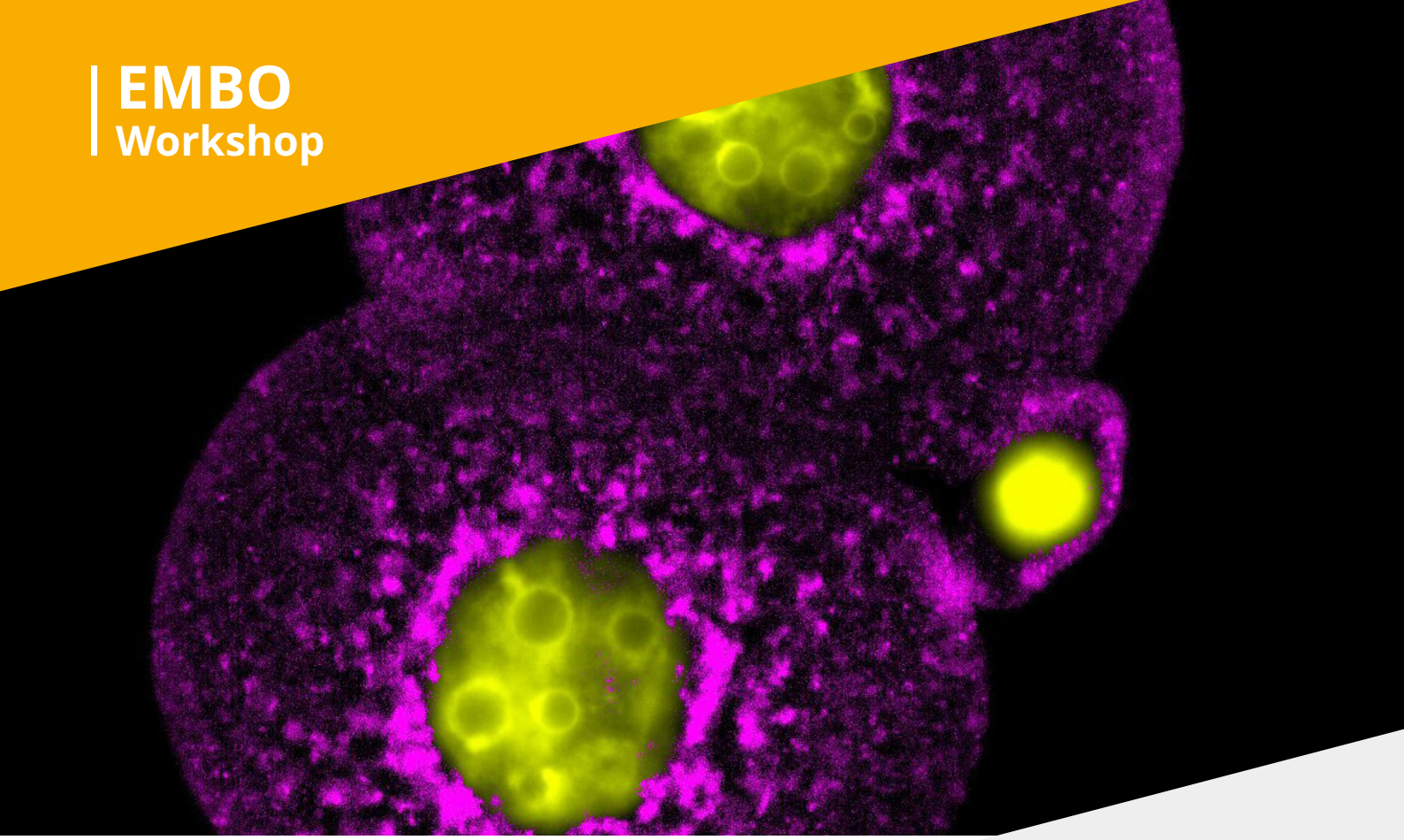
EMBO
Workshop



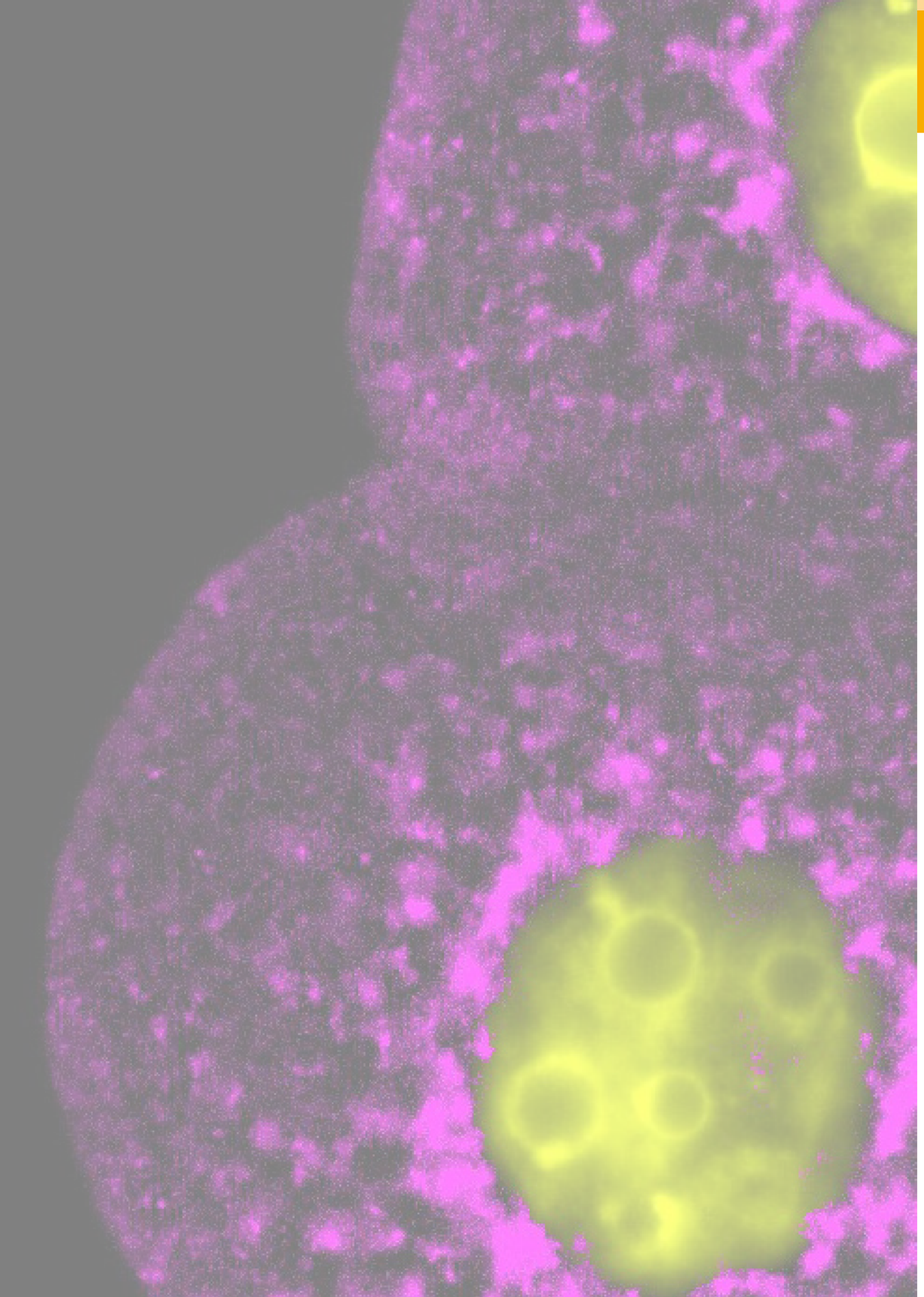
abstract book

Awakening the Genome During Developmental Reprogramming:

