA. Removal of H1 and the subsequent local chromatin decompaction are therefore critical early steps in the transcriptional activation of lineage-specific genes during cell fate specification and cellular differentiation. ADP-ribosylation, the transfer of adenosine diphosphate (ADP)-ribose moieties from NAD+ onto H1 by poly-ADP-ribose polymerase 1 (PARP1/ARTD1), decreases H1 affinity for DNA and thereby initiates its eviction from the chromatin fiber. Considering this important role of PARP1 in transcription regulation, surprisingly little is known about how the enzyme is targeted to the physiologically correct gene loci.

Focusing on the first hours of neuronal differentiation of primary adult subventricular zone (SVZ)-derived progenitor cells, we here describe a sequence of events by which the atypical homedomain proteins PBX1 and MEIS2 cooperate to rapidly and specifically recruit PARP1 to the promoters of some neuron-specific genes: PBX1 is bound to H1-compacted chromatin in undifferentiated cells, de facto priming these sites for later activation. As soon as differentiation is induced, MEIS associates with chromatin-bound PBX1, recruits PARP1 and initiates PARP1-mediated eviction of H1 from the chromatin. These results establish a previously unrecognized role for MEIS proteins in the orchestration of chromatin dynamics and suggest a novel mechanism for how selectivity can be achieved in targeting PARP1 to the promoters of downstream genes.

81 | A new image analysis tool for monitoring chromatin organization in live cells.

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Transcriptionally silent heterochromatin is preferentially localized in the nuclear periphery and transcriptionally active euchromatin in the nuclear interior. To monitor dynamic spatiotemporal changes in live cells, we have developed a novel image analysis tool, that lets us compare the distribution of Histone3.3-GFP (marker for euchromatin) and Histone2B-mCherry (marker for total chromatin) in a quantitative manner. As a proof of concept, peripheral heterochromatin was reduced in cells after treatment with known histone deacetylase inhibitor, trichostatin A, or after overexpression of the truncated lamin A protein, progerin. Interestingly, depletion of the transmembrane inner nuclear membrane protein Samp1 resulted in formation of more heterochromatin in the nuclear periphery. This suggests that Samp1 either promotes euchromatin formation or prevents heterochromatin formation. This unexpected result indicates an interesting role for Samp1 in organization of peripheral chromatin. The method we have developed will allow us to screen other likely proteins for their involvement in chromatin organization at the nuclear envelope, as well as investigate pathological changes in chromatin distribution relating to neurodegenerative diseases.

82 | Cell type-specific assessment and site-specific manipulation of DNA modification during mouse corticogenesis.

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The switch of neural stem cells from proliferation towards differentiation is a highly regulated process essential for proper brain development. Cytosine modifications are well-known to play a crucial role in developmental processes. However, the cell type-specific patterns and function of cytosine modifications during corticogenesis are poorly understood mainly due to the difficulty to isolate individual cell types from the cortex. To overcome this limitation, we used the Btg2-RFP/Tubb3b-GFP double reporter mouse line, which allows the isolation of proliferating and differentiating progenitors as well as neurons. The genome-wide distribution of methylcytosine and hydroxymethylcytosine was mapped resulting in the identification of differentially (hydroxyl-)methylated regions (DHMR) between the three linage-related cell types. DHMR were detected preferably within or in close proximity to neurogenesis-related genes and showed a high enrichment of basic loop helix transcription factor binding motives. To investigate the molecular function of these DHMR we sought to site-specifically manipulate these regions during corticogenesis using a catalytically inactive Cas9 fused with the catalytic domain of Tet-methylcytosine-dioxygenase 1 (Tet1). Manipulation of DHMR in vivo by in utero electrophoration of the dCas9-Tet1 construct leads to an aberrant corticogenesis in the electrophorated area confirming the regulatory role of the identified DHMR, which I will further investigate.

83 | Ribosome hydroxylase Mina53 is required for Glioblastoma and is involved in regulation of translation rate by regulating ribosomal biogenesis

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Glioblastoma multiforme (GBM) is one of the most aggressive types of tumors with a median 5-year survival of less than 5%, highlighting an urgent need for new treatments. Using a mouse neural stem cell based GBM model, we performed shRNA screens to identify epigenetic regulators required for the tumorigenicity. Among

these regulators is a ribosome hydroxylase Mina53 which hydroxylates His-39 of ribosomal protein, RPL27a. We have found that Mina53 and its catalytic activity is required for in vitro proliferation and colony forming ability of mouse glioma initiating cells (mGIC). Mina53 knock-down (KD) in mGICs resulted into their reduced ability to form tumors and thus marked an increased survival of injected mice with mGICs. Mina53 overexpression correlates with poor survival for glioma patients and its ablation strongly reduced the in vitro proliferation and colony forming ability of cells derived from the glioma patients. Mina53 KD resulted into a lower level of its substrate, RPL27a and most of the ribosomal proteins, a finding substantiated further by global proteome analyses. A proteome analyses using Mina53 KD and a CRISPR/Cas9 mediated Mina53 knock-out (KO) revealed that Mina53 KD/KO resulted into lower amounts of ribosomal proteins. We also confirm that Mina53 is indeed the main ribosome hydroxylase responsible for RPL27a at His39 hydroxylation. Mina53 KD resulted into a small but significant reduction in the global protein synthesis rate and this was dependent on catalytic activity of Mina. Taken together, we report that Mina53 is required for glioblastoma and it regulates translation through regulation of ribosomal biogenesis.

84 | Insights into NeuroD1 binding and function during neurogenesis.

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NeuroD1 is a potent neuronal transcription factor that has been successfully used to reprogram other cell types into neurons. We find that an ectopic expression of NeuroD1 is able to specify neuronal fate in pluripotent embryonic stem cells. This is further accompanied by induction of a large number of neuronal development genes. A genomewide binding analysis revealed that a significant fraction of these genes are direct targets of NeuroD1 at promoters or enhancers and this binding was very sequence-specific. Interestingly, using Bayesian modelling we show that prior to NeuroD1 binding, its target sites are embedded in a heterochromatin environment which gets remodeled to euchromatin following NeuroD1 expression and targeting to these sites. A number of these findings were further validated during cortical development. We next examined into principles that govern NeuroD1 access on the genome, and we find that genetic landscape across motif site plays a crucial role in governing selection of motif for binding. By integrating epigenomic datasets and motif information, we also identified potential NeuroD1 cofactor NCC1 (NeuroD1 cofactor candidate 1). Our current work is further investigating molecular mechanisms of their cooperation and its role in neurogenesis during cortical development.

85 | let-7 regulates adult neurogenesis through positive regulation of autophagy

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During adult neurogenesis, neural stem cells (NSCs) reside in the subventricular zone (SVZ) where they continuously give rise to neuroblasts that migrate along the rostral migratory stream (RMS) into the olfactory bulb (OB). There they radially migrate into the cellular layers of the OB, differentiate to mature interneurons and integrate into the existing neuronal circuitry. Despite extensive research, molecular mechanisms underlying radial migration, maturation and integration of new-born neurons are still not fully elucidated. Recently microRNAs (miRNAs) have been implicated as important regulators of adult neurogenesis. miRNAs are small, non-coding, single stranded RNAs that regulate mRNA activity by associating with a protein called Argonaute2 (AGO2).

To identify miRNAs with a potential role in adult neurogenesis, we performed a large-scale analysis of active miRNAs in new-born OB interneurons by using AGO2-immunoprecipitation, followed by small RNA sequencing. Using this approach, we identified let-7 as the most abundant miRNA family in new-born neurons. Knockdown of let-7 in migrating neuroblasts led to an impairment in radial migration and to an immature morphology of newly formed interneurons. This phenotype was accompanied by a decrease in autophagic activity. Activation of autophagy in neuroblasts lacking let-7 restored the ability of new-born neurons to radially migrate into the cellular layers of the OB but not their maturation. Taken together, our data demonstrates a novel miRNA-dependent link between autophagy and adult neurogenesis, which has important implications for neurodegenerative diseases, where these processes are impaired.

86 | Cell-intrinsic regulation of interneuron migration controls cortical neurogenesis

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Interneurons navigate along multiple tangential paths to settle into appropriate cortical layers. They undergo saltatory migration, which is paced by intermittent nuclear jumps whose regulation relies on interplay between extracellular cues and genetic-encoded information. However, it remains unclear how cycles of pause and movement are coordinated at the molecular level. Post-translational modification of proteins contributes to cell migration regulation. The present study uncovers that carboxypeptidase 1, which promotes deglutamylation, is a pivotal regulator of pausing of migrating cortical interneurons. Moreover, we show that pausing during migration attenuates movement simultaneity at the population level thereby controlling the flow of interneurons invading the cortex. Interfering with the regulation of pausing not only affects the size of the cortical interneuron cohort but also impairs the generation of age-matched upper layer projection neurons.

