Epichrom 2025 | Abstract book

Talks

T01
Fereshteh Dorazehi
Lund university

MORC2 directs transcription-dependent CpG methylation of the repetitive human genome in early neurodevelopment.

DNA methylation is essential for silencing genomic repeats such as LINE-1 retrotransposons (L1s) in the germline and the soma. Emerging evidence suggests that L1s are transcribed and functional in human pluripotent stem cells, but how methylation is patterned to human L1s upon exit of pluripotency is unknown. Here we investigate the critical role of chromatin regulator MORC2 in directing CpG methylation over its transcribed repetitive targets in cellular models of early human brain development. Reversible ATP-dependent dimerization is required for MORC2 accumulation over L1s but not gene promoters. Mutations in the ATPase module, causative for neurodevelopmental disorders, severely disrupt the distribution of MORC2 chromatin binding, leading to simultaneous loss of L1 transcriptional control and hyper-repression of zinc finger genes in iPSC models. Upon neural differentiation these phenotypes persist due to targeted defects in CpG methylation patterning. Together our results define the vital role of MORC2 in safeguarding the somatic human genome upon exit of pluripotency by ensuring CpG methylation patterning over repeats, in a manner analogous to the piRNA pathway in the germline.

T02
José Ramón Bárcenas Walls
Stockholm University

Epigenomic atlas of the human forebrain at single-cell resolution

Epigenetic mechanisms play a fundamental role in early brain development. Previous studies in the human developing brain have profiled mostly one epigenomic mechanism, the chromatin accessibility by sc-ATAC-seq. These studies provide a rich resource of precise regulatory elements, with temporal and regional resolution during brain development. Nevertheless, sc-ATAC-seq can not distinguish accessible and primed cis-regulatory elements (CREs, non-coding DNA that regulates gene expression) against active CREs.
Profiling histone marks can facilitate the annotation of CREs activity.

Therefore, the goal of this project is to reveal the dynamics of active and repressive histone modifications at the developing brain. For this purpose, we are currently profiling both H3K27ac and H3K27me3 simultaneously with nanobody-based CUT&Tag (nano-CT). Our current atlas spans 32,364 (H3K27ac) and 12,109 (H3K27ac &H3K27me3) single-cells from five different cortical samples. Profiling H3K27ac enables cell type clustering, revealing a cell type specific and dynamic acetylation across human forebrain through time. By leveraging H3K27ac as a proxy for gene expression, we observe high gene activity scores for canonical markers on their respective cell types. With H3K27me3 profiling we observe opposite and mutually exclusive signal against H3K27ac. For the excitatory neuron population, we are able to build a pseudotime trajectory, revealing cell type histone dynamics. For example, we observe a granular and step wise acquisition of H3K27ac across cell-subtypes, but broader changes from H3K27me3 on progenitors against early differentiated excitatory neurons. Our epigenomic atlas of human cortex provides a unique resource for multimodal epigenomic profiling.We envision an atlas of histone marks at single-cell resolution will reveal novel key drivers for cell fate determination in the fetal nervous system. Together with disease association datasets, our resource will highlight genetic loci likely affected in neurodevelopmental diseases such as autism or neurodevelopmental disorders.

T03
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Deciphering neuron-to-glioma synapses through 3D genomics and epigenetics data

Neuron-glioma interactions are critical drivers of glioma progression, with neuronal activity promoting tumour growth and invasion through paracrine signalling and direct synaptic input. Beyond well-established glutamatergic synapses, recent discoveries revealed that GABAergic interactions also contribute to glioma proliferation. Here, I will focus on how glioma cells decode neuronal cues via epigenetic mechanisms, including enhancer reprogramming, chromatin remodelling and rewiring of 3D genome organization, with transcriptions factors such as SMAD3 and PITX1 orchestrating transcriptional programs that sustain neuron-to-glioma communication. Additionally, recent integration of multi-omics data highlights gene regulatory networks linked to GABAergic signalling as contributors to glioblastoma (GB) pathogenesis. I will also underscore the distinct roles of GABAergic signalling across glioma subtypes, noting that, in GB, GABA-related metabolic and paracrine mechanisms, rather than synaptic input, may drive tumour progression. Understanding how epigenetic reprogramming facilitates glioma integration into neural circuits opens new avenues to disrupt these malignant neuron-glioma interactions by targeting the epigenetic machinery.

T04
Vivien Horvath
Lund University

Age-associated DNA methylation alterations as a potential causal mechanism of a transposon-mediated brain disorder

X-Linked Dystonia-Parkinsonism (XDP) is an age-related neurodegenerative disorder caused by a polymorphic retrotransposon insertion belonging to the SVA family. This insertion was identified in the 32nd intron of the TAF1 gene. Using patient-derived cell models and Oxford Nanopore sequencing we previously showed that the XDP-SVA is covered by epigenetic repressors, namely DNA and histone methylation. CRISPR-mediated removal of these repressive marks exacerbated the molecular disease phenotype, suggesting a protective role for these epigenetic modifications.
Since XDP is an adult-onset disease, we hypothesize that DNA methylation decreases with age, leading to disease manifestation. To understand how this process works in the brain, we did Oxford Nanopore sequencing coupled with single cell and bulk RNA sequencing on post-mortem prefrontal cortex samples from XDP patients and control individuals. The generated datasets were used to examine disease-related DNA methylation patterns and TAF1 alterations.
By analyzing CpG methylation we found that the XDP-SVA is partially demethylated, and that the methylation pattern is diverse across patients. Interestingly, this hypomethylation was coupled with high levels, albeit diverse accumulation of the pathogenic 32nd intron retention. Furthermore, we found that this intron retention is more prominent in glial cells that also showed an inflammatory response.
These findings underscore the pivotal role of age-related DNA methylation changes over retrotransposons in triggering neurodegenerative disorders and move the field forward towards translational research efforts. This work also serves as a roadmap for the study of other TE-mediated age-related disorders, as it shows the importance of using appropriate human models and long-read sequencing techniques to study retrotransposons.

T05
Astradeni Efthymiadou
Karolinska Institutet

Non-redundant roles of the HDAC3 corepressor complex subunits SMRT and NCOR in controlling inflammatory and metabolic macrophage pathways

In macrophages, transcription factors and transcription coregulators regulate metabolic and inflammatory pathways through transcriptional and epigenetic mechanisms. Specifically, the HDAC3 corepressor complex has pivotal roles in coordinating these mechanisms, with the homologous subunits NCOR and SMRT being fundamental for the assembly of the complex and its interactions with chromatin and transcription factors. However, the relative contribution of NCOR and SMRT in regulating macrophage pathways are still incompletely understood. In this study, we assessed their genome-wide roles in the mouse macrophage cell line RAW264.7 and in primary bone marrow-derived macrophages. Transcriptome analysis following corepressor depletion revealed six clusters of differentially expressed genes. NCOR depletion mainly upregulated pathways related to metabolism, whereas SMRT depletion mainly upregulated pathways related to inflammation. Epigenome analysis showed that corepressor depletion differentially changed H3K27 acetylation and chromatin accessibility, in agreement with the observed transcriptome changes. Cistrome analysis indicated that both corepressors differentially influence each other at chromatin. SMRT controls the nuclear localization and chromatin binding of NCOR, HDAC3 and GPS2, acting as the chromatin anchor for the corepressor complex. Finally, corepressor depletion differentially modulated macrophage reprogramming in response to IL4, TLR4 and LXR signaling. Overall, this study reveals the previously underappreciated, non-redundant roles of NCOR and SMRT in regulating H3K27 acetylation, chromatin accessibility, enhancer activity and transcription to differentially regulate metabolic and inflammatory pathways in macrophages.

T06
Berta Garrido Zabala
Uppsala University

Dual targeting of G9a and DNMTs effectively induces tumour cell death in multiple myeloma

Multiple myeloma (MM) is a haematological disease of the plasma cell that remains clinically challenging despite the development of novel therapies. Large-scale efforts have been conducted to investigate the genome-wide distribution of chromatin modifications in MM, as well as the associated epigenetic modifying enzymes. Comprehensive studies into the links between different epigenetic regulatory systems in myeloma progression and drug resistance are clinically relevant, yet largely lacking. G9a and the DNMTs are epigenetic modifiers that exhibit increased activity in MM, which is associated with poor prognosis. To investigate the partnership between G9a and DNMTs, we used a combinatorial treatment approach involving small molecule inhibitors. In-depth molecular analysis of the H3K9me2 distribution, DNA methylome and transcriptome of MM revealed a synergetic silencing mechanism involving G9a and DNMTs, which regulates the expression of the tumour suppressor genes PAX5, DCN, PLEKHO1 DENND2D, PTPRT, GATA4 and IPO13. Moreover, dual inhibition of G9a and DNMTs reduced cell viability, induced apoptosis, and reduced proliferative capability of MM cells and reduced tumour burden in xenograft MM models. Specifically, co-inhibition of G9a and DNMTs reduced the abundance of the oncoproteins IRF4, XBP1 and MYC. Taken together, our data provides novel insights into the molecular mechanism behind the synergistic effects of G9a and DNMT1 inhibition in MM.

T07
Vladislav Kuzin
Karolinska Institutet

Unraveling the Role of TOP2A and Repetitive Elements in Chromosomal Translocation

Topoisomerase II alpha (TOP2A) favors transcription elongation by removing opposing DNA supercoiling. TOP2 inhibitors trap TOP2 in a protein-DNA cleavage complex (TOP2cc), preventing re-ligation of DNA ends and causing DNA damage that triggers apoptosis. Despite their efficacy in

anticancer treatment, TOP2A inhibitors lead to side effects, including therapy-related secondary malignancies triggered by genomic translocations. We aim to understand the mechanism of TOP2A-associated translocations to improve treatments efficacy while reducing side effects.
Upon mapping TOP2Accs using our TOP2A CAD-seq, we surprisingly discovered that TOP2Accs were highly enriched at repetitive elements called Alu. Alu elements are retrotransposons that comprise 11% of the human genome and influence gene expression, genome evolution, and diseases. Alus evolved in primates into three subfamilies: the best conserved AluY, AluS, and AluJ, with more frequent mutations.
We observed that TOP2Accs were higher at AluY than AluJ, which could not be purely explained by sequence differences since AluY sequences with the highest and lowest TOP2Accs are almost identical. In fact, we found that TOP2Accs at Alus are context-dependent. Even though both AluY and AluJ are transcribed by RNA polymerase II and show accumulation of negative supercoiling (-sc), AluY show a defined nucleosome positioning and enrichment in non-B DNA structures, compared to AluJ. This suggests that -sc at AluY might promote non-B formation positioning flanking nucleosomes.
Due to its central AT-rich motif flanked by GC-rich sequence, we are investigating the hypothesis that AluY have the propensity to act as “supercoiling sink”. Specifically, we propose that the non-B DNA pins the formation of a plectonemic -sc at AluY. Given their recombinogenic nature, we are also testing whether Alu elements are involved in TOP2A-related translocations. The mechanistic characterization of TOP2A activity at Alus might provide foundational knowledge for the development of new anticancer therapeutics.