From Embryos to Organoids

4 – 7 May 2026 | Seville, Spain

EMBO



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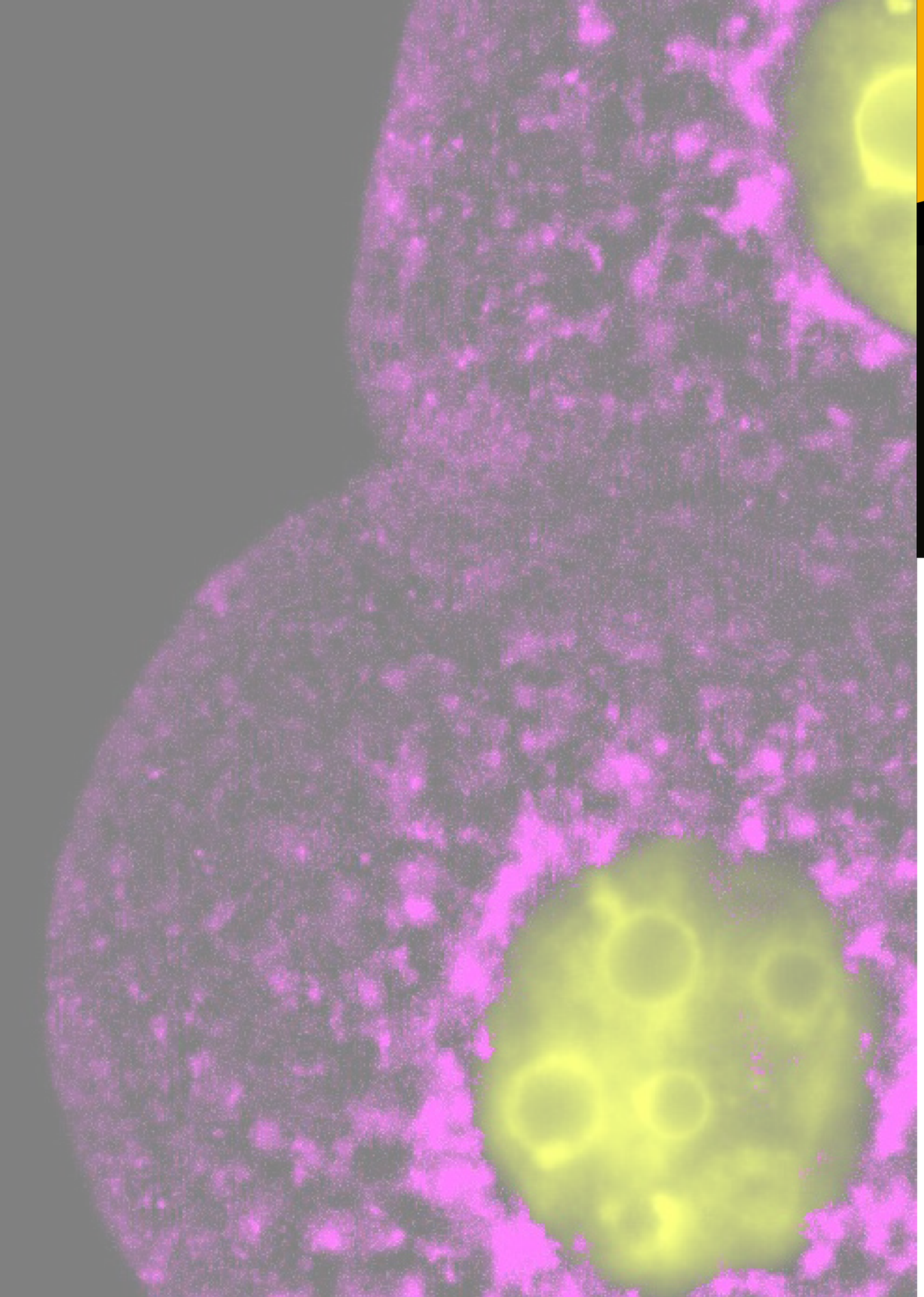