87 | IgLON family proteins Lsamp and Neurotrimin in neuronal development and behavior in mice

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Cell surface neural adhesion proteins are critical components in the complex orchestration of cell proliferation, apoptosis, and neuritogenesis essential for proper brain construction and behaviors. We focused on the impact of two plasticity-associated IgLON family neural adhesion molecules, Ntm and Lsamp, on the hippocampal neural development. Phenotyping neurons derived from the hippocampi of Lsamp-/-, Ntm-/- and Lsamp-/-Ntm-/- mice was performed in parallel with behavioural testing to study behavioural correlates for neuronal alterations.

Ntm-/- hippocampal neurons exhibited premature sprouting of neurites 6 hrs after plating and manifested accelerated neurite elongation and branching 3 days later (at DIV3), indicating that Ntm is an inhibitory factor for neuritogenesis. The robust effect of deleting Ntm alone disappeared if Lsamp was deleted as well, suggesting regulatory interactions between Lsamp and Ntm. We also show interplay between Lsamp and Ntm in regulating tissue homeostasis: the impact of Ntm on cellular proliferation was dependent on Lsamp, and Lsamp appeared to be a positive regulator of apoptosis in the presence of Ntm. Similar to the neuronal phenotype, the behavioural profiles of the mutant mice demonstrated parameter-specific in-

teractions between Lsamp and Ntm, suggesting their role in establishing specific neuronal projections.

Altogether, evidence both from cultured hippocampal cells and from behavioural experiments show combined and differential interactions between Ntm and Lsamp in the formation of hippocampal circuits and behavioural profiles. We demonstrate that mutual actions between IgLON molecules can regulate the initiation of neurite sprouting at very early ages, even cell-autonomously, without existing adhesion between the cells.

88 | A mouse model of DYRK1A-related intellectual disability syndrome shows altered gliogenesis and defects in myelination

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Individuals with de novo mutations in DYRK1A present microcephaly and intellectual disability (ID), in most cases accompanied with astrogliosis and hypomyelination. Human DYRK1A is located in chromosome 21 and its triplication contributes to the neurological alterations associated to Down syndrome (DS). DYRK1A encodes a protein kinase with a conserved function across evolution in the developing brain where it regulates neurogenesis and neuronal survival and differentiation. There is evidence that the overexpression of DYRK1A contributes to the astrogliosis associated to DS. However, the role of DYRK1A in gliogenesis or glial cell function has not been studied. Here we show that adult haploinsufficient Dyrk1a+/- mice phenocopy the ID-related DYRK1A syndrome exhibiting an increased number of astrocytes in the telencephalon and altered myelination of the corpus callosum, alterations that arise early in development. Neural progenitors isolated from Dyrk1a+/- embryos show an altered capacity to differentiate into astrocytes and oligodendrocytes. In vivo, an excess of cortical astrocytes in the Dyrk1a+/- model is evident one week after birth. In contrast, at perinatal stages, there is a deficit of oligodendrocyte precursors in the corpus callosum, which is due to a reduced embryonic oligodendrogenesis. After the second postnatal week the population of oligodendrocytes reaches normal numbers in Dyrk1a+/- mice. However, they show a defect in myelination that persists in the adult. Our results suggest a new role of DYRK1A in glial cell development that could contribute to the ID and other neurological problems in patients carrying heterozygous mutations in the DYRK1A gene.

89 | MicroRNAs' synergy is critical for adult neural stem cells fate commitment

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Adult neurogenesis requires the precise control of neuronal vs. astrocyte lineage determination in neural stem cells (NSCs). While microRNAs (miRNAs) are critically involved in this step during development, their actions in hippocampal adult NSCs has been unclear.

By specific ablation of Dicer (a ribonuclease essential for miRNA biogenesis) in aNSCs in vivo and in vitro, we demonstrate that DICER function is crucial for the generation of new neurons, but not astrocytes in the adult murine hippocampus.

Through miRNAs profiling, we study dynamics of miRNAs expression in aNSCs along the neurogenesis process, and identify a new set of 11 miRNAs (out of which 9 not previously characterized in adult neurogenesis) that are sufficient and required for neural lineage fate choice at the expense of astrogliogenesis. Remarkably, these miRNAs rescue impaired neurogenesis in Dicer-cKO aNSCs to WT levels only when administered as a "pool", not individually.

Proteomics expression and GO analyses revealed that the 11 miRNAs sustain adult hippocampal neurogenesis through synergic modulation of 26 putative targets, from different pathways, but sharing similar biological processes such as neurogenesis, nervous system development and neuronal differentiation.

Our study provides evidence for the emerging notion of miRNA "convergence" that, by synergistically enforcement of gene-regulatory networks, allows the acquisition of neurogenic fate program in aNSCs.

The identification of a new set of miRNAs determining neuronal fate of aNSCs raises interesting perspectives with regard to cell reprogramming. We aim to discuss the use of the miRNA-pool as possible strategy to improve neural stem cells-based regenerative therapies.

90 | Cellular principles of adult neurogenesis revealed by chronic in vivo imaging

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Neural stem/progenitor cells (NSPCs) generate new neurons throughout life in the mammalian hippocampus. However, the cellular principles of cell division and neural cell birth remain largely unknown due to a lack of longitudinal observations of individual NSPCs within their endogenous niche. We established a chronic in vivo imaging approach using 2-photon microscopy and followed single Achaete-scute homolog 1 (Ascl1)-expressing NSPCs and their progeny in the mouse hippocampus for up to 2 months. We provide direct evidence for extended, yet limited self-renewal and direct neurogenic cell divisions of radial glia-like NSPCs. Further, our data reveal unexpected self-renewing cell divisions of non-radial glia-like NSPCs. We show that fate specification and cell cycle length depend on previous cell division history of individual cells and that individual lineages show a high variability of cell death, suggesting a possible asymmetric distribution of survival determinants within sister cell clones. Thus, the data presented here reveal the cellular principles of NSPC division in the adult hippocampus allowing for life-long neurogenesis in the mammalian brain.

91 | The medial habenula axons are guided caudally by DCC-Netrin1 mechanism

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The guidance of the growing axons is one of the main processes during the development of the central nervous system. In the last years, we have focus our research in the retroflex tract. This fascicle is originated in the habenula. In order to reach their target, they display a complex trajectory. They navigate ventrally along the dorsoventral axis, bend caudally in the proximity of the floor plate and grow caudally towards their target. We and others have elucidated partially the molecular mechanisms that control this complex process. Recently, we have demonstrated that the Sustantia nigra pars compacta plays a role as an intermediate target of the medial habenula axons. Therefore, this neural population is our principal candidate to control the caudal bending of these axons. Using the mouse as a model we have

identify Netrin1 as the signal and Dcc as the receptor related for this process. The distribution of the dopaminergic neurons of the Substantia nigra generates a natural Netrin1 gradient. In open neural tube explant cultures, we have demonstrated that netrin1 is sufficient to derail the medial habenula axons form their trajectory. The block of Dcc receptor in habenular neurons by antibodies avoid this deviation. The analysis of Dcc and Netrin1 lack of function in mouse models revealed strong alterations in the retroflex tract trajectory. Therefore, we have demonstrated that the caudal bending of the habenular axons due to the influence of the Substantia nigra pars compacta involve the Dcc-Netrin1 molecular mechanism.

92 | Tissue-specific regulation of gene expression by Pax6 in the developing mouse forebrain

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The development of complex brain structures requires the coordination of multiple molecular networks, which are controlled by a relatively small group of transcription factors. How a given transcription factor regulates the development of different brain territories is still far from being understood.

Pax6 is a pleiotropic transcription factor and a key regulator during embryonic development. In the developing cortex and thalamus Pax6 is expressed by neural progenitors forming a gradient and it is downregulated as cells exit from the cell cycle. However in the prethalamus it is highly expressed by all progenitors and also by a subset of postmitotic neurons. Although its functions in the cortex have been extensively studied, very little is known about its molecular actions in the diencephalon. Is Pax6 regulating gene expression in the same way in these different tissues? On the contrary, are Pax6 actions tissue-dependent? In this project we aimed to explore the molecular actions of Pax6 during the development of those three different territories. Using RNA-seq we have analysed the changes in gene expression after acute, tamoxifen-induced deletion of Pax6 in cortex, thalamus and prethalamus of E13.5 embryos. The computational comparison of the data across tissues showed a major contrast in the regulation of many genes between the cortex and diencephalic tissues, especially those related to cell cycle regulation, differentiation and axon guidance. This work provides insights on the common actions of Pax6 across the brain and, maybe more importantly, on the pathways that Pax6 might be controlling in a tissue-specific manner.