T08
Yuri Schwartz
Umeå University

Polycomb repression works without Siesta

Epigenetic repression by Polycomb group proteins is crucial for the development of multicellular organisms. These proteins form multi-subunit complexes, some of which share subunits and exhibit similar enzymatic activities. The specific roles of these partially overlapping complexes in epigenetic repression remain an intriguing question. Utilizing Drosophila genetics, we discovered that Polycomb Repressive Complex 1 (PRC1) and related Siesta-RING1 complexes monoubiquitylate histone H2A at lysine 118 (H2AK118) in distinct genomic regions. Our findings indicate that the Siesta gene regulates larval locomotion independently of H2AK118 ubiquitylation and is not necessary for the epigenetic repression of Drosophila homeotic genes. By exploiting the functional division between PRC1 and Siesta-RING1 complexes, we used thousands reporters integrated in parallel to demonstrate that H2AK118 ubiquitylation alone does not significantly repress transcription.

T09
Serhat Aktay
KTH

Mapping the functional genomic landscape using divergent transcription analysis of PRO-seq data

Divergent transcription, where transcription occurs bidirectionally from promoters and enhancers, is widespread in multicellular organisms, revealing active genes and enhancers while driving genome evolution and gene origination. To explore this phenomenon, we developed TrackTx, a computational framework that processes precision run-on sequencing (PRO-seq) data to identify functional genomic regions in animals and plants. TrackTx is highly automated, user-friendly, and quantifies RNA synthesis across functional regions. The beta-version is available on GitHub (SerhatAktay/TrackTx).

TrackTx begins by creating a reference genome index and loading data from public or private sources. It then aligns sequenced reads to the reference genome and outputs files with genomic coordinates and counts of actively engaged RNA polymerases. The framework identifies functional genomic regions

such as active promoters, gene bodies with productive elongation, enhancers, termination windows, and unannotated genes. TrackTx quantifies transcriptional activity and compares it between samples, allowing for insights into gene expression regulation.

The framework has been applied to PRO-seq data from species including human, mouse, dog, fruit fly, and plant. These analyses revealed similarities and differences in gene expression across organisms and underscored the need for updated reference genomes. Additionally, TrackTx facilitated the discovery of unannotated genes and enhancers, contributing to a more complete understanding of transcriptional landscapes. In summary, TrackTx provides a powerful tool for mapping functional genomic regions, quantifying gene expression, and exploring regulatory mechanisms of transcription across diverse species.

T10
Mikhail Panfilov
Uppsala University

Mechanistic insights into DNA movement during chromatin remodeling

ATP-dependent chromatin remodeling enzymes (remodelers) regulate DNA accessibility in eukaryotic genomes. Many remodelers reposition (slide) nucleosomes, yet how DNA is propagated around the histone octamer during this process is still incompletely understood. Given that the DNA substrate is tightly constrained within the nucleosome, sliding requires overcoming barriers of the nucleosome's energetic landscape, which requires multiple consecutive catalytic cycles of the remodeler. We have recently demonstrated that during sliding by Chd1 and SNF2h remodelers, movement of entry-side DNA precedes that of exit-side DNA. This temporal delay indirectly implied the transient absorption or 'buffering' of DNA inside the nucleosome. Here we combine a new method for controlling NTP-driven reactions in single-molecule experiments via the local generation of NTPs (LAGOON) with 3-color single molecule FRET measurements for more detailed characterization of the buildup and propagation of unstable 'buffering' intermediates during nucleosome remodeling. Single-molecule measurements allowed us to monitor nucleosomes sliding under single-turnover conditions by representative remodelers from two different families, SNF2h and Chd1. Our new data shed new light on the formation of nucleosome sliding intermediates across multiple sequential translocation steps and allow us to estimate the efficiency of ATP usage by the remodeler. Moreover, the quantification of 'buffering' intermediates for nucleosomes assembled on two distinct DNA sequences allows us to investigate how the nucleosome's energetic landscape influences different aspects of remodeling process.

T11
Maria Needhamsen
Karolinska Intitutet

Long-read Sequencing: Virus Characterization, HLA haplotyping and Hydroxymethylation Profiling of Human Neurons

Long-read sequencing, such as Oxford Nanopore Technology (ONT), can be applied to native, genomic DNA to distinguish hydroxy- from conventional DNA methylation. We applied ONT to post-mortem, sorted neuronal nuclei (n=2) and demonstrated high sample-wise correlations when compared with array-based data from the same samples. Assessment of brain chromatin states from the International Human Epigenome Consortium (IHEC) revealed that hydroxymethylated sites predominantly overlapped with transcribed regions and enhancers. Pathway analysis further confirmed associated genes to be enriched in neurological processes.

In addition to epigenetic profiling of human neurons, we utilized ONT for Epstein Barr Virus (EBV) characterization and Human Leukocyte Antigen (HLA) haplotyping. For this, we applied the adaptive sampling feature of ONT to selectively enrich genomic regions of interest. As a proof-of-concept we

used the human Raji Burkitt lymphoma-derived cell line, which carries EBV. The high sequencing depth enabled de novo assembly of the EBV genome for strain calling and further facilitated identification of EBV integration sites in the human host. We plan to further investigate EBV latency states based on ONT-derived DNA methylation profiles. As a proof-of-concept for HLA haplotyping, we utilized the human monocytic THP1 cell line. Through de novo assembly, we identified a novel HLA haplotype and next steps involve characterization of HLA haplotype-specific DNA methylation across different cellular conditions.

In summary, we applied long-read sequencing across a range of biological questions - from neuronal epigenomics to viral strain and HLA haplotype characterization - facilitating simultaneous genetic and epigenetic assessment.

T12
Bastien Hervé
Karolinska Institutet

Deciphering multiple sclerosis lesions via multiomics and DBiT sequencing

Multiple Sclerosis (MS) is a chronic autoimmune disease targeting oligodendrocytes myelin, wrapped into sheaths around axons of neurons.
Affecting the central nervous system, this inflammatory disease is characterized by active and inactive lesions.
In this study, we explored lesion types heterogeneity in time and space in the human spinal cord in both cervical and lumbar regions.
The spatial resolution of cells in tissue is essentially focusing on shining light on a sole biological modality.
To overcome this limitation, we applied Deterministic Barcoding in Tissue for Spatial Omics Sequencing (DBiT) maneuvering both chromatin accessibility dynamics, via nanoCutTag technology associated with transcriptome sequencing.
The capture of multiple histone modifications, such as H3K27me3 and H3K27ac, in combination with transcriptome profiling, can alleviate cell types diversity inside and outside spinal cord lesions, as well as human brain anatomical regions.
In a nutshell, the multi-layer capture of biological material unravels unique insights to lesion types environment to improve the characterization of MS lesions.

T13
Yorick van de Grift
Linköping University

Intestinal Cell Identity Decisions: a WNT-WNT situation

WNT signalling is an ancient cell-cell communication mechanism that regulates virtually all processes during embryonic development and organismal homeostasis. This pathway has also emerged as the main driver of the self-renewal of adult tissues, including the intestinal epithelium, whose turnover relies on rapidly dividing intestinal stem cells (IESC) and their adjacent secretory Paneth Cells (PC): WNT drives both IESC stemness and PC differentiation. Mechanistically, WNT activates the protein ß-catenin, capable of associating to specific locations across the genome to activate WNT-target genes. Given the many roles in diverse cellular contexts attributed to WNT/ß-catenin, one key question remains unanswered: how can ß-catenin transform the positional information – the signals from the extracellular environment – into the appropriate cell-specific gene expression programs? In this project we will address this question by comparing the two cell types IESCs and PCs: these cell types constitute an ideal model to understand this, for they are both strictly dependent on WNT signalling and, despite being physically adjacent at the bottom of the crypt, display two divergent identities. We identify GATA transcription factors as (co)-regulators that finetune the balance between the divergent gene expression programs mediated by ß-catenin in the intestinal crypt.

T14
Alek Erickson
Stockholm University

Atlas of human facial development reveals enhancers of mesenchymal patterning genes

Facial development relies on precise spatial patterning of neural crest–derived mesenchyme, yet the regulatory logic linking position, chromatin state, and morphology remains elusive. We generated a multi-modal atlas of human craniofacial development, integrating single-cell RNA and ATAC sequencing, spatial transcriptomics, and other published datasets. LinkPeaks mapping, joined with epigenomic annotation, linked thousands of predicted enhancer elements to their target genes, cell types, and spatial niches. Autocorrelation analysis revealed combinations of 'spatial genes' defining regional identities, and cell interaction analysis was used to explore potential upstream inductive signals. Among these spatial genes, FOX and PAX transcription factors exhibit motif enrichment in differentially accessible and linked peaks across mesenchymal subtypes. We identified a deeply conserved region linked to PAX1, overlapping a genetic variant associated with facial shape. Mice deficient for this region exhibited defects in craniofacial bone formation and growth. Together, our work defines how chromatin accessibility, enhancer activity, and signaling converge to encode positional identity in the developing human face.

T15
Alessandro Gozzo
Linköping University

Paternal Argonaute proteins control sperm-borne mitochondrial small RNA and embryonic metabolism

The paternal germline serves as a repository of intricate genetic information, contributing to shaping the development and fitness of the offspring. Beyond transmitting genomic DNA, spermatozoa also carry small non-coding RNAs (sncRNAs). These undergo notable shifts during spermatogenesis, resulting in a highly specific mature sperm sncRNAs profile. Among these, mitochondrial-derived small non-coding RNAs (mitosRNAs) represent a poorly understood class yet are abundant in the male gonads. We characterize Drosophila sperm mitosRNAs, finding the majority mapping to piRNAs, exhibiting similar lengths and nucleotide biases. Previously, we showed the plasticity of sperm mitosRNAs in response to diet. Using RNA interference experiments we now prove Piwi and Aubergine involvement in the biogenesis of mitosRNA and finally, find mitosRNA to be involved in the intergenerational metabolic reprogramming of the early embryos.