EMBO Workshop

Awakening the Genome During Developmental Reprogramming:

From Embryos to Organoids

4 – 7 May 2026 | Seville, Spain

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Registration
2 February 2026

Sustainable event

CONTACT
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PROGRAMME

DAY 1 | 4-May-26

14:30- 16:00 **REGISTRATION**

16:00- 16:15 **WELCOME PARTICIPANTS AND SUSTAINABILITY REMARKS**
Ana Boskovic, Antonio Giraldez, Melissa Harrison, Miguel Moreno-Mateos

SESSION 1: MATERNAL AND GERMLINE REGULATION

Session chair: Howard Lipshitz

16:15- 16:40 **EPIGENETIC MECHANISMS OF CELLULAR PLASTICITY**
Maria Elena Torres Padilla

16:40- 16:55 **UNCOVERING ESSENTIAL REGULATORS DURING THE MATERNAL-TO-ZYGOTIC TRANSITION BY CRISPR-RFXCAS13D SCREENINGS**
Ismael Moreno-Sanchez

16:55- 17:30 **COFFEE BREAK**

17:30- 17:45 **PREVENTING CPG HYPERMETHYLATION IN OOCYTES SAFEGUARDS MOUSE DEVELOPMENT**
Antoine Peters

17:45- 18:00 **INTEGRATOR MASSIVELY REPRESSES GENE TRANSCRIPTION DURING ZYGOTIC GENOME ACTIVATION**
Mary Mullins

18:00- 18:15 **SINGLE-MOLECULE M6A MAPS UNCOVER HOW POSITION AND STOICHIOMETRY SHAPE MATERNAL MRNA FATE**
Jean-Denis Beaudoin

18:15- 18:30 **SPATIALLY PATTERNED ZYGOTIC GENOME ACTIVATION FULFILLS EMBRYO QUALITY CONTROL**
Matthew Good

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18:30- 18:45 **THE MATERNAL PADI6 CONTROLS DNA METHYLATION AND GENOMIC IMPRINTING MAINTENANCE IN PREIMPLANTATION MOUSE EMBRYOS**
Andrea Riccio

19:00- 21:00 **COCKTAIL RECEPTION**

DAY 2 | 5-May-26

SESSION 2: EPIGENETIC REGULATION DURING ZGA

Session Chair: Miguel Manzaneres

09:00- 09:25 **ADJUSTING THE TIMING OF MAMMALIAN DEVELOPMENT**
Aydan Bulut-Karslioglu

09:25- 09:40 **IDENTIFICATION OF PIONEER FACTOR INTERACTING PROTEINS TO ELUCIDATE THE MOLECULAR MECHANISMS THAT REGULATE ZYGOTIC GENE EXPRESSION**
Mina Kojima

09:40- 09:55 **MECHANISMS OF POLYCOMB DOMAIN INHERITANCE AND ESTABLISHMENT ACROSS THE MATERNAL-TO-ZYGOTIC TRANSITION**
Shelby Blythe

09:55- 10:10 **HISTONE GENE REPLACEMENT DURING THE MATERNAL TO ZYGOTIC TRANSITION IN DROSOPHILA**
Dan McKay

10:10- 10:45 **COFFEE BREAK**

10:45- 11:10 **SHORT RNAS, LONG MEMORIES: WHEN GENOME DEFENCE BECOMES GENE REGULATION**
Alejandro Burga

11:10- 11:35 **ESTABLISHING CHROMATIN TOPOLOGY AND ENHANCER FUNCTION DURING ZYGOTIC GENOME ACTIVATION**
Eileen Furlong

SESSION 3: MZT IMPACT ON HUMAN DEVELOPMENT AND FERTILITY

Session Chair: Sanna Vuoristo

11:35- 12:00 **DECOUPLING PRINCIPLES OF MAMMALIAN BODY PLAN DEVELOPMENT**
(Sponsored by ISDB)
Berna Sozen

12:00- 12:15 **A HUMAN-SPECIFIC MECHANISM DRIVEN BY RETROTRANSPOSONS IMPACTS PREIMPLANTATION DEVELOPMENT IN A 3D MODEL OF THE HUMAN BLASTOCYST**
Raquel Fueyo

12:15- 12:30 **DGCR8 HAPLOINSUFFICIENCY IMPAIRS PLURIPOTENCY VIA PRIMATE-SPECIFIC RNA DYSREGULATION**
Sara Heras

12:30- 12:45 **BEYOND MARKERS: SR-FTIR MICROSPECTROSCOPY DEFINES MACROMOLECULAR SIGNATURES OF PRIMED-TO-FORMATIVE TRANSITIONS IN OOCYTE-INFORMED HUMAN REPROGRAMMING**
Elena Gonzalez-Munoz

12:45- 13:10 **SYNTHETIC EX UTERO EMBRYOGENESIS: FROM NAIVE PLURIPOTENT CELLS TO COMPLETE EMBRYO MODELS**
Jacob Hanna

13:10- 13:40 **FLASH TALKS**
Cielo Centola, Annemarie Branks, Pavel Kravchenko, Sara Formichetti, Jasmina Al-Mousawi, Evgeniy Ozonov

13:40- 15:00 **LUNCH (MEET THE SPEAKERS LUNCH)**

15:00- 15:20 **SPEED NETWORK:**
Session to introduce each other, switching every minute