93 | Proteogenomic insights into 3'-UTR-associated RNA-binding proteins during neural differentiation

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Recent studies indicate that mutations in RNA-binding proteins (RBPs) may lead to neurodegeneration. RBPs fulfil their functions by tuning post-transcriptional regulations of gene expression. Here, I studied the dynamics of the recruited RBPs to the 3'-untranslated regions (3'-UTRs), which are strikingly diverse in nervous system. Indeed, the diversity of 3'-UTR-isoforms in neurons implies that the recruited RBPs are involved in different aspects of post-transcriptional regulation, and their malfunction may cause neurological disorders. Interestingly, transcription and processing happen simultaneously, hence, RNA processing is mostly co-transcriptional rather than post-transactional. In other words, many RBPs bind to their target readily during the transcription. Therefore, to study RBPs associated with the 3'-UTRs I targeted specifically phosphorylated RNA-polymerase II using ChIP-SICAP, a method I developed for studying chromatin-associated proteins. Targeting RNA polymerase II revealed 210 proteins of which 108 proteins are known RBPs. This includes RBPs involved in mRNA transcription, splicing and transport. Altogether, using this approach I could reveal novel aspects of post-transcriptional machinery during neural differentiation, and shed a new light on how 3'-UTR-associated RBPs regulate gene expression.

94 | Investigating lineage decisions and translocation phenomena during cone photoreceptors cell emergence

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Until recently, the retina was believed to develop from a pool of equipotent progenitors that commit to specific fates after their last apical division. However, our lab showed that a significant population of retinal neurons is generated by committed precursors that morphologically differ from apical progenitors. To understand how different types of committed precursors aid efficient retinal neurogenesis and lamination, we here study the emergence and development of photoreceptor cell precursors (PRCprs), that later give rise to cone photoreceptors. We use the zebrafish as a model organism due to its unmatched imaging potential. Using lineage analysis, we showed that the vast majority of PRCprs divide symmetrically within the developing PRC layer. These cells reach apical positions by undergoing an unexpected migratory pattern. Light sheet fluorescence microscopy revealed that PRCprs are born from apical progenitors and first undergo a directional and rapid basal trans-

location. Unlike progenitor cells that move apically in G2, they subsequently move back to the apical side during S phase in a stepwise manner and undergo G2 and mitosis apically. Since basal translocation of PRCprs is more efficient than of progenitor cells, we asked whether they employ different cytoskeleton components to move. Genetic interference with microtubule stability blocked PRCpr translocation, whereas it had no effect on progenitor cells. Nevertheless, arrested PRCprs managed to terminally divide, indicating that in these cells translocation and cell cycle are uncoupled. Next, we aim to explore the molecular basis of PRCpr behavior to understand how fate decision transforms progenitors into committed precursors.

95 | ECtomics: Dissecting spinal cord ependymal cell heterogeneity using single-cell transcriptomics

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Cells with stem cell potential, this is with the ability to self-renew and generate specialised progeny, exist in the spinal cord throughout life. Previous studies localised this stem cell potential within the ependymal cell (EC) population in axolotls, zebrafish, mice, and humans. Despite holding great promise for repair, the precise identity of the spinal cord stem cell remains elusive and little is known about why ECs in mammals are much worse than in axolotls at resolving spinal cord injuries.

In mouse, morphological features and the expression of a handful of neural stem cell markers suggest that ECs are rather heterogeneous. Due to limitations of traditional methods (requiring large numbers or cells or based on a limited set of markers genes), it is not known whether this morphological and molecular heterogeneity reflects functional differences or different maturation states. To tackle these questions, we are using single-cell RNA-sequencing technology and advanced computational methods to generate and analyse the transcriptomes of individual ECs from the adult mouse spinal cord. Our goal is to establish EC subtypes and cell states and use pseudotemporal ordering to elucidate potential lineage relationships among ECs. In parallel, we will validate EC subtypes in the tissue context using high-resolution confocal microscopy. This first comprehensive characterisation of spinal cord ECs in the mouse will provide novel and fundamental insights into how ECs possess and maintain their unique self-renewing properties. In the future, this study will facilitate realisation of the therapeutic potential of spinal cord stem cells.

96 | Gene regulatory mechanisms required for neuronal chemosensitivity

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Chemosensitive areas of the mammalian brainstem regulate breathing and are stimulated by small increases in carbon dioxide levels in arterial blood. The genetic and molecular bases for this type of chemosensitivity are poorly understood. C. elegans is an excellent model for probing mechanisms of gas-sensing because it possesses CO2 sensitive neurons (BAG neurons) that mediate stereotyped behavior and whose cell physiology can be studied in vivo. Proper expression of a BAG cell fate requires the ETS-domain-containing transcription factor ETS-5. Strikingly, its mammalian homolog Pet1 is required for the development of CO2-chemosensitive brain regions, and Pet1 mutants have defects regulating breathing in response to CO2 challenges. To characterize in vivo the DNA binding properties of ETS-5, I have performed a ChIP-seq study to identify loci occupied by ETS-5. I will identify which of these loci are ETS-5 gene targets that are functionally important for CO2-chemosensitivity. Furthermore, a comparison of the DNA sequences bound by ETS-5 in vivo to those bound by ETS-5 in vitro indicates that other DNA-binding factors cooperate with ETS-5 to activate gene expression in CO2-chemosensitive neurons. These studies will determine mechanisms by which a conserved gene-regulatory system promotes a specific neural fate and advance understanding of neuronal CO2-chemosensitivity.

97 | Zeb1 potentiates genome-wide gene transcription in glioblastoma via a novel Lef1-dependent mechanism

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Glioblastoma is the most common and aggressive brain tumor, with a subpopulation of stem-like cells thought to mediate its invasive growth and therapeutic resistance. The EMT inducing factor Zeb1 was linked to tumor initiation, invasion and resistance to therapy in glioblastoma, but how Zeb1 functions at molecular level and what genes it regulates remains poorly understood. Contrary to the common view that EMT factors act as transcriptional repressors, here we show that genome-wide binding of Zeb1 associates with both activation and repression of gene expression in glioblastoma stem-like cells. Transcriptional repression requires direct binding of Zeb1, while indirect recruitment to regulatory regions by the Wnt pathway effector

Lef1 results in gene activation, independently of Wnt signaling. Amongst genes activated by Zeb1 are predicted mediators of tumor cell migration and invasion, many of which correlate with Zeb1 expression in patient tumor samples, including the guanine nucleotide exchange factor Prex1. Prex1 promotes migration of glioblastoma cells and is predictive of low patient survival, highlighting the importance of the Zeb1/Lef1 gene regulatory mechanism in this brain tumor.

98 | MyT1 counteracts the neural progenitor program to promote vertebrate neurogenesis

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The generation of neurons from neural stem/progenitor cells requires large scale changes in gene expression. These are controlled to large extent by proneural transcription factors such as Ascl1, and include the repression of the Notch transcriptional program. While recent studies have focused on characterizing the differentiation genes activated by proneural factors, less is known on the mechanisms that suppress progenitor cell identity. Here, we show that Ascl1 induces the zinc-finger transcription factor MyT1 while promoting neuronal differentiation. We combined functional studies of MyT1 in the embryonic brain with the characterization of its transcriptional program, to address the role of MyT1 in neurogenesis. We found that MyT1 binding is associated with genome-wide repression of gene transcription in neural stem/progenitor cells. It promotes neuronal differentiation by counteracting the inhibitory activity of Notch signaling at multiple levels, targeting the Notch1 receptor and many of its downstream targets. These include regulators of the neural progenitor program such as Hes1, Sox2, Id3 and Olig1. Thus, Ascl1 suppresses Notch signaling cell-autonomously via MyT1, showing how activation of neuronal differentiation is tightly coordinated with repression of the progenitor program.

99 | Molecular control of self-renewal and neurogenic characteristics of cortical progenitors

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Self-renewing progenitors acquire their neurogenic competency at the expense of the progressive loss of their expansive capacity. We are investigating fate-switching mechanisms that change self-renewing progenitors into asymmetrically dividing progenitors, namely from neuroepithelium cells (NEs) into radial glia (RGs). Despite the importance of the transition in balancing self-renewing proliferation and differentiation of neural progenitors, the mechanisms underlying NE to RG transition are only partially understood.

Our previous studies have identified that Fgf10 regulates this transition process. In order to identify the downstream targets that may be involved in the switch from NE to RG, we have performed RNA seq analysis of Fgf10 gain- and loss of function mutant cortices. Through the functional analysis of these candidate genes in ES cell cortical progenitor differentiation assays, we have identified Tuba8 as an Fgf10 downstream gene target regulating cortical progenitor differentiation. Interestingly Tuba8 was recently identified as a causal gene of polymicrogyria, emphasizing its importance in cortical development. We will discuss the role of Tuba8 in corticogenesis, in particular from the aspect of cortical progenitor differentiation and cell division modes.