T16
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Uppsala Universitet

Genetic and germ line methylomic consequences following a multigenerational exposure related to metabolic diseases in mouse.

In recent years, transgenerational phenomena have been described in a variety of experimental models following exposure to certain environmental insults. However, not much is known regarding the dynamics followed by the genome and the germ line epigenome, in the generations when such exposure takes place. This is relevant for understanding the inheritance and effects of diseases across generations. In the present study, we exposed a population of mice to excess calorie intake during early development by reducing litter size at birth. Control females reared 8 pups, whereas females of the small litter group nursed 4 pups throughout lactation. Mice reared in small litters developed metabolic disease with ageing. The exposure was multigenerational and lasted for 3 generations. The control lineage was maintained in parallel and not exposed to this treatment. The adult sperm of the males was investigated across these 3 generations, in both lineages. Therefore, the collection of

samples took place after each generational exposure when sexual maturation occurred. Because the mothers of each generation were introduced from outside the lineages, we investigated the effects transmitted via the paternal germ line only.
We observed that SNPs progressively emerged, generational after generation, and associated with the separation of two populations in the multigenerational exposure group, while a third population emerged in association with the control lineage. The SFS test showed no signs of cofounder's effect, a usual critique to this type of experiment as the initial exposed generation is smaller than the consequent ones. An interesting pattern was also observed in the sperm methylome, where significant differences emerged already in the first generation, diminished in the second generation, and then increased to higher levels in the third generation compared to both previous generations. In this study we tracked hypermethylated regions which emerged in the F0 that loose progressively their methylation and linked it with emergence of SNPs in their progeny. Due to the nature of the stressor, we found a differential behavior of the emergence of SNPs in these regions when compared with the control lineage.

T17
Artemy Zhigulev
KTH / SciLifeLab

Rare gain-of-function regulatory mutations explain the missing heritability of bicuspid aortic valve

Bicuspid aortic valve (BAV) is a congenital heart defect affecting ~1% of the population, often leading to severe complications. Despite its high heritability, genetic studies have identified only a few coding or common GWAS variants, explaining a minority of cases. Recognizing the gap in understanding the disease etiology, we hypothesized that less common regulatory variants in promoters and enhancers might play a critical role in BAV pathogenesis.
To investigate this, we integrated whole-genome sequencing with high-resolution three-dimensional genome organization profiling in sixteen patients with BAV and tricuspid aortic valves (TAV). We identified BAV- and TAV-specific promoter- enhancer interactions disrupted by rare mutations in interacting regulatory regions (RMiRRs). We further prioritized RMiRRs predicted to alter transcription factor binding and found a 1.5-fold enrichment of gain-of-function RMiRRs in previously implicated genes among BAV patients.
Using human fetal heart scRNA-seq datasets spanning aortic valve development, we observed that moderately rare RMiRRs (allele frequencies <3%) were predicted to alter the transcriptome of specific developmental valve mesenchymal cell and fibroblast populations. Affected genes were enriched for pathways linked to BAV pathogenesis, aortic valve development, EMT, and TGFBR signaling. Interestingly, fetal heart spatial transcriptomics data revealed discrepancies: traditionally implicated genes affected by RMiRRs show weak correlations with aortic valve regions, while related pathways, such as EMT and TGFBR, demonstrate much stronger associations.
Expanding the BAV pathway network with newly implicated genes uncovered substantial genetic heterogeneity at the single-patient level. These findings position rare regulatory mutations as key contributors to missing BAV heritability and highlight the need for further mechanistic studies.

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P01
Ram Prakash Yadav
Uppsala University

Unlocking single cell chromatin accessibility in formalin-fixed paraffin-embedded samples by scFFPE-ATAC

Background: In clinical laboratory and biological research, formalin-fixed paraffin-embedded (FFPE) samples are considered to be a gold standard for tissue preservation and histological analysis.

Chromatin accessibility governs gene expression by modulating the interaction between transcription factors and DNA. Single-cell chromatin accessibility profiling technology is a key tool for studying epigenetic regulation in human disease at the single-cell level. Due to excessive DNA damage during FFPE sample processing, it is impossible to capture single-cell chromatin accessibility in FFPE tissue samples with preexisting single-cell techniques.
Method: To address the above technical challenge, we develop a unique nuclei isolation protocol for FFPE samples, a newly designed FFPE-Tn5 transposase, and high-throughput DNA barcoding with 56,623,104 cell barcodes per run that profiles single-cell chromatin accessibility in FFPE samples via T7 promoter-mediated DNA damage rescue and in vitro transcription.
Results and conclusions: We are first to decode single-cell chromatin accessibility in mouse and human FFPE tissue samples. We benchmarked our approach using mouse FFPE spleen samples by comparing them to fresh mouse tissue. We successfully used scFFPE-ATAC on clinical FFPE human lymph nodes archived for 8-15 years and discovered different epigenetic regulators in tumor centers and invasive edge epithelial cells in human lung cancer. Furthermore, we examined paired primary and relapsed follicular lymphoma (FL), as well as FL transformed into diffuse large B-cell lymphoma from the clinical samples archived for 6-13 years, revealing key epigenetic drivers of tumor progression. Overall, scFFPE-ATAC opens the door for spatial and retrospective epigenetic research by enabling high-throughput, high-sensitivity chromatin accessibility profiling in clinical and biomedical specimens that have been archived for a long time. In addition, this technology offers a powerful tool for studying tumor recurrence and metastasis, with broad applications in basic research and personalized medicine.

P02
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Exploration of small non-coding RNAs in Multiple Sclerosis

Introduction: Small non-coding RNAs (sncRNAs) have emerged as promising biomarkers due to their high stability and accessibility. In a previous study, we profiled sncRNAs across immune- related cells and fluids revealing Multiple Sclerosis (MS)-associated changes and opposing patterns in the central nervous system (CNS) compared to the periphery.
Aims: Replication of previous findings and further exploration of sncRNAs as markers of MS disease activity and progression.
Methods: The Small-seq protocol was applied to profile sncRNAs in nearly 800 samples, with ~300 individuals with matching plasma and cerebral spinal fluid (CSF). The cohort encompassed various MS disease trajectories, assessed based on clinical information, as well as 91 non- inflammatory neurological disorders controls (NINDCs) and 48 healthy individuals. The stability of sncRNAs was assessed by leaving blood and CSF from three subjects for an extended period of time in room temperature (0, 1, 3, 6, 24, and 48 hours), followed by exposure to freeze/thawing conditions. Processing of the Small-seq data included filtering, mapping, deduplication, annotation, counting and normalization.
Results: Detected sncRNAs were categorized into microRNAs (miRNAs), tRNA-derived fragments (tRFs), small nucleolar RNAs (snRNAs), snoRNA-derived RNAs (sdRNAs), yRNA-derived fragments (ysRNAs). We found significant differences in sncRNA distribution between plasma and cerebrospinal fluid (CSF). Thus, miRNAs (88%) dominated in plasma, with smaller fractions of tRFs (10%) and ysRNAs (2%), while CSF displayed a more balanced profile of entities with tRFs at 47%, miRNAs at 45%, and ysRNAs at 7%. Importantly, sncRNA profiles remained generally stable across preanalytical conditions, including prolonged bench time (up to 48 hours) and repeated freeze-thaw cycles.
Conclusion: This multi-compartment study, new insights into distinct molecular and tissue- specific signatures linked to MS immunopathology, particularly the divergence between CNS and peripheral compartments. Additionally, the stability of sncRNA profiles under common preanalytical conditions supports the reliability of Small-seq for large-scale, translational applications.

P03
Sanghita Banerjee
Karolinska Institutet

Identifying the chromatin configuration associated with the diversification of enteric neurons.

Enteric neuron types are generated in a temporally defined manner, with different neural phenotypes arising within defined time windows. Moreover, at a given stage scRNA-seq data suggests that stem cells initially adopt one out of two prototypic states during neurogenesis before differentiating to their final identities. The developmental trajectory of these neurons is precisely regulated at different stages. Here we profiled the chromatin accessibility derived from mouse embryos at two distinct developmental stages from embryonic day (E) 13.5 and E18.5 by scATAC-seq. We aimed to identify the developmental stage-specific gene regulatory programmes within and across different cell types and identify potential molecular switches throughout lineage development. Earlier study from the lab has demonstrated that binary neurogenic branching is a fundamental and persistent feature throughout development. Taking advantage of the chromatin assay, we sought to delineate the transcriptional networks and enhancer elements that orchestrate branch formation during development. Analysis of gene activity scores from the scATACseq datasets enabled the identification of major cell populations—including progenitors, neuroblasts, and mature neurons — at both early (E13.5) and late (E18.5) embryonic stages. Notably, we observed a temporal shift in cell type composition, characterized by a progressive decline in progenitor cells accompanied by an increase in neuroblasts and mature neurons at E18.5. Additionally, at E13.5, cells derived from the small intestine and colon exhibited distinct chromatin accessibility landscapes, suggesting region-specific epigenetic regulation of developmental programs. These findings highlight dynamic changes in cellular states and underscore stage-specific transcriptional and chromatin regulatory landscapes that govern neurodevelopmental progression. Collectively, our study shows that neuron subtype specification in the ENS arises from a combination of a non-temporal binary fate split and time-restricted chromatin-driven transcriptional landscapes. These postulated mechanisms explain how the developing ENS achieves cell diversify without apparent spatial patterning codes in the small intestine.

P04
Mohammad Alzrigat
Karolinska Institutet

EZH2 inhibition sensitizes retinoic acid-driven senescence in synovial sarcoma

No abstract submitted

P05
Ana Varas Sánchez
Karolinska Institute

tTEscanR: an advanced R-based package to quantify and visualize translation efficiency from sequencing data.