15:30- 17:30 **POSTER SESSION I (COFFEE AND REFRESHMENTS) - SUBMISSION ID#3-38, 54**

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SESSION 4: IMAGING MECHANISMS OF MZT

Session Chair: Melissa Harrison

17:30- 17:55 **MOLECULAR KINETICS OF GENE REGULATION DURING EMBRYONIC DEVELOPMENT**
Mustafa Mir

17:55- 18:10 **CHROMATIN TRACING REVEALS GENOME ORGANISATION FROM SUB-MEGABASE TO CHROMOSOME SCALES IN EARLY MOUSE EMBRYOS**
Franziska Kundel

18:10- 18:35 **A ROLE FOR NUCLEAR ORGANIZATION IN TRANSCRIPTION REGULATION**
Nadine Vastenhouw

18:35- 18:50 **NANOSCALE IMAGING REVEALS RNA-DEPENDENT CHROMATIN COMPACTION DURING EARLY HETEROCHROMATIN FORMATION**
Mark Pownall

18:50- 19:15 **SPATIOTEMPORAL REGULATION OF TRANSLATION DURING ZYGOTIC GENOME ACTIVATION**
Mounia Lagha

19:15- 22:00 **DINNER ON YOUR OWN AND CITY EXPLORATION**

DAY 3 | 6-May-26

SESSION 5: RNA REGULATORY MECHANISMS DURING MZT

Session Chair: Miguel Moreno-Mateos

09:00- 09:25 **SPATIO-TEMPORALLY REGULATED MATERNAL RNA DECAY FACTOR ESSENTIAL FOR ZEBRAFISH DEVELOPMENT**
Ariel Bazzini

09:25- 09:40 **NUCLEAR SPECKLES LINK NUCLEAR ORGANIZATION TO GENE REGULATION IN THE EARLY EMBRYO**
Joanna Jachowicz

09:40- 10:05 **AWAKENING DORMANT RIBOSOMES**
Andrea Pauli

10:05- 10:20 **MATERNAL RNA LOCALIZATION THROUGH INTRINSICALLY DISORDERED PROTEINS**
Andrea Putnam

10:20- 10:45 **CONTROL OF HISTONE BIOGENESIS BY A SPERM-BORNE TRNA-FRAGMENT**
Ana Boskovic

10:50- 11:20 **COFFEE BREAK**

SESSION 6: ENHANCER REGULATION DURING GENOME ACTIVATION

Session Chair: Chris Rushlow

11:20- 11:35 **FUNCTIONAL CHARACTERIZATION OF DUX-INDUCED TRANSCRIPTIONAL CLUSTERS DURING THE EXIT FROM TOTIPOTENCY**
Sergio Ruiz Macias

11:35- 12:00 **MATERNAL CTNNB1 AND CTNNB2 PLAY DISTINCT FUNCTIONS DURING EARLY ZEBRAFISH EMBRYOGENESIS**
Anming Meng

12:00- 12:15 **PRECISION VS PLASTICITY IN ENHANCER-DRIVEN CELL FATE SPECIFICATION**
Theodora Koromila

12:15- 12:40 **A SINGLE MOLECULE VIEW OF ZYGOTIC GENOME ACTIVATION**
Mike Levine

12:40- 13:05 **EPIGENETIC INHERITANCE AND REPROGRAMMING IN EARLY MAMMALIAN DEVELOPMENT**
Wei Xie

13:05- 13:35 **FLASH TALKS**
Sergei Pirogov, Antoine Canat, Jenna Bergmann, Marina Makharova, Caroline Hoppe, Curtis Boswell, Eleonora Perego

13:35- 15:00 **LUNCH (MEET THE SPEAKERS LUNCH)**

15:00- 15:20 **SPEED NETWORK SESSION**

15:30- 17:30 **POSTER SESSION II (COFFEE AND REFRESHMENTS) - SUBMISSION ID#40-77**

20:00- 21:00 **VISIT TO THE REALES ALCAZARES UNESCO WORLD HERITAGE SITE**

21:00- 22:30 **DINNER ON YOUR OWN AND CITY EXPLORATION**

DAY 4 | 7-May-26

SESSION 7: STRUCTURAL REMODELING IN RNA AND CHROMATIN DURING MZT

Session Chair: Antonio Giraldez

09:00- 09:25 **THREE-DIMENSIONAL GENOME REORGANIZATION FORESHADOWS ZYGOTIC GENOME ACTIVATION IN DROSOPHILA**
Juanma Vaquerizas

09:25- 09:40 **CHROMATIN GOLD ELECTRON MICROSCOPY (CHROMGEM) REVEALS ULTRASTRUCTURAL HALLMARKS OF TRANSCRIBING AND SILENT CHROMATIN DURING MAMMALIAN GENOME ACTIVATION**
Alice Sherrard

09:40- 09:55 **PARTITIONING OF TRANSPOSON-RICH MATERNAL DNA VIA CHROMATIN COMPACTION IS A PREREQUISITE FOR EMBRYONIC GENE ACTIVATION**
Patrick Murphy

09:55- 10:10 — **UNCOVERING THE ROLE OF CHROMATIN MATERIAL PROPERTIES IN ZYGOTIC GENOME ACTIVATION**
Joana M N Vidigueira

10:10- 10:35 — **ROLE OF RNA GRANULES IN THE MATERNAL TO ZYGOTIC TRANSITION**
Geraldine Seydoux

10:40- 11:10 — **FLASH TALKS**
Hui Chen, Anna Hakes, Samantha Fallacaro, William Hamilton, Matthias Tisserand Erpeldinger