100 | Dynamics and function of distal regulatory elements during neurogenesis and neuroplasticity

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Gene regulation in mammals involves a complex interplay between promoters and distal regulatory elements that function in concert to drive precise spatio-temporal gene expression programs. However, the dynamics of distal gene regulatory landscape and its function in the transcriptional reprogramming that underlies neurogenesis and neuronal activity remain largely unknown. Here, we performed a combinatorial analysis of genome-wide datasets for chromatin accessibility (FAIRE-seq) and the enhancer mark H3K27ac that reveal the highly dynamic nature of distal gene regulation during neurogenesis, which gets progressively restricted to distinct

genomic regions as neurons acquire a post-mitotic, terminally differentiated state. We further find that the distal accessible and active regions serve as target sites for distinct transcription factors that function in a stage-specific manner to contribute to the transcriptional program underlying neuronal commitment and maturation. Mature neurons respond to a sustained activity of NMDA receptors by epigenetic reprogramming at a large number of distal regulatory regions as well as dramatic reorganization of super-enhancers. Such massive remodeling of distal regulatory landscape in turn results in a transcriptome that confers a transient loss of neuronal identity and gain of cellular plasticity. Furthermore, NMDA receptor activity also induces many novel pro-survival genes that function in neuroprotective pathways. Taken together, these findings reveal the dynamics of the distal regulatory landscape during neurogenesis and uncover novel regulatory elements that function in concert with epigenetic mechanisms and transcription factors to generate the transcriptome underlying neuronal development and activity.

101 | A zebrafish chemical genetics screen identifies distinct contributions of epigenetics in dopaminergic neurogenesis

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During neurogenesis neural progenitor cells recruit distinct gene expression programs to specify different developmental trajectories and eventually attain specific cellular identities.

Chromatin regulators act in concert with developmental signals and transcription factors to establish and maintain these specific gene expression profiles. Development of dopaminergic neurons from neural progenitor cells has emerged as a relevant paradigm for the study of cellular differentiation in the nervous system.

Recent studies have revealed organization of distinct dopaminergic subgroups both in zebrafish and rodents. Yet, less is known about chromatin regulation in dopaminergic progenitors and in process of their terminal differentiation. To identify epigenetic mechanisms that function during dopaminergic neurogenesis, we performed a small molecule screen in zebrafish embryos and compared the development of dopaminergic to other neuronal types in situ in treated embryos. We designed a library of 40 small molecule compounds that targets a broad range of epigenetic protein classes and mechanisms. Our screen identified several small molecule compounds associated with histone acetylation as well as H3K4 methylation affecting dopaminergic neurogenesis in zebrafish. Our findings also include regulators that have previously not been implicated in embryonic neurogenesis in vivo. Subsequent analysis targeted distinct processes during which the small molecule exposure mi-

ght interfere, ranging from neural stem cell/progenitor proliferation to their differentiation and cell survival. Our chemical screen provides a resource of chromatin regulatory mechanisms involved in dopaminergic neurogenesis. To further investigate the specific contributions of each of these chromatin regulatory processes identified in our screen, we are now undertaking a molecular genetics approach.

102 | On demand optogenetic activation of human stem cell-derived neurons

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Human stem cell-derived neurons offer various possibilities to study neuronal function in health and disease. A powerful tool to optically activate and silence neurons is to utilize light-gated ion channels. However, the lack of robust optogene expression in human iPS cell-derived neurons impedes functional studies. Transgenic iPS cell lines lose optogene expression within few passages and viral delivery of optogenes to differentiated neurons has been shown to be very variable. By using the piggyBac Transposon system for gene delivery, we have generated stable human iPS cell lines, which harbor optogenes that can be activated on demand in postmitotic neurons. The cells are easily differentiated into neurons by transcription factor overexpression. Whereas activation in stem cells caused optogene expression to be rapidly lost, induction in differentiated neurons was stable over several passages. Without further selection, our method allows the activation of optogenes in more than 40% of the neurons compared to 1% efficiency by AAV delivery. Robust photocurrents and action potentials were evoked by light stimulation in optogene-expressing cells compared to control cells.

In summary, our inducible neuronal cell lines are an advanced and user-friendly system allowing the widespread application of optogenetics in stem cell-derived neurons and cerebral organoids.

103 | The radial fiber-mediated FGF signal ensures the feedback signaling from neurons to radial glial cells in the developing cerebral cortex

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The temporal control of the balance of self-renew and differentiation of radial glial (RG) cells is crucial to determine the number of different types of neurons in the cerebral cortex. Recently, we revealed that the inheritance of the radial fiber is important for the RG cells self-renewal. Although it is well known the radial fiber function as the scaffold of migrating neurons, the role and mechanism of radial fiber in the controlling of RG cells self-renewal is largely unknown. Here, we showed that the radial fiber is critical for the receiving neuron derived FGF18 signal and transport of FGF activated ERK MAPK, which promotes RG cell self-renewal. In the radial fiber, the activated ERK MAPK is localized on the endosomal vesicles and transported by the dynein motor system. Furthermore, we examined FGF18 gene function. FGF18 is transiently expressed the early born neurons and its temporal signal contributes the production of late born neurons by promoting the RG cells self-renewal. From these results, we propose a temporal feedback signaling system from neurons to RG cells mediated by the radial fiber, in which the fine tuning of proportion between the early born neurons and the late born neurons.

104 | Transcriptomics reveal an integrative role for maternal thyroid hormones during zebrafish embryogenesis

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Thyroid hormones (THs) are essential for embryonic brain development, but the genetic mechanisms involved in the action of maternal THs (MTHs) are still largely unknown. Here to begin to understand the underlying genetic mechanisms under MTHs regulation we used an established zebrafish MCT8 knockdown model and performed transcriptome analysis in morphant and control zebrafish embryos. Subsequent mapping of differentially expressed genes in Reactome pathway analysis together with in situ expression analysis and immunohistochemistry allowed to unravel the genetic networks and cells under MTHs regulation in zebrafish embryogenesis. We found 4,343 differentially expressed genes and Reactome pathway analy-

sis revealed TH is involved in 1681 pathways. Notably, MTHs regulate the expression of core developmental pathways, such as WNT and NOTCH in a cell specific context. Cellular distribution of neural MTHs-target genes demonstrated cell specific action for MTHs in neural stem cells maintenance and fate decision of neuron classes. Together our data shows that MTH acts as an integrator by regulating the expression of genes involved in the cross-talk between key pathways in neural development during zebrafish embryogenesis. All in line with observed knockdown phenotype and the phenotype of human AHDS patients our data is able to clarify the genetic origins behind this human congenital condition.

105 | De novo ganglion cell genesis by targeted expression of KLF4 in retinal progenitors with restricted neurogenic potential

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The most prevalent cause of irreversible blindness worldwide is the degeneration of retinal ganglion cells (RGCs) in glaucoma. One relevant strategy to restore vision is the stimulation of self-repair mechanisms, such as the endogenous reprogramming of Müller glia cells (MGCs). However, in mammals, MGCs are similar to postnatal retinal progenitor cells (RPCs), both exhibit restricted neurogenic competence in such a way that MGCs regenerate rod photoreceptors (RP) when stimulated, but they are not competent to generate RGCs. In this study, by targeting the expression of Klf4, a key regulator of stemness and iPS generation, we reprogrammed the competence of postnatal RPCs in vivo. First, we tested whether KLF4 is required for RGC genesis during retinal development. Conditional inactivation of Klf4 in mice (cKO) did not affect visual acuity nor the generation of RGCs. To test if KLF4 is sufficient to reprogram restricted RPCs, we performed gain-of-function experiments in vivo at late stages of retinal development. KLF4-overexpressing cells prematurely exit the cell cycle, fail to generate RPs and to express RP specification genes. Strikingly, KLF4 overexpression induced the activation of genes essential for RGC development. In this condition, a significant number of cells migrated to the ganglion cell layer and projected axons towards the optic nerve head, which reinforces that they adopted the RGC fate. These results show that it is possible to induce RGC genesis outside of their developmental window by a single reprogramming factor. Therefore, this discovery has potential for development of new glaucoma therapies via reprogramming of MGCs.

106 | Screening for novel binding partners of the developmental transcription factor Isl1

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LIM-homeodomain (LIM-HD) transcription factors act as key developmental regulators, both through their ability to bind DNA through homeodomain:DNA interactions, and through their ability to form larger complexes through protein:protein interactions. The LIM-HD protein Islet-1 (Isl1) has been shown to be involved in the development of many crucial tissues, including motor neurons, the striatum, retina, and pituitary gland in the brain, and several other tissues throughout developing mammals. However, many of the mechanisms behind its action have yet to be determined.

The Isl1 homeodomain has low intrinsic DNA-binding specificity. Thus, its ability to influence gene expression in different cell types is highly dependent on cofactor proteins. Although the Isl1-containing transcriptional complex found in developing motor neurons is well characterised, the key partners of Isl1 in other tissues are less well described.

In order to identify partners of Isl1 that could form transcriptional complexes relevant to development, yeast two-hybrid mating screens have been used, utilising the N-terminal LIM domains of Isl1 as bait, as these are known protein:protein interaction domains. Both the isolated LIM domains, and the LIM domains bound to the LIM-interacting region of their primary cofactor, Ldb1, have been screened.