Protein translation unfolds in three sequential stages: initiation, elongation and termination. Elongation is dependent on codon-anticodon interactions, with suitable nucleotide pairing being essential for efficient translation. To quantify translation efficiency, it is crucial to assess the availability of each mRNA codon and its corresponding tRNA anticodon. However, this process is challenging due to fluctuations in mRNA and tRNA levels across tissues and cell types, which may introduce biases and hinder accuracy. Moreover, investigating tRNAs presents experimental difficulties because of gene code degeneration, complex tRNA secondary structure and their extensive post-transcriptional modifications.

In our previous work, we developed a computational pipeline to quantify and analyze mRNA codon usage relative to the availability of pairing tRNA anticodons as a proxy to determine its translation efficiency. We termed that score, theoretical translation efficiency (tTE). Here, we extend its potential by introducing tTEscanR, a powerful and user-friendly R-based package designed to quantify translation efficiency from bulk and single-cell sequencing data. tTEscanR offers a comprehensive approach to quantify translation efficiency by integrating gene expression data and chromatin accessibility data. The modular design of tTEscanR ensures flexibility, allowing users to either run independent components or a complete pipeline based on their research needs. Its user-friendly R-based interface simplifies the analysis of complex data, even for researchers with minimal computational experience. Additionally, tTEscanR includes an advanced visualization module that generates high-quality plots, aiding in interpretation of results and enhancing the ability to communicate findings effectively. Applicable to both bulk and single-cell sequencing data, tTEscanR provides a versatile tool for a wide range of experimental setups and biological contexts, enabling deeper exploration of translation efficiency and its role in cellular processes, disease mechanisms, and therapeutic development.

P06
Ziwei Liu
Karolinska Institutet

Investigation into neuronal diversification mechanisms in the enteric nervous system

The enteric nervous system (ENS) is a subdivision of the autonomic nervous system. It is organised into two plexuses: the myenteric plexus residing between the outer longitudinal and inner circular muscle layer of the gastrointestinal tract, and the submucosal plexus lying below the mucosa. ENS plays a crucial role in regulating peristalsis, blood flow, and secretion in the gastrointestinal tract. Selective enteric neuron classes (ENCs), mainly nitrergic neurons, are affected in some gut disorders, including gastroparesis, oesophageal achalasia, and Chagas disease. Previously, our group has characterised 12 murine myenteric ENCs and 3 submucosal ENCs using single-cell RNA sequencing technology (scRNA-seq). In this study, we aim to understand the regulatory mechanisms underlying progressive neuronal development and maturation in the ENS using single-cell multiomics(scRNA-seq, scATAC-seq and nanoCUT&Tag).

P07
Stefania Iliana Tziola
Uppsala University

The lncRNA PCAT1 recruits polycomb repressive complex 2 to target genomic regions and induce H3K27me3-mediated gene silencing in multiple myeloma

Multiple Myeloma (MM) is a heterogeneous hematological malignancy defined by the intertwined dysregulation of genetic and epigenetic mechanisms. To date, no single genetic alteration has been described as disease driver thereby highlighting the essential role of epigenetic modifications in the establishment and progression of MM. Despite the advances of novel therapies, MM remains largely incurable. We have previously shown that EZH2, the catalytic subunit of PRC2 responsible for H3K27me3 deposition, is upregulated in MM leading to the silencing of key tumor suppressors. Although the polycomb repressive complex 2 (PRC2) lacks sequence specificity, the presence of an RNA-binding domain on the EZH2 subunit suggests that lncRNAs may serve as potential interactors guiding PRC2 recruitment to specific genomic regions. Herein, we demonstrate for the first time that the lncRNA PCAT1 directly interacts with EZH2 and recruits the PRC2 to target genomic locations. Furthermore, we identified that the genes SWAP70, MYOF and NOS3 were regulated by the PRC2-PCAT1 axis in MM cells. Interestingly, inhibition of PCAT1 and EZH2 induced the expression of the predicted target genes SWAP70, MYOF and NOS3. Moreover, we found in multiple independent MM patient cohorts that overexpression of PCAT1 and low expression of the PRC2 target genes is associated with poor prognosis and disease progression. Collectively, our findings reveal that lncRNA

PCAT1 directly binds to the PRC2 via EZH2 to guide the silencing of SWAP70, MYOF and NOS3, potentially promoting myeloma progression and suggesting a regulatory axis not previously described in cancer.

P08
Adriana Carvajal-Jimenez
Karolinska Institutet

High-Throughput Profiling of Transcription Factor Binding Dynamics in Response to Epigenetic Modulation

Epigenetic drugs (or epidrugs) can reshape transcriptional programs by altering the chromatin landscape, yet their global impact on transcription factor (TF) binding remains poorly understood. While we know that epidrugs affect chromatin association and transcription factor (TF) function, we lack detailed information on specific TF changes when chromatin is disrupted by epidrugs. This issue is more complex for TFs with similar motifs or those not directly binding DNA. However, current methods are inadequate to test individual TFs at the necessary scale.

In this project, we aim to systematically evaluate how a selected epidrug influences TF-DNA interactions across the genome. To achieve this, we employ a cell library produced by High-throughput Insertion of Tags Across the Genome (HITAG) comprising more than 250 HEK293T derived clones. Each clone expresses a unique FLAG-tagged transcription factor (TF), facilitating the parallel analysis of binding profiles for multiple individual TFs within a single experiment.

Our experimental approach will combine in situ reverse transcription with nanoCUT&Tag5 on the 10x Genomics platform. This strategy will provide single-cell-level data for FLAG-TF identification and TF occupancy in a high-throughput manner. Multiplexed profiling of TF binding at this scale has not been previously achieved and holds the potential to reveal the effects of chromatin remodelers on TF occupancy across a broad range of specific targets.

P09
Dominika Drapała
Linköping University/Jagiellonian University

Targeting histone acetylation to restrain P. gingivalis-triggered inflammatory activation of macrophages

The epigenetic impact of pathogenic bacteria remains largely unexplored, even though these microbes strongly influence cellular behavior. Periodontitis is a chronic inflammatory disease that arises from microbial dysbiosis in the oral cavity, primarily driven by Porphyromonas gingivalis (Pg). Roughly 24% of adults suffer from the severe form of periodontitis, which results in significant tissue damage and eventual tooth loss. Moreover, periodontitis has been implicated in systemic diseases through dissemination of both Pg and inflammation. We propose that Pg may manipulate the macrophage epigenetic landscape to promote a proinflammatory M1 phenotype, thereby sustaining chronic inflammation in periodontitis. The hypothesis will further investigated through genome-wide epigenomic studies in Prof. Claudio Cantù's lab at Linköping University. Preliminary findings support this approach, indicating that Pg infection induces significant changes in the macrophage epigenome, as evidenced by elevated mRNA levels of histone acetylation and methylation enzymes 24 hours post-infection. Comparative transcriptomic analysis between M1 and wound-healing M2 phenotype at 4 hours post-infection revealed distinct transcriptional responses to Pg. Pharmacological inhibition of histone acetylation attenuated production of key proinflammatory cytokines (IL1B, IL6, IL8, CCL2, CCL5) without affecting MAPK or NF-κB signaling pathways. Notably, histone acetylation inhibition reduced Pg internalization and enhanced reactive oxygen species production, suggesting a potential mechanism for inflammation resolution. These findings provide initial evidence that Pg can modulate

the host epigenome in macrophages and highlight histone acetylation as a potential therapeutic target for mitigating infection-driven inflammation.

P10
Letian Zhang
Stockholm University

Transposase-assisted profiling of DNA methylation (TRADE-seq)

DNA methylation is a key epigenetic modification that interacts with histone marks to regulate gene expression and cellular identity. Conventional single-cell DNA methylation profiling methods rely on cytosine-to-uracil conversion, requiring high sequencing depth per nucleus (typically several million mapped reads). Here, we introduce TRADE-seq (Transposase-assisted profiling of DNA methylation), which utilizes fusion proteins for in situ DNA methylation profiling at single-cell resolution. TRADE-seq streamlines the workflow into a single-tube reaction and is compatible with droplet-based platforms such as 10x Chromium. Bulk TRADE-seq generates DNA methylation profiles comparable to WGBS and MeDIP-seq, while single-cell TRADE-seq resolves cell identities in heterogeneous populations.

P11
Simon Perrin
Karolinska Institute

Injury-Induced Epigenetic Memory Enhances Neural Stem Cell Plasticity

Mammals have limited regenerative capacity, particularly in the central nervous system. The spinal cord contains quiescent neural stem cells, ependymal cells (EpCs) which expand after injury to contribute to the glial barrier before returning to quiescence. Yet, they fail to sufficiently contribute to neuro/oligodendrogenesis. Expression of developmental transcription factors, including Olig2, allows EpCs to generate oligodendrocytes and improve axon conduction after injury. This process depends on tissue damage, suggesting that injury regulates EpC lineage plasticity. Whether injury endows resident EpCs with long-term plasticity remains unknown. Here, we investigated whether Olig2 overexpression can induce oligodendrogenesis when EpCs return to quiescence after injury. Using Foxj1CreERT, R26Olig2-Tom mice, we overexpressed Olig2 in quiescent EpCs in uninjured spinal cord or 28 days post-injury (dpi). While inducing Olig2 expression in uninjured spinal cord did not induce response from EpCs, its induction at 28 dpi led to EpC expansion and differentiation into oligodendrocytes. To explore the basis of this increased plasticity, we analyzed single-cell mulitomics from uninjured and injured spinal cord. While few transcriptomic changes were observed, numerous chromatin regions were specifically accessible at 28dpi compared to uninjured, indicating that EpCs acquire an epigenetic memory from injury. We identified more than 500 memory chromatin regions, linked to genes such as Runx1 or Bcl3, involved in neurogenesis and immune response. Using deep-learning models, we compared the transcription factors regulating these memory regions to those of regions transiently open in response to injury. Transient regions were predominantly enriched in injury-responsive AP1 motifs, while memory regions were enriched for Krüppel-like factors (KLF) / specificity protein (SP) motifs, revealing a novel role of KLF/SP in shaping the chromatin landscape of EpCs. In conclusion, our findings uncover epigenetic memory as a key regulator of neural stem cell plasticity, opening paths to engineer epigenetic programs unlocking the regenerative potential of neural stem cells.