11:10- 11:40 — **COFFEE BREAK**

SESSION 8: EVO DEVO AND NOVEL MODEL SYSTEMS IN THE MATERNAL TO ZYGOTIC TRANSITION

Session Chair: Ferenc Mueller

11:40- 12:05 — **NON-CANONICAL HISTONE MODIFICATION DURING THE MATERNAL-TO-ZYGOTIC TRANSITION**
Miler Lee

12:05- 12:20 — **A BALANCING ACT BETWEEN A TRANSCRIPTION FACTOR AND ITS BINDING SITE FACILITATES CHROMATIN ORGANIZATION**
Haley Brown

12:20- 12:35 — **A COMPARATIVE EPIGENOMIC ANNOTATION FOR REGULATORY ELEMENTS IN THE ALLOTETRAPLOID GENOME: MAPPING OF THE NON-CODING FUNCTIONAL ELEMENTS DURING CYPRINUS CARPIO EMBRYO DEVELOPMENT**
Damir Baranašić

12:35- 12:50 — **BEYOND THE CPG: DISSECTING THE FUNCTIONS OF ATYPICAL DNA METHYLATION DURING EARLY VERTEBRATE DEVELOPMENT**
Ozren Bogdanovic

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12:50- 13:15 — **EVOLUTIONARY LANDSCAPES OF THE ZYGOTIC GENOME ACTIVATION IN ANIMALS**
(Sponsored by Spanish Genomics Network)
Manuel Irimia

13:15- 14:45 — **LUNCH (MEET THE SPEAKERS LUNCH)**

14:45- 16:45 — **POSTER SESSION III (COFFEE AND REFRESHMENTS) - SUBMISSION ID#79–126**

SESSION 9: GENE REGULATION DURING GENOME ACTIVATION IN MAMMALS

Session Chair: Antoine Peters

16:45- 17:05 — **BRIDGING THE GAP BETWEEN OOCYTE DEVELOPMENT AND MZT**
Ed Grow

17:05- 17:20 — **A ZGA CHECKPOINT REVEALED BY MAMMALIAN INTERSPECIFIC HYBRIDS**
Yang Yu

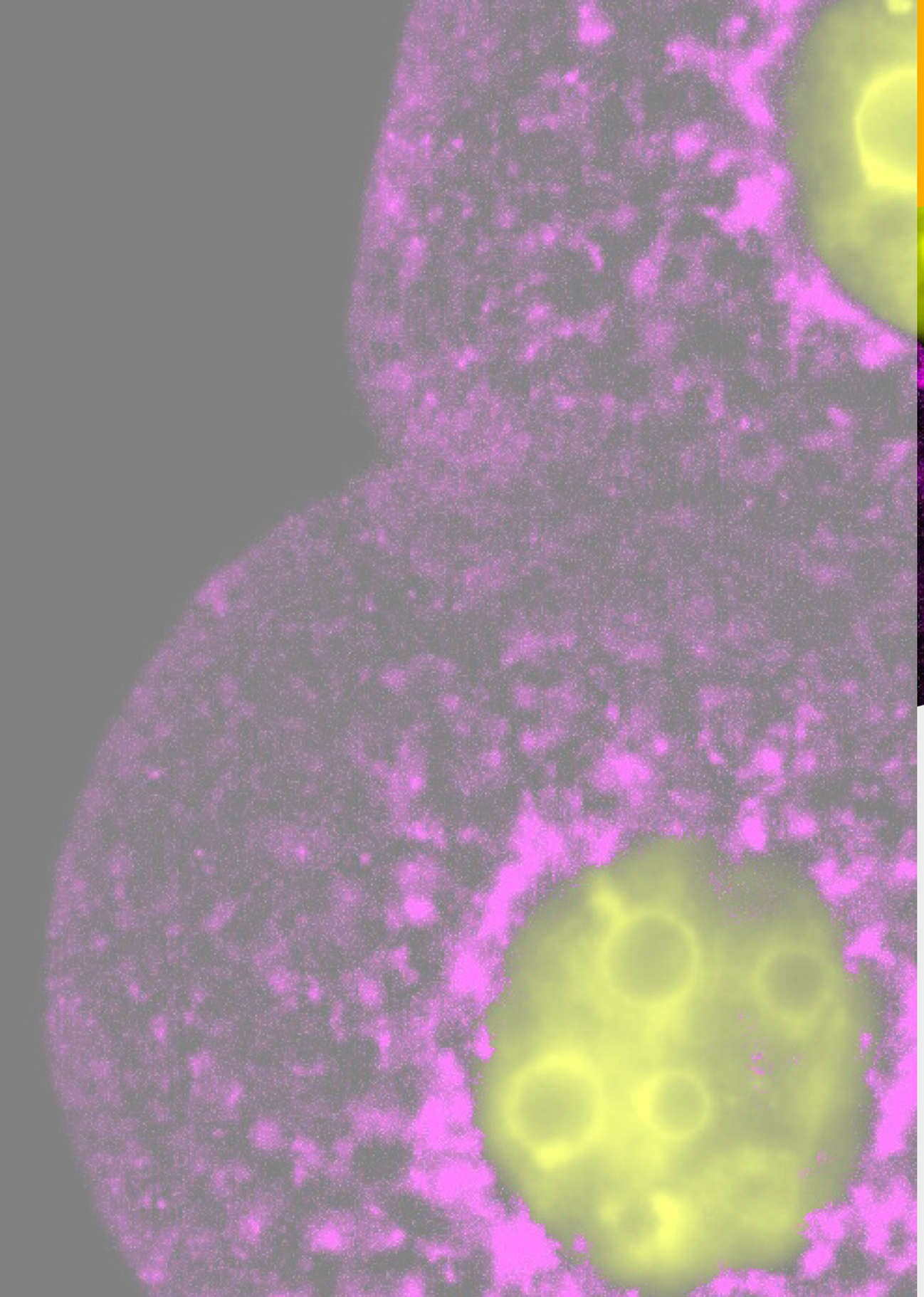
17:20- 17:45 — **DNA DAMAGE RESPONSE IN THE EARLY MAMMALIAN EMBRYO: FRIEND OR FOE?**
Barbara Pernaute