Screening has so far resulted in a pool of approximately 100 Isl1-interacting proteins. Once these interactions are confirmed to be biologically relevant, they may provide understanding into the mechanism of action of Isl1 in distinct tissues, giving more insight into how Isl1 plays such a diverse role in development.

107 | The Super Elongation Complex interacts with Notch signaling to drive neural stem cell fate commitment

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Asymmetric stem cell division establishes an initial difference between a stem cell and its differentiating sibling, critical for maintaining homeostasis and preventing carcinogenesis. Yet the mechanisms that consolidate and lock in such initial fate bias remain obscure. Here, we use Drosophila neuroblasts to demonstrate that the super elongation complex (SEC) acts as an intrinsic amplifier to drive cell fate commitment. SEC is highly expressed in neuroblasts, where it promotes self-renewal by physically associating with Notch transcription activation complex and enhancing

HES (hairy and E(spl)) transcription. HES in turn upregulates SEC activity, forming an unexpected self-reinforcing feedback loop with SEC. SEC inactivation leads to neuroblast loss, whereas its forced activation results in neural progenitor dedifferentiation and tumorigenesis. Our studies unveil an SEC-mediated intracellular amplifier mechanism in ensuring robustness and precision in stem cell fate commitment and provide mechanistic explanation for the highly frequent association of SEC overactivation with human cancers.

108 | Her6_venus knock-in characterization and its dynamic expression during zebrafish neural development

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The discovery of short period (ultradian) oscillations in gene expression, has revolutionised our ideas of the mechanisms by which cells make choices in fields as diverse as neurogenesis, stem cell heterogeneity and inflammation.

Our previous work had shown that miR-9 is essential for neurogenesis, an effect mediated by targeting hairy1 (Xenopus). We have also showed that mir-9 controls the ultradian oscillations of Hes1. Based on RT-PCR data, we have shown that pri-miR-9 is also likely to oscillate in progenitors and that mature miR-9 builds over time, eventually terminating Hes1 oscillations. Therefore, we have proposed that a mir-9/Hes1 regulatory loop controls oscillations in neural progenitors and can form a tunable timer for cell differentiation.

To understand the mechanism and significance of such oscillations one must be able to study dynamic regulation of gene expression in vivo. The zebrafish embryo is a system of choice for live imaging that allows the analysis of cellular dynamics in the context of the whole organism.

In order to address our hypothesis we generated a Her6_venus knock-in fish. An intensive characterization of this line was carried out including double whole-mount FISH to correlate venus expression to endogenous her6 expression, mRNA half life and protein half life.

We are currently investigating dynamic expression of her6 at a single cell level in the tissue context during embryonic development. In addition, we will correlate dynamics with cell state by analyzing her6_venus expression in conjunction with a GFAP progenitor reporter or elavl3 neuronal reporter.

109 | Uncovering gene regulatory function of a novel zinc finger protein in embryonic neurogenesis

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Neuronal development is a complex process that relies on a precisely controlled transcriptional program as well as the genomic integrity, wherein transcriptional regulators such as zinc finger proteins play a critical role. Despite independent characterization, zinc finger factors involved in regulation of cellular differentiation during neurogenesis remain largely unknown. In this study, we employed extensive bioinformatics approaches to retrieve a collection of known and novel zinc finger proteins encoded in the murine genome. Combining this prediction with gene expression profiling of tissues across lineages during embryonic development, we identified a set of zinc finger factors that are restricted to neuronal tissues and highly induced during neuronal development. We further extensively characterized an essential role of one such unexplored protein MAJ1 during neurogenesis. To uncover whether this protein directly regulates gene expression, we next attempted to identify genomic targets of this protein and at the same time performed gene expression profiling following its depletion during neurogenesis. Interestingly further, immunoprecipitation assay in combination with mass-spectrometry revealed that it associates with repressive epigenetic machinery and thus potentially functions in gene repression during cortical development. We are currently analyzing these data and further performing biochemical assays to gain deeper insights into the mechanism of how this factors influences the gene regulatory program underlying cortical development.

110 | Genome Stability by DNA polymerase ß is critical for neuronal differentiation in cortical development

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Maintenance of genomic stability is thought to be crucial for brain morphology and function. DNA polymerase ß (Polß) is a core DNA repair enzyme in base excision repair (BER) and is also involved in epigenetic DNA demethylation processes. Its deficiency has been shown to induce widespread neuronal apoptosis in the developing

nervous system. Here, to investigate the underlying mechanism of this apoptosis, we studied the spatiotemporal roles of Polß in the developing cortex using two distinct forebrain specific conditional knockout mice, Emx1-Cre/Polßfl/fl in which the floxed Polß allele is deleted in neural progenitors and Nex-Cre/Polßfl/fl in which the allele is lacked in postmitotic neurons. In Emx1-Cre/Polßfl/fl mice, we found that DNA double-strand breaks (DSBs) formed during replication in cortical progenitors remained unrepaired in postmitotic neurons, which led to p53-mediated apoptosis and axonal growth defects. In contrast, these phenotypes were not observed in Nex-Cre/Poßfl/fl mice. Moreover, DNA base-damaging agents remarkably enhanced DSB formation in cultured Polß-deficient cortical progenitors. Similar effects were observed with vitamin C treatment, which induces TET1-mediated active DNA demethylation. In support of this view, gene expression analysis showed that a group of genes were aberrantly expressed in the developing cortex of the Emx1-Cre/Polß fl/fl mice. These findings suggest that Pol ß-dependent BER contributes to neuronal differentiation during cortical development by maintaining genome stability in active DNA demethylation of neural progenitors.

111 | Development of an autonomous platform for high throughput manipulation of single stem and progenitor cell in intact tissue

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A central goal in developmental neuroscience is understanding the genetic logic of brain development and evolution and the role of single neural stem and progenitor cells in brain tissue formation in ontogeny and phylogeny. To answer this question, we have previously developed microinjection into single stem cells as a means to track and manipulate stem cells in tissue (Taverna et al, 2012; Wang et al, 2014; Kalebic et al, 2016)). Here we report recent advances in the development of a computer vision guided, fully autonomous microinjection platform capable of manipulating single neural stem and progenitor cells in tissue. We will use this tool to reconstitute specific neural stem cell types and to reprogram neural stem cells in developmental and evolutionary space.

112 | Non-canonical features of the Golgi apparatus in bipolar epithelial neural stem cells

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Apical radial glia (aRG), the stem cells in developing neocortex, are unique bipolar epithelial cells, extending an apical process to the ventricle and a basal process to the basal lamina. Here, we report novel features of the Golgi apparatus, a central organelle for cell polarity, in mouse aRGs. The Golgi was confined to the apical process but not associated with apical centrosome(s). In contrast, in aRG-derived, delaminating basal progenitors that lose apical polarity, the Golgi became pericentrosomal. The aRG Golgi underwent evolutionarily conserved, accordion-like compression and extension concomitant with cell cycle-dependent nuclear migration. Importantly, in line with endoplasmic reticulum but not Golgi being present in the aRG basal process, its plasma membrane contained glycans lacking Golgi processing, consistent with direct ER-to-cell surface membrane traffic. Our study reveals hitherto unknown complexity of neural stem cell polarity, differential Golgi contribution to their specific architecture, and fundamental Golgi re-organization upon cell fate change.

113 | Mnb/Dyrk1A orchestrates a signaling network linking the mechanisms that regulate neurogenesis, cell cycle, and terminal differentiation of neuronal progenitors

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The Down syndrome and microcephaly related gene Mnb/Dyrk1A encodes an evolutionary conserved protein kinase subfamily which plays diverse functions in brain development. Its founding member was identified in Drosophila melanogaster and was named minibrain (mnb) due to the reduced brain size associated to its loss of function. This phenotype was originated by altered proliferation in the developing larval brain, suggesting key functions for Mnb/Dyrk1A in the control of neural proliferation and neurogenesis (Tejedor et al., 1995). Previous works of our lab have shown that Mnb/Dyrk1A is sequentially expressed in neural progenitors at the proliferation to neurogenesis transition, in cycling neurogenic progenitors and in newborn neurons at the developing Drosophila and vertebrate CNS (Hammerle et al, 2002, Hammerle et al, 2008; Hammerle et al, 2011; Shaikh et al, 2016). This complex

but precise developmental expression pattern reveals sequential functions of Mnb/Dyrk1A in the regulation of the neurogenic switch, the cell cycle, and in the terminal differentiation of neuronal progenitors. The dissection of the molecular mechanism underlying these functions display a complex signaling network in which Mnb/Dyrk1A regulates several transcription factors (Ase/Mash1/Ascl1; Dpn/Hes; Pros), various cell cycle regulators (p27kip1, CycD1) and signaling cascades (Notch). We propose that Mnb/Dyrk1A helps to coordinate these mechanisms along the cellular processes of neurogenesis ensuring the precise timing of neuronal production.