P12
Eva Brinkman
Karolinska Institutet

Unravelling gene regulation: Exploring transcriptome and protein interactions at single-cell resolution

Gene expression is a fundamental process that dictates cellular identity and plasticity by translating genetic information into functional molecules. Remarkably, even within genetically identical cell populations, gene expression often exhibits significant heterogeneity. This variability is thought to be crucial for cell survival, particularly in challenging environments. In cancer, such fluctuations can lead to the emergence of rare cells capable of withstanding treatment. Since drug resistance is a major obstacle in cancer therapy it is essential to understand how gene expression heterogeneity arises.

Transcription is orchestrated by transcription factors (TFs), which recruit cofactors (CoFs) to modulate gene expression. However, the impact of co-acting TF/CoF pairs on transcriptome at single cell resolutions is still an enigma. This project aims to address this question by developing a method that simultaneously quantifies co-occurring TF/CoF pairs and the transcriptome, at the single-cell level. In this way, we aim to characterize the correlation between the TF/CoF pair interactions and the resulting transcriptomic changes. Our approach has the potential to reveal interactions that occur in subpopulations of cells, which are obscured in bulk studies. Apart from its application on TF/CoF pairs, this approach can be extended to investigate the impact of other protein-protein interactions of interest, opening new avenues for future research.

Here, we will present the first data showing the heterogeneity of c-MYC/MAX interactions and the nuclear RNA expression patterns in single colon cancer cells. In future research we will examine these cells under challenging conditions, such as exposure to c-MYC antagonists or inhibitors of the c-MYC/MAX complex. As these cooperating transcription factors are key regulators of cell proliferation, we are particularly interested in subpopulations that emerge.

P13
Lara Kular
Karolinska Institute

A genetic-epigenetic interplay at 1q21.1 locus underlies CHD1L-mediated vulnerability to primary progressive multiple sclerosis

Multiple Sclerosis (MS) is a heterogeneous inflammatory and neurodegenerative disease with an unpredictable course towards progressive disability. Treating progressive MS is challenging due to limited insights into the underlying mechanisms. We examined the molecular changes associated with primary progressive MS (PPMS) using a cross-tissue (blood and post-mortem brain) and multilayered data (genetic, epigenetic, transcriptomic) from independent cohorts. In PPMS, we found hypermethylation of the 1q21.1 locus, controlled by PPMS-specific genetic variations and influencing the expression of proximal genes (CHD1L, PRKAB2) in the brain. Evidence from reporter assay and CRISPR/dCas9 experiments supports a causal link between methylation and expression and correlation network analysis further implicates these genes in PPMS brain processes. Knock-down of CHD1L in human iPSC-derived neurons and knock-out of chd1l in zebrafish led to developmental and functional deficits of neurons. Thus, several lines of evidence suggest a distinct genetic-epigenetic-transcriptional interplay in the 1q21.1 locus potentially contributing to PPMS pathogenesis.

P14
Mattia Zaghi
Karolinska Institutet
Liquid biopsy epigenomics in Multiple Sclerosis

Introduction:
The varying nature of Multiple Sclerosis (MS) highlights the need for good biomarkers to follow effectively the disease progression. Current diagnostic approaches are limited to symptom evaluation and imaging. These premises make the finding of informative and reliable fluid biomarkers very appealing.

Objectives/Aims:
To test the use of cell-free Chromatin (cfChromatin) profiling in plasma as a new biomarker to monitor the progression of MS pathology and response to treatment. The project focuses on profiling histone modifications, specifically H3K4me3 and H3K27ac, from MS patients, naive and following B cell depletion therapy (Rituximab), and healthy controls.
Methods:
We have modified previously developed protocol for chromatin immunoprecipitation followed by sequencing (ChIP-seq) to profile cell-free chromatin and compared data quality with other published data. Using both large publicly available datasets (Roadmap Epigenomics) and inhouse single-cell epigenomics datasets, we have performed cell type deconvolution.
Additionally, we conducted parametric statistical analysis to find differentially enriched loci between groups, comparing the average signal found in controls to each MS samples. All tests were corrected for multiple hypothesis using FDR and a p and q-value was computed. The clinical material consisted of people with MS, both treatment naïve (n=6) and exposed to B celldepleting
therapies (BCDT; n=11), as well as healthy controls (n=20).
Results:
Preliminary results indicate that cfChromatin modifications, such as H3K4me3 and H3K27ac, can be successfully profiled using ChIP-seq with quality levels comparable to previous publications. Our data show differences between the MS and control samples in terms of cell/tissue type composition, with a higher level of brain-related genomic loci being enriched in MS. More specifically some of the enriched loci are associated with neuronal genes and glia (Astrocytes and Oligodendrocytes) associated genes. Interestingly, some of this brain enriched loci show a higher signal enrichment in MS samples suggesting that indeed cfChIP-seq can effectively detect meaningful differences between disease & control conditions.
Conclusion:
Our results suggest that cfChromatin has a potential to be used as a biomarker in MS. A higher number of samples and a pathology-related dataset for cell type deconvolution will be helpful in the future to better understand the differences between different disease stages and treatments.

P15
Chandana Rao Prakash
Karolinska Institutet

Joint epigenome profiling using 3DRAMseq in human memory B cells

Epigenetic mechanisms such as DNA methylation are fundamental for gene regulation gene expression, influencing cell fate decisions during development and in disease contexts. DNA methylation is a stable and sensitive epigenetic mark that is known to play an important role in several autoimmune diseases like Multiple Sclerosis.

Traditional multi-omics approaches have typically been limited to analyzing one or two epigenetic modalities simultaneously, hindering a comprehensive understanding of the complex interplay among various regulatory layers. To address this limitation, this project in collaboration with Boyan Bonev's group, utilizes 3DRAM-seq, an innovative method that concurrently profiles spatial genome organization, chromatin accessibility, and DNA methylation at high resolution across the genome. Integrating 3DRAM-seq with RNA sequencing offers powerful tool for gaining deeper insights into the regulatory landscape of the genome.

In this project, we apply 3DRAMseq on sorted human memory B cells to understand the regulatory landscape of this cell type. Additionally, we have used two different restriction enzymes for the Hi-C, to overcome the challenges of low input and yet provide higher resolution contact maps. We have performed deep sequencing on the 3DRAMseq libraries and the analysis is ongoing. Applying this

method on rare and low input patients samples can shed light on multi layer epigenetic regulation in the context of Multiple Sclerosis.

P16
Morad Kamand
Stockholm University

Shaping the Brain: How Key Morphogens Rewrite the Epigenetic Code During Early Development

Understanding how the human brain forms its complex regional architecture requires more than transcriptomic data alone. While single-cell RNA sequencing (scRNA-seq) reveals gene expression states, it captures only the endpoint of regulatory processes. Epigenetic profiling—specifically of DNA methylation and histone acetylation—offers upstream insights into regulatory events that precede and shape transcription. This is particularly critical during early brain development, where dynamic signaling environments orchestrate lineage decisions before genes are actively transcribed. This project investigates how key developmental morphogens—Wnt, Shh, FGF, and EGF—modulate the epigenetic landscape prior to transcriptional activation. Using brain organoids and fetal tissue models, we apply single-cell epigenomic techniques to determine how morphogen exposure alters chromatin states in region-specific manner. Our aim is to identify morphogen-driven epigenetic signatures that direct the formation of forebrain, midbrain, and cerebellar regions. By focusing on epigenetic regulation, we seek to uncover early, instructive mechanisms that shape brain patterning—offering a more predictive and mechanistic understanding than transcriptomics alone

P17
Lovisa Örkenby Kämpe
Linköping University

Sperm-borne miR-309 cluster miRNAs regulate maternal mRNA decay in fly embryo

miRNAs exhibit tissue- and developmental-specific functions by regulating mRNA stability and stalling translation. In early Drosophila embryogenesis, miRNAs from the miR-309 cluster are key embryonic contributors to the maternal-to-zygotic transition (MZT), targeting a substantial fraction of the maternally loaded genes and facilitating the zygotic gene activation. Although their expression is well-characterized in embryos and larvae, their presence and functions in other contexts are less explored.

Here, we address this gap using the XP-seq protocol, which bypasses the 5’ phosphate (P) bias to capture small RNAs with diverse 5’ modifications. Typically, active miRNAs are 5’ phosphorylated, which is required for the recognition of the Ago protein. However, un-phosphorylated –and inactive- miRNAs have been reported, representing an additional regulatory layer of miRNA activity. We found that miRNAs from the miR-309 cluster are present in sperm in an un-phosphorylated form. These miRNAs were not detected in 5’ P-tagged form or when using the commercial NEBNext small RNA library kit. To assess their function in the embryo, we sequenced embryos from fathers depleted of the miR-309 cluster. Surprisingly, these offspring exhibited a transient but decreased general mRNA expression, suggesting the involvement of intermediate regulatory factors.

Analysis of the reduced transcripts revealed that the embryonic deadenylation (EDEN) motif, recognised by the RNA-binding protein Bruno-3, was overrepresented within their 3’ UTRs. Notably, Bruno-3 also has a target site for dme-mir-5-5p, a member of the miR-309 cluster found in sperm. Using published data on poly(A) tail lengths, we found that transcripts reduced after loss of paternal miR-309 miRNAs typically undergoes poly(A) tail elongation between egg activation and early embryogenesis - a process that may be counteracted by an excess of Bruno-3.

Our findings suggest that paternal miRNAs from the miR-309 cluster influence the regulation of MZT, and that dynamic miRNA 5’ end modifications help regulate when and where they are

functionally active.

P18
Artem Ilin
Stockholm University, MBW

Exploring single-cell epigenetic landscape in Drosophila embryogenesis
Cell-fate specification during embryogenesis depends on the coordinated activation of lineage genes and Polycomb-mediated repression of alternative programs, yet the single-cell choreography of these antagonistic chromatin states is poorly defined. Here we deploy nano-CUT&Tag, which tags H3K27ac and H3K27me3 simultaneously in the same nucleus, to profile thousands of cells from mid- to late-stage Drosophila melanogaster embryos. By integrating these dual-mark maps with published single-cell RNA-seq, we build a joint chromatin–transcriptome interaction model that reconstructs developmental trajectories and quantifies how activating and repressive signals converge to restrict potency. Preliminary analysis reveals a progressive, cell-type-specific expansion of H3K27me3 accompanied by contraction of H3K27ac, while many developmental regulators display adjacent or overlapping active and repressive domains, indicating that Polycomb often establishes dynamic boundaries rather than acting as a binary off-switch. To test causality, we combine tissue-specific knock-down of the PRC2 methyltransferase Enhancer of zeste with single-nucleus RNA-seq, directly linking altered chromatin architecture to transcriptional outcomes in myogenic lineages. Our work converts Waddington's epigenetic landscape from a metaphor into a quantitative, data-driven map of chromatin-controlled cell-fate decisions, offering a general framework to probe how the interplay of activation and repression shapes lineage commitment and to potentially evaluate disease-related cellular heterogeneity.