17:45- 18:00 — **A MATERNAL CHEK1 VARIANT DELAYS MITOTIC ENTRY AND IMPAIRS EMBRYONIC GENOME ACTIVATION**
Xiao Xu

18:00- 18:25 — **RISE AND SINE: MECHANISMS OF ZGA IN MOUSE EMBRYOS**
Kikue Tachibana

18:30- 19:00 — **COMMUNITY DISCUSSION AND CLOSING REMARKS**
Ana Boskovic, Antonio Giraldez, Melissa Harrison, Miguel Moreno-Mateos

20:30- 24:00 — **CONFERENCE CELEBRATION AND GALA DINNER PARTY**



ABSTRACTS
(in order of appearance)

EMBO

SESSION 1 MATERNAL AND GERMLINE REGULATION

Epigenetic mechanisms of cellular plasticity

Maria-Elena Torres-Padilla (torres-padilla@helmholtz-muenchen.de)

Institute of Epigenetics and Stem Cells, Helmholtz Munich, Germany. Faculty of Biology, Ludwigs Maximilians University of Munich, Germany

After fertilisation, a new developmental programme starts, whereby a single cell, the one-cell embryo or zygote, will form an entire new organism. The period that follows fertilisation is characterised by major changes in cellular plasticity and potency. Coinciding with these changes in cellular potency, massive alterations occur in the nucleus, with major transcriptional, epigenetic and architectural remodelling, as well as the establishment of the DNA replication landscape. In particular, replication timing is a major epigenetic fingerprint, which is linked to nuclear organisation. Understanding how nuclear organisation and the replication timing programme are first established at the beginning of development is essential to decipher how the first epigenetic regulatory layers are set in place during development. I will discuss recent advances in this area, which have informed us on how the early embryo controls its DNA-based processes, the regulation of DNA replication and its molecular interdependencies with epigenetic regulation and nuclear organisation.

#22 • Uncovering Essential Regulators During the Maternal-to-Zygotic Transition by CRISPR-RfxCas13d Screenings

Ismael Moreno-Sanchez[1, 2], Luis Hernandez-Huertas[1], Daniel Nahon-Cano[1], Jesus Crespo-Cuadrado[1], Alejandra Cano-Ruiz[1], Gabriel da Silva Pescador[3], Anthony J Treichel[3], Gopal Kushawah[3], Ariel A Bazzini[3, 4], Miguel A Moreno-Mateos[1]

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[2] *Universidad Loyola Andalucía, Av. de las Universidades, 2, 41704 Dos Hermanas - Seville, Spain*
[3] *Stowers Institute for Medical Research, 1000 E 50th St, Kansas City, MO 64110, USA*
[4] *Department of Molecular and Integrative Physiology, University of Kansas Medical Center, 3901 Rainbow Blvd, Kansas City, KS 66160, USA*

The Maternal-to-Zygotic Transition (MZT) reprograms early vertebrate development by coordinating zygotic genome activation (ZGA) with the systematic clearance of maternally deposited RNAs. Although several regulators of MZT have been described, the functional roles of most maternal transcripts remain largely unexplored. We recently established and optimized the use of CRISPR-RfxCas13d (CasRx) technology in animal embryos, demonstrating its high efficiency and specificity when applied through transient strategies such as ribonucleoprotein complexes or mRNA-gRNA. Using our optimized technology, we have performed large-scale functional screenings targeting hundreds of maternally supplied mRNAs in zebrafish embryos. As a proof of concept, we have completed a screening focused on transcripts encoding protein kinases and phosphatases in zebrafish, uncovering Bckdk as a novel post-translational regulator of MZT. Knockdown of bckdk mRNA resulted in early development defects, global ZGA deregulation, diminished H3K27ac levels, and partial impairment of maternal RNA clearance. Phospho-proteomic analysis upon Bckdk depletion revealed a decreased phosphorylation of Phf10, a chromatin remodeling factor also involved in ZGA. Importantly, reduced Phf10 phosphorylation was sufficient to account for the developmental and molecular phenotypes observed in the absence of Bckdk. In parallel, systematic screenings of post-transcriptional and post-translational regulators have provided new insights into their potential involvement in the MZT.

Altogether, our findings i) demonstrate the potential of CRISPR-RfxCas13d to reveal novel early developmental factors shedding light on the role of Bckdk as post-translational regulator of MZT and ii) contribute to enhance in vivo RNA-targeting CRISPR-Cas technology through transient approaches, promoting the expansion of CRISPR-Cas knockdown therapies.

#24 • Preventing CpG Hypermethylation in Oocytes Safeguards Mouse Development

Antoine Peters

Friedrich Miescher Institute for Biomedical Research (FMI)

In mammalian somatic and male germline cells, genomes are extensively DNA methylated (DNAme). In oocytes, however, DNAme is largely limited to transcribed regions. Most regulatory CpG-island (CGI) sequences are also devoid of repressive DNAme in somatic and germ cells of both sexes. The mechanisms restricting DNAme acquisition in oocytes, at CGIs and globally, and the relevance thereof for regulating gene expression and embryo development after fertilization have been largely unknown. We showed recently that the histone H3 lysine 36 dimethyl (H3K36me2) demethylases KDM2A and KDM2B prevent genome-wide accumulation of H3K36me2, thereby impeding DNMT3A-catalyzed de novo DNAme, including at CGI gene promoters. By recruiting variant Polycomb Repressive Complex 1 (vPRC1), they further control H2A mono-ubiquitin deposition and vPRC1-dependent gene repression. We demonstrated that aberrant Dnmt3a-dependent DNAme established in Kdm2a/Kdm2b double mutant oocytes represses transcription from maternal loci in two-cell embryos. The lethality of Kdm2a/Kdm2b maternally deficient pre-implantation embryos is suppressed by Dnmt3a deficiency during oogenesis. Hence, KDM2A/KDM2B are essential for confining the oocyte DNA methylome, conferring competence for early embryonic development. Our research implies that the reprogramming capacity eminent to early embryos is insufficient to erase aberrant DNAme from maternal chromatin, and that early development is vulnerable to gene dosage haplo-insufficiency effects.