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114 | Early-onset dystonia gene Kmt2b is selectively required for reprogramming cell fate to the neuronal lineage

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Transcription factor-driven reassignment to the neuronal fate has defined the paradigm of direct lineage conversion, opening new vistas on the mechanisms that enable cell fate transitions across germ layers. Yet, while both pioneer factors and their target chromatin configuration are well defined, the chromatin regulatory mechanisms that mediate the dynamic acquisition of neuronal fate remain elusive. Here we defined the functional role of two methyltransferases of lysine 4 on histone H3, KMT2A and KMT2B (also called, respectively, MLL1 and MLL2), founding mammalian members of the Trithorax family of epigenetic regulators of cell identity, and causative genes of, respectively, two neurodevelopmental disorders, Wiedemann-Steiner syndrome and early onset dystonia. Through both single and compound conditional inactivation of the two enzymes during induced neuronal (iN) cell reprogramming, we discovered that KMT2B is selectively required for neuronal transdifferentiation, with its loss impacting not only the efficiency of lineage conversion but also, specifically, the unfolding of neuritogenesis. In particular, by integrating imaging, transcriptomic and epigenomic profiling at distinct stages of transdifferentiation upon KMT2B inactivation, we uncovered: i) conspicuous transcriptional changes in great excess of those observed in mature cells, underscoring the power of this approach in detecting fate switch vulnerabilities; ii) a prevalent dyregulation in the maturation transdifferentiation subnetwork, providing a molecular blueprint for impaired neu-

ritogenesis; iii) a failure to fully suppress alternative fates; iv) dysregulation in key dystonia causative genes, establishing the value of cell fate conversion systems for the elucidation of disease-relevant pathways.

115 | The Gli3 zinc finger transcription factor controls cortical neurogenesis by regulating the cell cycle

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The cerebral cortex consists of an enormous number of neuronal subtypes and this diversity underlies its ability to perform highly complex neural tasks. How these neurons develop at the correct time and space and in sufficient numbers constitutes a major challenge in Developmental Neurobiology. We are studying the molecular basis of this process by investigating the function of the Gli3 zinc finger transcription factor, a key regulator of cortical development. We provide evidence for Gli3's novel role in controlling the early neuronal differentiation of radial glial cells. In Gli3 mutant mice, early neurogenesis is delayed leading to the failure to form subplate neurons and to an increased formation of upper layer neurons. Gene expression profiling indicated that altered expression of cell cycle control genes precedes this neurogenesis defect. Indeed, the cell cycle length of cortical progenitors is reduced due to shorter G1 and S-phases. Expression of the cell cycle dependent kinase Cdk6, which regulates the G1-S transition, is up-regulated in Gli3 mutants. Gli3 binding to the Cdk6 promoter represses Cdk6 expression suggesting that Gli3 regulates the cell cycle of cortical progenitors by controlling Cdk6 expression. Finally, we will present data from ongoing pharmacological rescue experiments which aim at reducing Cdk6 activity in Gli3 mutants. Taken together, our data reveal a link between Gli3, the cell cycle and control of neurogenesis in cortical development. These findings will contribute to our understanding of the molecular mechanisms that allow cortical progenitors to be maintained but also to produce neurons in sufficient numbers.

116 | A novel regulatory function within an ancestral splicing factor underlies the emergence of the neuronal microexon programme

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Gene regulatory programmes are often organized in networks controlled by master regulators. Deciphering the key evolutionary steps leading to the formation of these networks can help us understand their functioning as well as their impact on the biological processes they control. Our work focuses on the evolution of a neuron-specific splicing programme: microexons. Microexons are tiny exons as short as 3nt that are selectively included in neurons compared to other cell types, often located in protein regions mediating protein-protein interactions. Importantly, microexon regulation shows a switch-like behaviour during neuronal differentiation and was found to be impaired in autistic patients. The inclusion of the majority of microexons depends on the vertebrate and neural-specific regulator SRRM4. However, by analysing tissue-specific RNA-seq data from several metazoan species we have uncovered an unprecedented degree of conservation for microexons, which share neural-specificity and SRRM4 cis-regulatory motifs across large evolutionary distances. These observations led us to examine in detail the SRRM2/3/4 locus in non-vertebrates, revealing extensive control at the level of alternative gene isoforms between tissues. Transphyletic swaps of these isoforms showed a conserved regulatory mechanism for microexon inclusion, which relies on a novel uncharacterized protein domain expressed only in neural tissues. We propose that restricting the usage of this novel regulatory function to neurons enabled the remodelling of the neuronal proteome in metazoans.

117 | Systematic comparison of brain organoids versus human fetal tissues transcriptomes reveals model-specific neural differentiation fates

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Three-dimensional cultures to model diseases and development are becoming of great interest in current research, especially in the context of neuronal maturation where, until now, our knowledge relies almost exclusively on animal models, whose brain development is very different from humans. In this context, several protocols using human induced pluripotent stem cells (hiPSCs) as a starting point have been set up to obtain brain organoids relying in two different approaches: self-patterning versus extrinsic cues-driven patterning. Here, we applied a patterning-driven approach to generate cortical spheroids from control-derived hiPSC lines and we analyzed transcriptomes from two different stages, finding, as expected, that our system pushes neuronal maturation towards dorsal telencephalon. Moreover, we generated transcriptomes from human fetal tissues from different regions of the brain, which represent a rare dataset that we used as reference to compare transcriptomes from (1) cortical spheroids, (2) two-dimensional glutamatergic neuronal

cultures differentiated from control-derived hiPSC and (3) published transcriptome datasets from brain organoids differentiated by different protocols,. Principal component analysis revealed that cortical spheroids cluster with fetal tissues as opposed to two-dimensional neuronal cultures, indicating that they recapitulate better the differentiation process. The comparison of multiple protocols revealed the transcriptional identities pushed by the different organoids differentiation approaches. Preliminary analysis already showed that around 70% of the transcriptome of cortical spheroids follows the same expression pattern found in fetal tissues, indicating that cortical spheroids are suitable for the accurate in vitro study of neurodevelopmental disorders involving cortical development.

118 | Epigenetic regulation by BAF (mSWI/SNF) complexes in cortical neurogenesis

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During early neocortical development, neural stem cells (NSCs) divide symmetrically to expand the progenitor pool, whereas in later stages, NSCs typically divide asymmetrically to self-renew and produce other cell types. Although the timing of the switch from symmetrical (proliferation) to asymmetrical (differentiation) division is critical for determining progenitor and neuron numbers, nevertheless epigenetic mechanisms that control symmetrical and asymmetrical divisions in early and late cortical development are not fully understood. In my talk, I will discuss our unpublished data in understanding of chromatin remodeling during cortical neurogenesis. I will highlight roles of chromatin remodeling SWI/SNF (BAF) complexes and their epigenetic cofactors in NSC proliferation, differentiation in early and late cortical development

119 | Nipbl interacts with Zfp609 and the integrator complex to regulate cortical neuron migration

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Mutations in NIPBL are the most frequent cause of Cornelia de Lange syndrome (CdLS), a developmental disorder encompassing several neurological defects, including intellectual disability and seizures. How NIPBL mutations affect brain development is not understood. Here we identify Nipbl as a functional interaction partner of the neural transcription factor Zfp609 in brain development. Depletion of Zfp609 or Nipbl from cortical neural progenitors in vivo is detrimental to neuronal migra-

tion. Zfp609 and Nipbl overlap at genomic binding sites independently of cohesin and regulate genes that control cortical neuron migration. We find that Zfp609 and Nipbl interact with the Integrator complex, which functions in RNA polymerase 2 pause release. Indeed, Zfp609 and Nipbl co-localize at gene promoters containing paused RNA polymerase 2, and Integrator similarly regulates neuronal migration. Our data provide a rationale and mechanistic insights for the role of Nipbl in the neurological defects associated with CdLS.

120 | Modeling neurological diseases using single cell genomics in brain organoids

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The central nervous system is the most complex organ in mammals. During embryogenesis, neural progenitor cells self-renew as well as differentiate into to give rise to neurons, astrocytes and oligodendrocytes at distinct stages of development. It is very clear that neuronal development is driven by a highly complex gene regulatory network involving transcription factors and epigenetic regulators. It is increasingly being appreciated that a defect in these programs during early development may underlie complex neurological disorder in humans. Using available datasets from genome-wide association studies (GWAS), we found 40 neurological diseases to be associated with 543 SNPs. Majority of those SNPs are localized in the non-coding regions, while the regulatory impact of these genomic elements is not known in the context of brain development and function. In this study, we will generate cerebral organoids (CO) from human induced pluripotent stem cells (hiPSC) derived from patients with SNP-associated neurological diseases. We will next decipher the function of these non-coding SNPs during neuronal development by an extensive phenotypic analysis of derived organoids. Ultimately, we will employ single-cell gene expression and accessibility analysis to decode region-specific misregulation in the gene regulatory program and relate this to better understand the disease.