P19
Gert-Jan Hendriks
Basic Genomics

Isoform-Resolved RNA-Sequencing with RNA BaseCode

RNA sequencing has transformed molecular biology, enabling deep insights into gene expression across tissues and individual cells. Recent advances in single-cell and spatial transcriptomics now allow researchers to resolve transcriptional activity with spatial and cellular resolution.
Reliable detection and quantification of distinct RNA isoforms however remains a key limitation of short-read sequencing. Despite their scalability, current methods often struggle to distinguish closely related transcript variants, hindering our understanding of Biological and disease-related processes where specific isoforms, or splicing more generally can play important roles.
With the development of the BaseCode chemistry and software platform, Basic Genomics offers researchers a tool to leverage the power of short-read sequencing to produce high-quality and highly quantitative data using their existing platforms and workflows. In doing so, BaseCode enables a deeper understanding of alternative splicing, isoform switching, and transcript diversity, offering a scalable solution for transcriptomic studies across fields—from cancer biology to developmental genomics.

P20
Caterina Francesconi
Lund University

Using DiMeLo-seq to probe the epigenetic control of polymorphic repetitive elements

Although repetitive elements (REs) constitute more than 50% of the human genome and are known to affect gene expression, both by acting as cis-regulatory elements and by altering the chromatin environment, they remain greatly understudied. Due to their high disruptive potential if deregulated, they are subjected to several layers of control, often entangled. In particular, DNA methylation,

histone modifications and transcriptional/post-transcriptional silencing mediated by complexes such as HUSH and piwi-interacting RNAs are known to be involved in silencing REs, in ways often specific to the developmental stage and RE subfamily. Probing the regulation and regulatory effects of young RE subfamilies is particularly challenging, as the standard epigenetic profiling methods are based on short reads. The youngest LINE1 and SVA elements, present within the genome as multiple indistinguishable copies, are often unmappable. Additionally, polymorphic insertions, i.e. variable across individuals and not present in the reference genome, and length polymorphisms, i.e. somatic repeat expansions variable across cells and tissues, represent an additional hurdle. A recently developed molecular tool (DiMeLo-sequencing) allows epigenetic profiling through Oxford Nanopore long-read sequencing, circumventing the mappability challenges intrinsic to short-read approaches. Additionally, through the tool it is possible to obtain combined information on histone modifications and DNA methylation on an individual-read level, with the potential of detangling the epigenetic regulation of polymorphisms. Here, we applied the DiMeLo-sequencing, targeting H3K4me3, to epigenetically profile the activation of LINE1 in human stem cells lacking a critical player in their silencing, MORC2. To validate the methodology, we compared the obtained histone methylation profile with those produced through cleavage under targets and release using nuclease, i.e. CUT&RUN, the golden standard of epigenetic profiling. Furthermore, we applied adaptive sampling, a targeted Oxford Nanopore sequencing approach, to improve sequencing depth over REs. We then assessed if the higher sequencing depth can improve the reliability of the DiMeLo-sequencing, as well as lead to a more efficient expenditure of time and computational resources.

P21
Diego Henrique Fagundes Macedo
Stockholm University, MBW

Exploring the epigenetic regulation of sexual commitment in Plasmodium falciparum

Sexual differentiation in Plasmodium falciparum is essential for malaria transmission, yet the epigenetic mechanisms initiating gametocyte commitment remain poorly resolved. We propose to integrate high-resolution chromatin profiling via NanoCUT&Tag and ATAC-seq to dissect the regulatory landscape underlying this developmental switch. NanoCUT&Tag will map histone modifications (e.g., H3K9me3, H3K4me3) at key loci such as pfap2-g, while ATAC-seq will provide genome-wide chromatin accessibility profiles across committed and non-committed subpopulations. Synchronized cultures and fluorescence-activated cell sorting (FACS) will enrich early sexual precursors for comparative analyses. This approach will elucidate whether changes in nucleosome positioning and histone marks precede transcriptional activation, revealing how chromatin state drives lineage commitment. By resolving these dynamics, our study aims to identify epigenetic determinants of sexual development and potential targets to block malaria transmission.

P22
Marcel Tarbier
Uppsala University / SciLifeLab

Inferring elusive cell features from single-cell gene expression

Family history and lifestyle are important factors to consider when a patient has a serious illness, since your lineage and environment are strong indicators for many common and rare diseases. Similarly every cell has a lineage and a specific cellular environment that is informative for its “behaviour”. Especially in cancer cells there has been an appreciation that it is crucial to understand and characterize lineage and environment relationships to better understand its potential to invade healthy tissue, metastisize and avoid treatment.
However, in patient biopsies it is challenging to assess the molecular state of single-cells while preserving information about their lineage and micro-environment, drastically limiting our ability to link these properties with cancer progression and treatment outcome. Recent developments in computational biology and AI, however, may aid in filling this critical gap.

We have already shown that small differences in the molecular composition of cells are informative of their lineage, and developed an algorithm (GEMLI) that can infer cell lineage relationships with high confidence. We are now extending this computational tool to additional data types to enable a better understanding of the interplay of cell lineage relationships and cancer heterogeneity.
Similarly, we found that different cellular environments leave distinct molecular footprints in transcriptomic data and are developing machine learning solutions that provide information about a cells environment purely based on its molecular composition, thus enabling unprecedented insights into the relationship of cancer heterogeneity and local cell environments.

P23
Christian Riedel
MBW, Stockholm University

LIN-39 functions as a neuron-specific developmental determinant of longevity in Caenorhabditis elegans with reduced insulin/IGF-like signaling

The nuclear chromatin landscape changes with age, a phenomenon that has been observed across many species including humans. Importantly, perturbation of this landscape can be sufficient to change the rate of aging, indicating at least a partial role in controlling this process. This raises the question of whether differences in the chromatin landscape are also a distinguishing feature of animals that age at unusual rates. Here we addressed this gap, by focusing on insulin/IGF-like signaling (IIS) as a well-characterized signaling pathway whose impairment drastically slows aging and extends the organism's lifespan. Specifically, we conducted ATAC- and mRNA-seq in Caenorhabditis elegans with reduced IIS, i.e. daf-2 mutants. Interestingly, we found that particularly enhancer regions become closed and transcriptionally repressed with age while they become opened and transcriptionally active under reduced IIS, indicating their potential relevance for lifespan regulation. By screening through proteins binding these regions, we identified LIN-39 as a new aging-preventive transcription factor (TF) required for the longevity of daf-2 mutants. LIN-39 performs this role specifically during development and specifically in neurons. Further investigation showed that LIN-39 was acting around the L3 stage and in the hermaphrodite-specific VC class of cholinergic motor neurons. Finally, aging-prevention by LIN-39 was dependent on DAF-16/FOXO, an established pro-longevity TF acting downstream of DAF-2 which may synergize with LIN-39 in VC neurons and/or act downstream of the emitted signal. We ultimately propose a model whereby longevity of daf-2 mutant hermaphrodites requires a longevity-promoting signal emitted by VC neurons – a signal that relies on correct VC neuron maturation around the L3 stage, which is assured by the actions of LIN-39 and resulting chromatin and gene expression changes. This renders LIN-39 a rare example of a developmental determinant of longevity.

P24
Michael Hawgood
Karolinska Institutet

Spatial mapping of DNA synthesis reveals dynamics and geometry of human replication nanostructures

DNA replication is essential to life and ensures the accurate transmission of genetic information, which is significantly disturbed during cancer development and chemotherapy. While DNA replication is tightly controlled in time and space, methods to visualise and quantify replication dynamics within 3D human cells are lacking. Here, we introduce 3D-Spatial Assay for Replication Kinetics (3D-SPARK), an approach enabling nanoscale analysis of DNA synthesis dynamics in situ. 3D-SPARK integrates optimised nucleotide analogue pulse labelling with super-resolution microscopy to detect, classify, and quantify replication nanostructures in single cells. By combining immunofluorescence techniques with click chemistry-based nascent DNA labelling and transfection of fluorescent nucleotide derivatives, we map multi-colour DNA synthesis events in relation to established replication proteins, local RNA-protein condensates or large subnuclear domains. We

demonstrate quantitative changes in size, relative abundance and spatial arrangement of nanoscale DNA synthesis events upon chemotherapeutic treatment, CDC6 oncogene expression and loss of chromatin organiser RIF1. The flexibility, precision and modular design of 3D-SPARK helps bridging the gap between spatial cell biology, genomics, and 2D fibre-based replication studies in health and disease.

P25
Claudia Kutter
Karolinska Institute – SciLifeLab

Hidden rearrangements and epigenetic shifts in CRISPR-Cas9-edited cells

CRISPR-Cas9 has revolutionized genome editing, yet its application can cause complex and unintended genomic alterations with profound consequences for chromatin organization and epigenetic regulation. We combined two complementary approaches to dissect these unintended outcomes. First, we applied a droplet-based target enrichment strategy with long-read sequencing and custom de novo assembly to resolve Cas9-induced rearrangements at kilobase resolution. We uncovered duplications, inversions, and interchromosomal DNA fragment integrations - all within the targeted locus - that generated aberrant DNA fragments and altered cell proliferation. Second, we identified a recurrent 283-kb deletion on chromosome 10q23.31 in CRISPR-edited HAP1 cells, affecting key regulatory genes including PTEN and KLLN. This large deletion triggered widespread changes in histone acetylation and gene expression, disrupting cell cycle and DNA replication programs. Notably, this deletion was also observed in cancer patient genomes, aligning with transcriptional dysregulation linked to chromatin state alterations as observed in CRISPR-edited HAP1 cells. Together, our findings demonstrate that CRISPR-Cas9 editing can reshape the chromatin landscape in unexpected ways, underscoring the need for thorough genomic validation but also offering new insights into the intersection of structural variation, chromatin dynamics and cellular identity.