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SESSION 1: MATERNAL AND GERMLINE REGULATION

#121 • Integrator Massively Represses Gene Transcription During Zygotic Genome Activation

William Jones, Mary Mullins

University of Pennsylvania

Maternal factors supplied to the egg during oogenesis control the earliest stages of embryonic development in all animals.¬ We identified key genes acting in the maternal control of vertebrate development in a maternal-effect forward genetic screen in the zebrafish. We identified an unexpected gene integrator subunit 6 (ints6) as functioning in dorsal-ventral patterning. We have now identified null alleles of ints6 that cause a maternal-effect mid-blastula arrest phenotype during the major wave of ZGA. Ints6 is one of 15-subunits of the Integrator complex, which binds paused RNA polymerase II and functions co-transcriptionally to cleave nascent transcripts or dephosphorylate the C-terminal domain of RNA Pol II to either repress or enhance transcription, as well as trim the 3' ends of snRNAs for their function in the spliceosome. We performed RNA-seq in ints6 maternal mutants and found thousands of differentially expressed genes, over 99% of which were upregulated in mutant embryos during the mid-blastula period. Interestingly, two-thirds of the genes upregulated are normally silent at these stages. Chromatin is known to be relatively open during these stages of animal development. We propose that Integrator plays a novel role in massively repressing gene transcription during mid-blastula stages when chromatin is relatively open and repressive chromatin is stilling being laid down. While ZGA studies have primarily focused on genome activation, our results highlight the importance of gene repression during this unique stage of development.

#47 · Single-Molecule m6A Maps Uncover How Position and Stoichiometry Shape Maternal mRNA Fate

Sarah Alshawi[1], Srihari Madhavan[1], Anna Delgado Tejedor[2], Cassandra Kontur[3], Antonio J. Giraldez[3], Eva Maria Novoa[2], Jean-Denis Beaudoin[1]

[1] *Department of Genetics and Genome Sciences, UConn Health, Farmington, CT, USA*
[2] *Centre for Genomic Regulation, Barcelona, Spain*
[3] *Department of Genetics, Yale University School of Medicine, New Haven, CT, USA*

The maternal-to-zygotic transition (MZT) shifts embryonic developmental control from maternally-provided mRNAs and proteins to the newly transcribed zygotic genome. Proper progression requires two coordinated outcomes for maternal mRNAs: activation of translation via cytoplasmic polyadenylation and timely clearance. Although N6-methyladenosine (m6A) has been implicated in maternal mRNA decay, antibody-based mapping limits site-level resolution and quantification of modification stoichiometry. We therefore use zebrafish embryos and Oxford Nanopore direct RNA sequencing to map m6A at base-level, per-molecule precision across MZT and relate modification location and stoichiometry to transcript abundance and poly(A)-tail length.

At MZT onset, genes producing at least one m6A-modified transcript exhibit reduced RNA abundance yet longer poly(A) tails and higher translation relative to genes producing only unmethylated transcripts. Within individual genes, m6A frequency decreases over MZT, and m6A-modified molecules generally carry shorter poly(A) tails than unmethylated counterparts. Effects are position-dependent: 5'-UTR and coding-sequence m6A sites associate with sustained tail shortening, whereas 3′-UTR sites show mixed tail-length effects early and shorter tails later. Machine-learning models identify DRACH motifs and proximity to stop codons and splice junctions as top predictors of m6A frequency, and co-occurrence analyses show m6A counts plateau per molecule with influential site pairs typically nearby.

Unexpectedly, loss of the m6A reader Ythdf2 causes global poly(A)-tail shortening independent of m6A status, revealing an m6A-independent role in tail metabolism. Together, our data establish m6A as a dynamic, position- and stoichiometry-dependent regulator of maternal mRNA fate and uncover Ythdf2 as a broader effector of poly(A) regulation during early vertebrate development.

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SESSION 1: MATERNAL AND GERMLINE REGULATION

#119 · Spatially Patterned Zygotic Genome Activation Fulfills Embryo Quality Control

Matthew Good

Early embryo development features autonomous, maternally driven cell divisions that self-organize the multicellular blastula or blastocyst tissue. Maternal control cedes to the zygote starting with the onset of widespread zygotic genome activation (ZGA), which is essential for subsequent cell fate determination and morphogenesis. Intriguingly, ZGA onset is often assumed to be uniform at the level of an entire embryo, we find instead that it can be non-homogenous and precisely patterned at the single-cell level. We previously demonstrated a stereotyped spatial and temporal ordering of ZGA in a model vertebrate embryo. Unknown, however, was whether this precise ZGA patterning was required for development. To address this fundamental question, we devised a strategy to spatially control cell divisions that perturb blastula embryo organization. We demonstrate the feasibility of spatially inverting the cell size pattern of embryos and find that these inverted embryos exhibit a re-oriented pattern of ZGA. Mispatterned ZGA along the animal-vegetal axis triggers embryo apoptosis, revealing that gastrula embryos have a built-in quality control system to sense inappropriate ZGA patterning, including regionalized defects in transcription onset. The quality control response is non-autonomous, dependent on an anti-apoptotic signal that suppresses cell death outside the animal hemisphere. These results reveal the requirement of properly patterned ZGA for normal development and the existence of a surveillance system for embryo quality control exquisitely tuned to the spatial and temporal ordering of genome activation and zygotic gene expression. The paper describing these results in under revision and a preprint has been posted to BioRXIV.