121 | The role of synaptic protein SV2B in embryonic development of the cerebral cortex

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The Outer Subventricular Zone (OSVZ) is a unique germinal layer crucial for the evolutionary expansion of the mammalian cerebral cortex, promoting gyrencephaly. By using ferret as animal model of folded brains, we have recently identified a critical period during early embryonic development when apical Radial Glia Cells (aRGCs) in the ventricular zone (VZ) undergo self-consuming divisions to produce massive amounts of basal Radial Glia Cells (bRGC). These early-formed bRGCs are founder progenitor cells seeding the OSVZ, and blockade of bRGC production during this critical period profoundly impairs the formation of the OSVZ. The gene encoding for Synaptic Vesicle Glycoprotein 2B (Sv2b) is differently expressed in the VZ before, during and after the critical period for bRGC formation and OSVZ seeding, and hence it is an attractive candidate to regulate this process. Importantly, Sv2b is expressed in the germinal layers of the developing ferret cortex but not in mouse, with a small and smooth cortex without bRGCs nor OSVZ. We have overexpressed Sv2b in progenitor cells of the embryonic mouse cortex by in utero electroporation at E14, and found that in the short-term (E17) it disrupted the distribution of electroporated cells. Sv2b overexpression disassembled the laminar organization of the VZ, induced the delamination of aRGCs to basal positions and altered their proliferation. At mid-term (P5), we observed the formation of folds in the electroporation site, with preserved lamination of cortical neurons. We propose that Sv2b may play important roles in the developmental formation of bRGCs and the OSVZ, and hence in the expansion and folding of the mammalian cortex.

122 | Splicing factors affect neural fate decisions by regulating TrkC splice variant balance

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An interplay between cell fate decisions in neural progenitor cells (NPCs) and neuron survival establishes the six-layered structure of the mammalian cerebral cortex during development. In turn, the correct distribution of projection neuron subtypes among these six layers dictates cognitive performance. We could previously show that the protein product of an alternative splicing event at the TrkC locus, TrkC-T1, is involved in the control of neocortical cell fate. Even though the high prevalence of alternative splicing events in the CNS is well-known, the role of transcript variants in the generation and maintenance of cortical layers has not been elucidated. We investigate how fine-tuning the balance between TrkC-T1 and the longer variant, TrkC-TK+, regulates neuronal differentiation and survival, which are essential for correct cortical development. Specifically, we are interested in the regulators of this transcript variant ratio, which we think enable cell-type-dependent functions of the

TrkC locus. We suggest that cell-type specific combinations of active splicing factors account for the change in TrkC variant balance during differentiation. We have bioinformatically identified exonic splicing enhancers in one of the cassette exons included in TrkC-T1, and showed that the splicing factor Srsf1 can bind these enhancers. Additionally, two of the kinases regulating the activity of Srsf1, Srpk1 and Srpk2, are differentially expressed in NPCs and neurons, pointing to different regulatory mechanisms in these cell types. In conclusion, our data indicate that Srsf1 is a strong candidate regulator of TrkC alternative splicing, and, consequently, that it may have a powerful impact on cortical structure.

123 | Investigating neural functions of imprinted, small nucleolar RNAs

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Small nucleolar RNAs (snoRNAs) are ubiquitously expressed and function in ribosomal RNA (rRNA) maturation through direct base pairing. One snoRNA class has conserved C/D box sequence motifs and recruits the Fibrillarin snoRNP complex to 2'-O-methylate complementary rRNA substrates. Interestingly, several C/D box snoRNAs are specifically expressed in neurons and lack rRNA complementarity. Even more peculiar, these snoRNAs are embedded within imprinted, long non-coding RNAs. The Snord116 and Snord115 snoRNAs are processed from the paternally expressed Ube3a antisense transcript, implicated in Prader-Willi syndrome. Another cluster of snoRNAs is processed from the maternally expressed Meg3 transcript. It has not been determined if these orphan snoRNAs are trans-acting RNAs that direct 2'-O-methylation of novel substrates and/or cis-acting RNAs that regulate allele-specific gene expression.

We established an in vitro system that permits allele-specific detection of neural gene expression. Hybrid embryonic stem cells (ESCs) from an M.m.musculus x M.m. castaneous cross were differentiated by Neurogenin-2 induction. Eighty percent of cells are TUBB3-positive by two days, and electrophysiological activity peaks by two weeks of induction. During differentiation the imprinted snoRNAs are up-regulated. We determined that these snoRNAs are enriched in the Fibrillarin snoRNP complex by RNA immunoprecipitation. Therefore, we hypothesize that imprinted snoRNAs direct 2'-O-methylation of unidentified RNA substrates. To identify potential substrates, we generated ESCs with deletion of the Meg3 snoRNAs. We are performing Fibrillarin CLIP-seq on differentiated neurons to identify putative RNA targets depleted upon snoRNA deletion. Successful target identification would provide novel insight into the potential functions of these unique neuron-specific, imprinted snoRNAs.

124 | Transcription factors binding to alternative promoters drive Rbfox1 expression in neurons

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The alternative splicing factor Rbfox1 controls neuronal excitation. The encoding gene contains a large noncoding part at its 5'end with four alternative promoters. These four promoters drive expression of Rbfox1 transcript isoforms that differ in their 5'UTR but not in their coding exons. Rare deletions in the 5' region of the human RBFOX1 gene were found in autism patients. The detected deletions include RBFOX1 intronic sequences as well as isoform specific 5'UTR exons and likely interfere with transcriptional regulation of individual RBFOX1 transcript isoforms. However, the pathogenic potential of the respective deletions is still unclear. This is due to a lack of understanding of RBFOX1 transcriptional regulation and of a suitable animal model.

We demonstrated that Rbfox1 expression in the mouse brain is driven by two out of the four conserved alternative promoters. Promoter 1 seems to be important to drive Rbfox1 expression in the developing neocortex and in most adult brain regions. Rbfox1 isoforms transcribed from Promoter 2 are highest in adult cerebellum. We have further identified transcription factors that bind to either the first or second promoter and drive expression of Rbfox1 in cortical neurons. Our results predict that reduced expression of only one of the two brain specific Rbfox1 transcript isoforms suffices to decrease Rbfox1 expression in different brain regions and may contribute to the pathogenesis of autism in RBFOX1 deletion carriers. To further study the causative role of reduced Rbfox1 expression in autism pathogenesis we are analyzing the Rbfox1 knockout mouse model in an autism battery.

125 | Cell non-autonomous role for the Lef1 transcription factor in the development of gabaergic domains in the visual system

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The Lef1 transcription factor, an effector of the canonical Wnt pathway, has been implicated in neurogenesis in some parts of the telecephalon. Lef1 is also an early marker of discrete populations of neurons in the dien- and mesencephalon, but its role in these brain regions was not established so far. To address this issue we used zebrafish as a model organism, and silenced lef1 with morpholinos.

Although the lef1 mRNA can be easily detected as early as at the stage of 10 somites, the Lef1 protein starts to appear in the dorsal diencephalon and mesencephalon (prospective thalamus and optic tectum) between 24 and 30 hrs post fertilization (hpf), in postmitotic glutamatergic neurons. In agreement, the induction of the mid-diencephalic organizer was not affected in morholino zebrafish embryos and progenitor domains of the thalamus and optic tectum were formed. However, the markers of gabaergic progenitors were not expressed in the adjacent gabaergic domains. Consequently, morpholino larvae lack gabaergic neurons in the thalamus, pretectum and optic tectum, i. e., the regions that are involved in visual processing. These results suggest that gabaerginc neurogenesis in the dorsal dien- i mesencephalon is regulated by external signals that are provided by newborn neurons in non-gabaergic niches, and that these signals are activated by Lef1. Our current research is directed towards explaining this non-autonomous effect of Lef1.

126 | Species-specific mechanisms of neuron subtype specification reveal evolutionary plasticity of amniote brain development

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Highly ordered brain architectures in vertebrates consist of multiple neuron subtypes with specific neuronal connections. However, the origin of and evolutionary changes in neuron specification mechanisms remain unclear. Here, we report that regulatory mechanisms of neuron subtype specification are divergent in the developing amniote brains. In the mammalian neocortex, the transcription factors (TFs) Ctip2 and Satb2 are differentially expressed in layer-specific neurons, whereas these TFs are co-localized in reptilian and avian neurons. We demonstrate a species-specific dependency in the fate restrictions of progenitors and the activity of cis-regu-

latory elements on the Ctip2 locus that accounts for the unique expression patterns of TFs in reptilian and avian brains. Furthermore, we revealed that the expression patterns of TFs and neuronal connections are not strictly conserved among amniotes. Our findings suggest that the regulatory mechanisms of neuronal specification are not closely associated with specific neuronal characteristics in amniotes and that the establishment of mammalian-specific regulatory elements has allowed for novel neuronal subtypes during neocortical evolution.