P26
Carlos Gallardo
Karolinska Institutet

Multi-dimensional analysis of MASLD signatures: discovering novel targets and biomarkers

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a growing global health concern affecting one in three adults worldwide. It occurs when excessive fat accumulates in the liver, often due to poor diet and lifestyle factors, increasing the risk of inflammation, fibrosis, and even liver cancer. Despite the growing repertoire of available diagnostic tools, there is a need for simple non-invasive tests that can accurately identify patients with MASLD, especially at the early stages of the disease. Moreover, while some drugs against obesity-driven MASLD have started to emerge, effective and long-lasting solutions are still lacking. To address this, we have conducted an in-depth characterization of gene expression changes throughout MASLD progression by combining data from both preclinical models and patients. We systematically evaluate the effect of hormone and small molecule inhibitor treatments, uncovering a novel mechanism by which lipid accumulation is regulated in liver cells. Furthermore, we establish a computational framework empowering large-scale integration of high-throughput sequencing data from over 1,000 patients with MASLD. We investigate gene biosignatures and alternative splicing events at various MASLD stages and assess their potential as biomarkers. Collectively, our findings reveal novel candidates for non-invasive MASLD diagnosis and pharmacological intervention.

P27
Qun Li
Karolinska Institutet

Deciphering cell states through mutations based on single cell data

The cell lineage of an organism is defined by the pattern of cell divisions during its development. Previous studies have used gene expression data to infer pseudo-time and branching trajectories from scRNA-seq data. While scRNA-seq gene expression data is valuable, it is also prone to noise. Some genes with low expression levels may not significantly impact overall expression but are crucial for cell cycle progression and neural commitment. Somatic mutations have been extensively examined in cancer research, providing insights into mutational processes and developmental cell lineages. Techniques such as WGS, WES, bulk RNA-seq, and single-cell DNA sequencing have been employed to analyze these mutations. However, similar to bulk RNA-seq, scRNA-seq can also be utilized to detect somatic mutations. Furthermore, scRNA-seq offers both expression and mutation information at single-cell resolution. Despite being sparser compared to other sequencing technologies, scRNA-seq data can be advantageous. Existing methods may not adequately address sequencing errors or be suitable for low -coverage regions and cells. Therefore, we propose developing a new model for identifying mutations using scRNA-seq data and tracing cell fate based on these mutations. We applied our model across multiple datasets, providing insights into the pathogenesis of transformation and the dynamic evolution of clonal complexity throughout disease progression.

P28
Claudio Cantù
Linköping University

The impact of Wnt signaling on the genome

We have developed a CUT&RUN Low-Volumes-Urea (LoV-U) protocol that enables the profiling of the genome-wide binding pattern of β-catenin, the key transducer of Wnt signaling, from low cell numbers. While CUT&RUN-LoV-U, as all protein-DNA interaction assays, aims at mapping the complete binding pattern of gene regulators, stringent statistics and limited experimental repetition too often causes to miss biologically relevant binding events. To solve this we developed ICEBERG, an experimental and informatic pipeline that harnesses numerous β-catenin CUT&RUN replicates to uncover the full spectrum of binding events and identifies previously unknown rare instances of β-catenin direct regulation. Integrating these technologies to detect β-catenin and other relevant targets, including the 3D-genome regulator CTCF, allowed us to measure how β-catenin binding impacts the 3D genomic structure. We discovered CTCF repositiongs under Wnt (RUW): upon Wnt activation, CTCF binds to many new genomic loci. We show that RUWs are characterized by both CTCF and TCF/LEF binding motifs, align with β-catenin binding patterns, and are dependent on the physical presence of β-catenin, underlying a previously neglected functional and physical interplay between β-catenin and CTCF. Our findings revealed that the RUWs mediate Wnt-dependent chromatin loops, and mutational assays indicated that they functionally contribute to Wnt target gene expression. RUWs, additionally, are part of a previously overlooked broad re-structuring of the chromatin interaction networks, which implicate the regulation of 3D genomic structure in the execution of the Wnt-induced transcriptional programs.

P29
Zhuang Liu
Karolinska Institutet

Spatiotemporal single-cell roadmap of human skin wound healing

Wound healing is vital for human health, yet the details of cellular dynamics and coordination in human wound repair remain largely unexplored. To address this, we conducted single-cell multi-omics analyses on human skin wound tissues through inflammation, proliferation, and remodeling phases of wound repair from the same individuals, monitoring the cellular and molecular dynamics of human skin wound healing at an unprecedented spatiotemporal resolution. This singular roadmap reveals the cellular architecture of the wound margin and identifies FOSL1 as a critical driver of re

epithelialization. It shows that pro-inflammatory macrophages and fibroblasts sequentially support keratinocyte migration like a relay race across different healing stages. Comparison with single-cell data from venous and diabetic foot ulcers uncovers a link between failed keratinocyte migration and impaired inflammatory response in chronic wounds. Additionally, comparing human and mouse acute wound transcriptomes underscores the indispensable value of this roadmap in bridging basic research with clinical innovations.

P30

Majid Pahlevan Kakhki
Department of Clinical Neuroscience, Karolinska Institutet, and Center for Molecular Medicine, Karolinska University Hospital, Stockholm, Sweden

Systematic comparison of dCas9-based DNA methylation epimodifiers over time indicates efficient on-target and widespread off-target effects

CRISPR/dCas9-based epigenome editing systems, including DNA methylation epimodifiers, have greatly advanced molecular functional studies revolutionizing their precision and applicability. Despite their promise, challenges such as the magnitude and stability of the on-target editing and unwanted off-target effects underscore the need for improved tool characterization and design. We systematically compared specific targeting of the BACH2 gene promoter and genome-wide off-target effects of available and novel dCas9-based DNA methylation editing tools over time. We demonstrate that multimerization of the catalytic domain of DNA methyltransferase 3A enhances editing potency but also induces widespread, early methylation deposition at low-to-medium methylated promoter-related regions with specific gRNAs and, interestingly, also with non-targeting gRNAs. A small fraction of the methylation changes associated with transcriptional dysregulation and mapped predominantly to bivalent chromatin associating both with transcriptional repression and activation. Additionally, specific non-targeting control gRNA caused pervasive and long-lasting methylation-independent transcriptional alterations particularly in genes linked to RNA and energy metabolism. CRISPRoff emerged as the most efficient tool for stable targeting of the BACH2 promoter, with fewer and less stable off-target effects compared to other epimodifiers but with persistent transcriptome alterations. Our findings highlight the delicate balance between potency and specificity of epigenome editing and provide critical insights into the design and application of future tools to improve their precision and minimize unintended consequences.

P31

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Uppsala University - Institute of Organismal Biology

Epigenetic changes in red blood cells during the life of chickens exposed to stressful or enriched environments

From an ecological perspective, many animals—particularly birds—are increasingly exposed to stress due to the ongoing climate crisis. These stressors include exposure to extreme temperatures during critical developmental periods, habitat loss, and increased interaction with urban environments. Developing tools that can reliably measure long-term stress exposure in animals is therefore essential in this context.
Currently, most approaches to assess long-term stress rely on measuring stress hormones. However, hormone levels typically reflect acute, recent stress rather than sustained or historical exposure. Epigenetic tools offer an alternative, as epigenetic marks in peripheral cells have been shown to reflect long-term stress history. This is particularly relevant for birds, which have nucleated red blood cells (RBCs)—a readily accessible cell type for field sampling.
In previous work, we demonstrated that epigenetic marks in chicken RBCs reflect early-life housing conditions (caged vs. aviary), which are associated with differences in fear responses and cognitive

performance. From this, two key questions emerged and are addressed in the present study: (i) Are RBC epigenetic marks associated with specific types of stress? and (ii) How long do these epigenetic alterations persist across the animal's lifespan?
To explore this, 4-day-old male chickens were subjected to incremental social isolation stress over three weeks—a stressor previously shown by our group to have long-term and transgenerational effects. We collected RBCs immediately after the treatment and again six months later. Given that the average RBC lifespan in chickens is one month, the second sampling reflects a fully renewed RBC population.
We performed reduced-representation DNA methylation analysis (using the GBS-MeDIP method) and investigated Differentially Methylated Regions (DMRs) across these two time points. Our results show that DMRs induced by stress or enrichment are predominantly hypermethylated. However, with age, enrichment-associated DMRs tend to lose methylation, while stress-induced DMRs tend to gain methylation. Consistently, the stress group showed more age-associated hypermethylated DMRs than controls and enrichment groups, with the latter exhibiting the fewest. Another interesting finding is the high emergence of DMRs on chromosome Z with age across all groups, a pattern particularly pronounced in the stress group.
This study demonstrates that early-life social stress in chickens leads to persistent and stressor-specific epigenetic changes in RBCs, with distinct age-related methylation trajectories—especially on ChrZ. These findings support the use of RBC epigenetic profiles as a promising biomarker system for long-term stress exposure in birds.

P32
John Lees
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Environmental Stress and the Mitochondrial Epigenome: Sex-dependent Developmental Programming of the Metabolic Phenotype

Mitochondria are central to key cellular functions including apoptosis, calcium regulation, and cell cycle control. They also serve as vital mediators in the cellular response to the external environment during development. A relatively unexplored component of this process, linking environmental exposures to mitochondrial function, is that of the mitochondrial epigenome. Although the presence and functional relevance of mitochondrial DNA methylation is still debated, recent findings point to its potential regulatory role. Across two studies, we show that developmental exposure to environmental stress has lasting effects on the avian mitochondrial epigenome—altering mitochondrial DNA methylation in parallel to changes in mitochondrial structure and function in a sex-specific manner. These findings open new avenues for understanding how the mitochondrial epigenome is associated with long-term physiological programming in males and females.

P33
Christopher Douse
Lund Stem Cell Center

Epigenetic control of nested retroelements in human brain development

Half of human intronic DNA is made from retroelements, sequences that have been reverse transcribed and integrated in new genomic locations. A key component of controlled gene expression is the accurate removal of introns from mRNA and protection against potential mis-processing induced by retroelements. While chromatin and epigenetic mechanisms are well known to influence the rate and amplitude of transcription, their influence on RNA processing events like splicing is relatively unexplored. Here we provide evidence that ZNF638, recently associated with multiple sclerosis severity, is a key factor linking epigenetic retroelement silencing to RNA processing in the neural lineage. We identify that ZNF638 recruits the HUSH-MORC2 epigenetic corepressor specifically to retroelements lying in introns of transcribed genes, and protects against cryptic termination and alternative splicing induced by these retroelements. Our work provides a mechanistic

framework to understand how RNA processing can be influenced by chromatin and impact molecular phenotypes important in neurological disorders.