127 | Sensory experience regulates cortical inhibition by inducing IGF1 in VIP neurons

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Inhibitory neurons regulate the adaptation of neural circuits to sensory experience, but the molecular mechanisms by which experience controls the connectivity between different types of inhibitory neuron to regulate cortical plasticity are largely unknown. Here we show that exposure of dark-housed mice to light induces a gene program in cortical vasoactive intestinal peptide (VIP)-expressing neurons that is markedly distinct from that induced in excitatory neurons and other subtypes of inhibitory neuron. We identify Igf1 as one of several activity-regulated genes that are specific to VIP neurons, and demonstrate that IGF1 functions cell-autonomously in VIP neurons to increase inhibitory synaptic input onto these neurons. Our findings further suggest that in cortical VIP neurons, experience-dependent gene transcription regulates visual acuity by activating the expression of IGF1, thus promoting the inhibition of disinhibitory neurons and affecting inhibition onto cortical pyramidal neurons.

128 | Candidate gene changes underlying the species-specific brain growth trajectory of *Homo sapiens*

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The well-known skull shape difference between modern humans and extinct Homo can be understood as the result of a change in brain growth trajectory early in life

(Hublin et al. 2015). This change, termed the 'globularization phase' (Gunz et al 2012), amounts to an extended period of growth around birth and delayed maturation of the brain structures of humans that likely contribute to their cognitive complexity, including our linguistic capacity (Boeckx and Benitez-Burraco 2014, Boeckx 2016). I provide a detailed examination of the paleogenomic record to highlight a few candidate changes (mostly regulatory) that the literature on cortical expansion gives us reasons to consider good causal candidates for an account of globularization. Among these are genes routinely associated with microcephaly and macrocephaly, and speficically, a high number of Outer-Subventricular Zone-related genes. The genes highlighted are known to interact with one another and thus provide a network that may have provided the seeds for the neurobiological basis of cognitive modernity.

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NOTES:

List of participants
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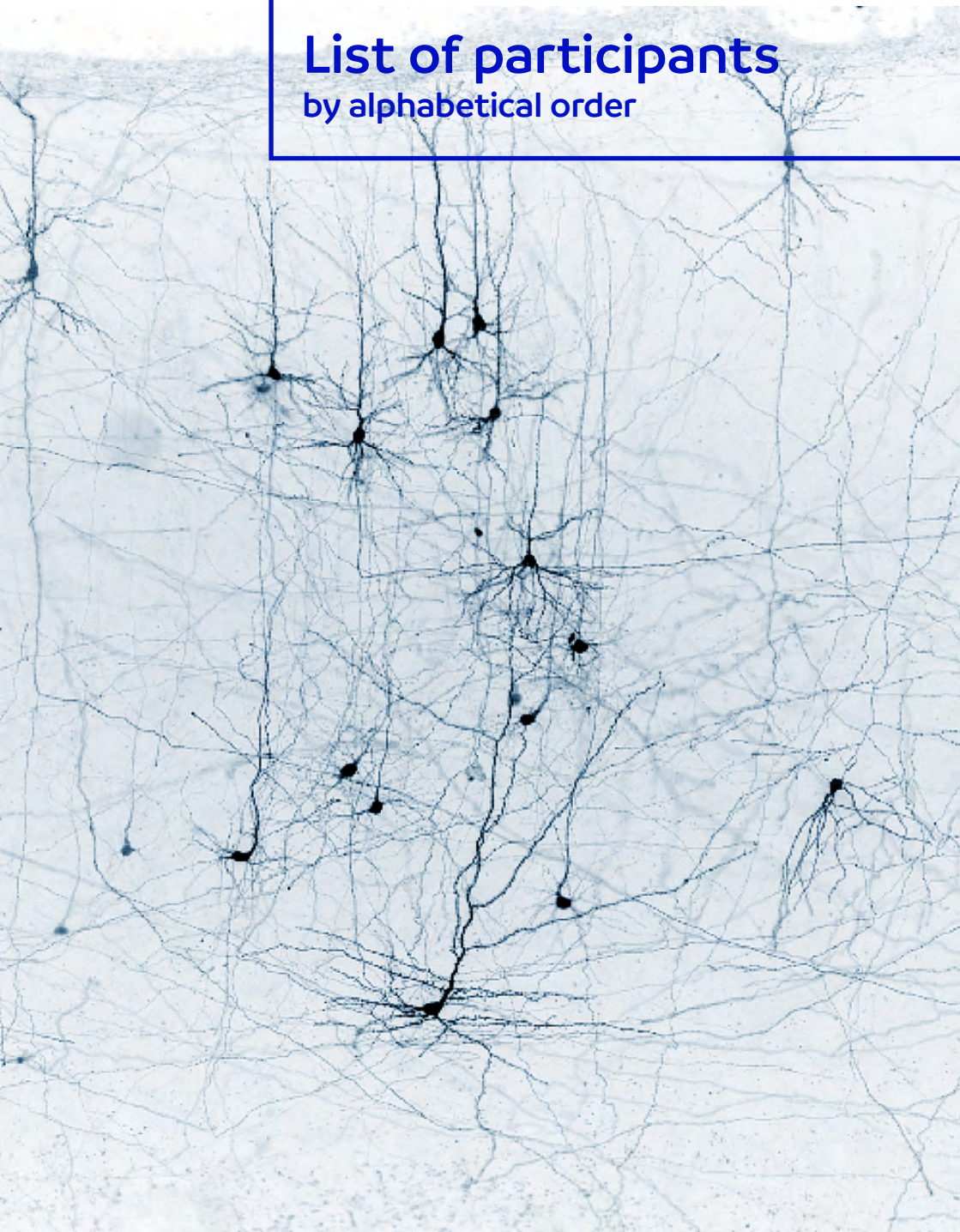

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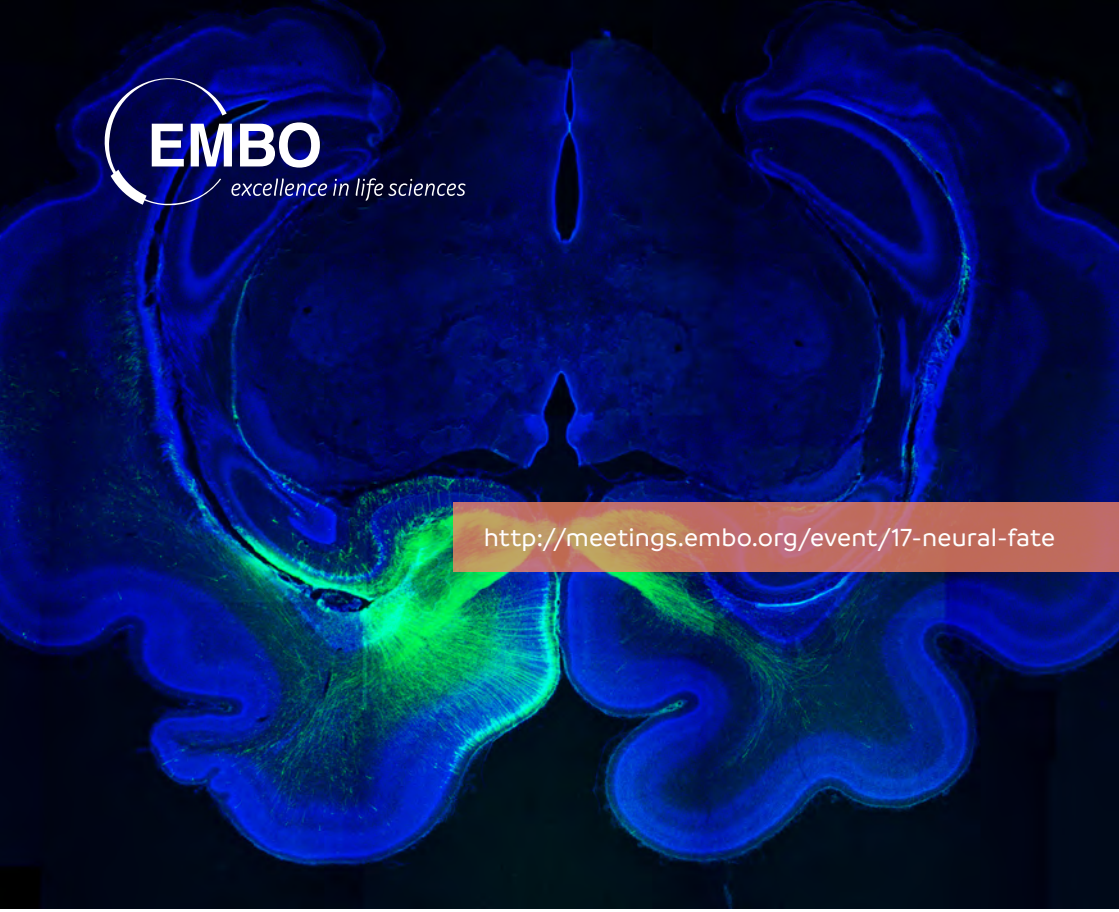

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