P34
Kathryn Jackson
Karolinska Institutet

MYC modulates TOP2A diffusion to promote substrate detection and activity

Topoisomerases are essential enzymes that alleviate DNA supercoiling by cleaving and resealing DNA strands. Regulation of their abundance and activity is crucial for maintaining genomic stability and supporting nuclear functions. Previously, we showed that the oncoprotein MYC recruits and stimulates topoisomerases to remove DNA entanglements generated by oncogenic transcription. Understanding this mechanism may suggest methods to inhibit MYC-driven topoisomerase activation, thereby selectively targeting tumor-specific transcription. Here, we demonstrate that the essential topoisomerase TOP2A in human cells exists in a dynamic equilibrium between sequestration in the nucleolus, substrate searching in transcription hubs, and active engagement on chromatin. This equilibrium is highly responsive to changes in DNA topology, allowing cells to regulate TOP2A levels in the nucleoplasm. Using single molecule tracking, we show that MYC significantly accelerates TOP2A diffusion in cells. In vitro, we confirm that MYC decreases the size of TOP2A condensates, likely by limiting non-specific self interactions. By increasing TOP2A diffusion, MYC promotes substrate binding and increases TOP2A engagement on the chromatin genome-wide, revealing the mechanism underlying MYC stimulation of TOP2A activity.

P35
Anaswara Sugathan
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Dysregulated ribosomal transcription due to reduced levels of WSTF in the chromatin remodelling complex B-WICH induces the stress response

Ribosomal gene expression is essential for cell growth and tightly regulated by environmental cues. The B-WICH complex, comprising WSTF, the ATPase SNF2h and nuclear myosin, activates rRNA transcription in response to glucose by modifying the chromatin state at the promoter. Here, we show that WSTF knock down reduced cell viability, without inducing a clear nucleolar stress and apoptosis. WSTF siRNA knock down did not lead to a p-53 dependent nucleolar stress, but instead resulted in reduced translation and induction of the Integrated stress response. In HeLa cells, which have a p53 deficiency, the integrated stress response with phosphorylation of EIF2α and HSP60 in the mitochondrial Unfolding stress response were activated. The mitochondria were not dysregulated; we observed a slight upregulation of mitochondrial oxygen response, similar to glucose starved cells, and the mitochondria responded to glucose refeeding equally well. In conclusion, WSTF knock down results in a p53-independend stress response mediated by integrated stress response pathway.

P36
Ilaria Piazza
Stockholm University

Decoding proteostasis through proteome-wide biophysics

Mass spectrometry-based proteomics typically provides snapshots of global protein abundances, assuming that clusters of co-regulated proteins accurately reflect cellular phenotypes. However, this overlooks critical aspects of protein homeostasis. Proteostasis— the equilibrium between properly folded proteins and misfolded aggregates—is a key regulator of cell function. Disruption of this balance leads to protein aggregation, which alters protein structure and function but is not detectable through conventional proteomics workflows that quantify protein abundance alone.

My group develops mass spectrometry-based technologies to monitor global changes in protein structure directly within cells. In this talk, I will show how we use these approaches to study proteome-wide events such as aggregation, phase separation, and protein unfolding under conditions that perturb proteostasis. I will highlight recent data where we map structural changes across the proteome in response to targeted chemical stressors, uncovering early signatures of protein destabilization. These tools expand the scope of proteomics beyond abundance, offering new insights into the cellular response to stress, and providing a complementary layer of information highly relevant to protein degradation pathways. This structural dimension of proteomics opens new possibilities for characterizing degrader activity, mode-of-action, and off-target effects in drug discovery.

P37
Chandrasekhar Kanduri
University of Gothenburg

Bifunctional tumor suppressor gene CACNA2D3 orchestrates adrenergic–mesenchymal transdifferentiation via RNA- and protein-mediated mechanisms.

Transdifferentiation between adrenergic and mesenchymal phenotypes in neuroblastoma drives tumor plasticity, therapeutic resistance, and relapse, however its molecular basis remains unknown. By integrating transcriptomic data from mesenchymal-like Schwann cell precursors and adrenergic-like neuroblasts with adrenergic-dominant low-risk and mesenchymal-enriched high-risk neuroblastomas, we identified CACNA2D3 as a key regulator of transdifferentiation. Genomic analysis of 818 neuroblastomas identified recurrent chromosomal alterations at the CACNA2D3 locus. Spatial transcriptomic analysis showed a positive correlation between CACNA2D3 expression and adrenergic signatures, while exhibiting a negative correlation with mesenchymal signatures. Single-cell RNA-seq analysis following CACNA2D3 knockdown uncovered a distinct mesenchymal-like sub-cluster, corroborated by a marked increase in MES-positive cells. Molecular investigation reveals the dual role of CACNA2D3 in transdifferentiation. CACNA2D3 RNA and chromatin regulators epigenetically silence mesenchymal genes via organizing nuclear phase condensates. In contrast, CACNA2D3 protein localizes to mitochondria, and preserves mitochondrial structure and cellular metabolism. Together, these findings provide molecular insights into CACNA2D3 driven transdifferentiation, opening new avenues for neuroblastoma-specific targeted therapies.

P38
Kanwal Tariq
Karolinska Institutet/Stockholm University

IGS38, a lncRNA transcribed from the human rDNA intergenic spacer, regulates rRNA transcription and rDNA chromatin organisation

The eukaryotic ribosomal genes are multi-copy genes, and approximately one third of them is actively transcribing in differentiated cells. A number of lncRNAs have been identified which are involved in silencing of rRNA gene copies by altering the chromatin configuration, but how activation of rRNA gene transcription is achieved is poorly understood. Here, we have identified lncRNAs that are transcribed from the human rDNA locus and we show that they have different functions: IGS38 positively regulates rRNA gene transcription by modulating the rRNA promoter accessibility while IGS32 antisense binds heterochromatin protein 1 (HP1α) and is involved in constitutive heterochromatin formation. IGS38 interacts with the Williams Syndrome Transcription Factor (WSTF), a component of the B-WICH chromatin remodelling complex, to keep the architectural protein Upstream Binding Factor (UBF) at the rRNA promoter, to facilitate the binding of the RNA pol I transcription factor RRN3 to stalled RNA pol II and thereby promote promoter escape. Furthermore, IGS38 knock down induces an indirect and mild innate immune-like response characterised by higher expression of OAS2, typically induced by interferon. Overall, the lncRNAs

IGS38 and IGS32as are involved in rDNA chromatin changes, and IGS38 isstimulating, together with WSTF, rRNA gene transcription in human cells.

P39
Shruti Jain
Dept of Molecular Biosciences, Stockholm University

A non-canonical role for U3 snoRNA in the regulation of antiviral immune response in Drosophila melanogaster

Small nucleolar RNAs (snoRNAs) are key components of the chromatin-associated transcriptome. In this study, we have shown snoRNA:U3:9B to be enriched in chromatin and its interaction with numerous protein-coding genes in the Drosophila melanogaster genome, including immune response genes. Using CRISPR/Cas9, snoRNA:U3:9B was deleted to assess its function in vivo. While snoRNA:U3:9B-knockouts remained viable under normal conditions, the larvae failed to pupate when challenged by Sindbis virus replicon, indicating that snoRNA:U3:9B is essential for eliciting an effective antiviral response. Additionally, the chromatin decompaction and immune gene activation observed during Sindbis virus infection were absent in snoRNA:U3:9B-knock-out larvae. With ChIRP-qPCR experiments, we have demonstrated that snoRNA:U3:9B localizes to immune genes in vivo, suggesting that the chromatin and gene expression changes in knockout strains result from direct regulatory effects. Furthermore, snoRNA:U3:9B associated with the ATP-dependent chromatin remodeling factor Brahma to recruit the complex to specific immune genes. Collectively, these findings reveal an antiviral defense mechanism in which a snoRNA facilitates immune gene activation by mediating the recruitment of a chromatin remodeling factor, thereby modulating local chromatin accessibility.

P40
Sindhu Vangeti
Stockholm University and Icahn School of Medicine at Mount Sinai

Mapping the epigenetic changes in the immune landscape following respiratory viral infections and vaccination

The mechanisms that determine the longevity of immune responses following infection, vaccination and durability of protection are not well understood. We performed several high-impact studies that utilized multiple single-cell immune monitoring approaches and sensitive bioinformatic modeling methods to develop robust predictive signatures. First, we relied on a controlled human influenza virus infection model and multi-omic analyses to describe changes in AP-1 gene expression, and accessibility at AP-1 targets 28 days after infection. We also found altered expression of histone deacetylase and MAP kinase genes, and increased accessibility at interferon-related gene promoter regions. Further, we identified several gene regulatory circuits (GRCs) within several immune cell types, but notably in CD14 and CD16 monocytes, we found GRCs involving the transcription factor ETS2. In the second study, we applied multiple high resolution omics technologies across various murine immune tissues to evaluate the long-term impact of vaccination in two quadrivalent influenza and two SARS-CoV-2 mRNA vaccination protocols (one “good”, one “bad”). CITE-seq analysis revealed that “good” vaccines which induced long-term protection resulted in the establishment of immune signatures in blood monocytes that had been previously associated with improved vaccine-induced antibody responses in humans when present at baseline. Paired single-nucleus gene expression and chromatin accessibility analysis identified long-term changes in spleen in red pulp macrophages (RPM) in all vaccinated groups. The spleen RPM changes, more evident in influenza vaccines, were related to myeloid differentiation processes, phagocytosis, lymphocyte proliferation and heme metabolism. Specific changes were seen in epigenetic regulatory circuits (ERC) involving the transcription factors encoded by Pparg, Spic, Tfec, Jund for influenza vaccines, and Ebf1 and Mef2 for SARS-CoV-2 vaccines. Multiple ERC involving Irf4 encoded transcription factors were identified to be altered in follicular B cells after influenza vaccination. Overall, we showed that in

addition to antigen-specific changes, infection and vaccination induce persistent epigenetic immune setpoint remodeling and mapping these changes could potentially improve development of future vaccinations or therapeutics for infectious exposures.