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SESSION 1: MATERNAL AND GERMLINE REGULATION

#64 • The Maternal PADI6 Controls DNA Methylation and Genomic Imprinting Maintenance in Preimplantation Mouse Embryos

Carlo Giaccari[1, 2], Francesco Cecere[1, 2], Angela Pagano[1, 2], Flavia Cerrato[1, 2], Andrea Riccio[1, 2]

[1] *University of Campania "Luigi Vanvitelli", Caserta, Italy*
[2] *Insitute of Genetics and Biophysics Adriano Buzzati-Traverso, CNR, Napoli, Italy*

In humans, variants of PADI6 are associated with female infertility and multilocus imprinting disturbance (MLID) in offspring. In the mouse, it was demonstrated that PADI6 is required for the storage and cytoplasmic localization of many proteins within the oocyte subcortical maternal complex, including the epigenetic factors UHRF1 and DNMT1. Moreover, maternal PADI6 depletion leads to defective epigenetic reprogramming and zygotic genome activation but not to an imprinting defect in two-cell mouse embryos, raising the possibility that imprinting disturbances arise later in development. By employing combined single-blastocyst RNAseq/BSseq and immunostaining validation in the embryos derived from Padi6P620A-mutant oocytes, we investigated the role of Padi6 in late preimplantation development. We demonstrated that embryos that overcame the two-cell stage block had a dramatic reduction in UHRF1 and DNMT1 protein levels, a decrease in H3K9me3, and whole-genome hypomethylation, including most imprinted loci and repetitive elements, at the blastocyst stage. Furthermore, these embryos showed deregulation of inner cell mass markers and defective blastocyst implantation, but no effect on trophoblast differentiation. Our results demonstrate that maternal PADI6 is a key regulator of the stability of epigenetic factors required to maintain repressive marks in late preimplantation mouse embryos. Its deficiency results in genomic imprinting defects that closely resemble those found in human patients and provide a mechanistic explanation for MLID caused by maternal PADI6 variants. Furthermore, the impairment of blastocyst implantation capacity, likely due to dysregulation of inner cell mass differentiation, provides new mechanistic insights into the control of female fertility and embryo development exerted by PADI6.

SESSION 2 EPIGENETIC REGULATION DURING ZGA

Adjusting the timing of mammalian development

Aydan Bulut-Karslioglu

Max Planck Institute for Molecular Genetics, Berlin, Germany

Pluripotency provides the foundation of embryonic development and regenerative medicine. Pluripotency is also remarkably dynamic, as it can alternate between different states and can be promptly resolved to allow lineage specification. As such, embryonic development relies on stem cell function in balance with differentiation. We investigate how this balance is maintained in coordination with the environment and the protective measures the embryos take at times of scarcity. I will talk about our efforts to decode the process of dormancy and identify the genetic, epigenetic, and metabolic mechanisms that govern this fascinating phenomenon.

#50 • Identification of Pioneer Factor Interacting Proteins to Elucidate the Molecular Mechanisms that Regulate Zygotic Gene Expression

Mina Kojima[1], Liyun Miao[1,2], Curtis Boswell[1], Antonio Giraldez[1]

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Upon fertilization, the zygotic genome undergoes transcriptional activation that coincides with developmental reprogramming. In zebrafish, pioneer transcription factors (TFs) Nanog, Pou5f3, and Sox19b (NPS) activate, and in some cases repress, many zygotic genes, but how exactly they do so remains unknown. To better elucidate mechanisms by which these TFs regulate gene expression, here we use proximity labeling and quantitative mass spectrometry to map NPS interactors in zebrafish embryos. Each TF interacts with ~80-150 proteins, and ~40 proteins interact with all three factors. Notably, chromatin remodeler complexes (including SWI/SNF and ISWI) are highly associated with NPS, and their localization depends on NPS function. This suggests that NPS make chromatin accessible for genome activation by recruiting chromatin remodelers. We also find that NPS interact with the H3.3-depositing HIRA histone chaperone and that NPS promote robust deposition of H3.3, which has been shown to promote chromatin accessibility and binding by other TFs. Furthermore, we find many other TFs interacting with NPS, including Sall4, which we also identified through an orthogonal proteomics approach to identify interactors of activating histone mark H3K27ac. Sall4 primarily co-binds to NPS-bound regions. Together with our observation that Sall4 interacts with repressive complexes, our finding that Sall4 depletion increases chromatin accessibility at many sites suggests that Sall4 serves as a repressor. Altogether, our work supports a mechanistic model in which NPS increases chromatin accessibility through chromatin remodeler recruitment and H3.3 deposition, and that subsequent Sall4 binding can counteract this activation to fine-tune the transcriptional program that controls early embryonic development.

EMBO Workshop

SESSION 2: EPIGENETIC REGULATION DURING ZGA

#80 • Mechanisms of Polycomb Domain Inheritance and Establishment Across the Maternal-to-Zygotic Transition

Shelby Blythe, Natalie Gonzaga-Saavedra, Eleanor Degen, Corinne Croslyn

Northwestern University

During early Drosophila embryogenesis, Polycomb Group (PcG) proteins establish chromatin domains that heritably mark developmental genes for long-term silencing. Although some epigenetic information is inherited from the maternal genome, PcG domains are established de novo in the zygote following the rapid cleavage cycles. Here, we integrate experimental and computational approaches to define how PcG-driven H3K27 methylation is regulated across the maternal-to-zygotic transition. Using ChIP-seq and live imaging of CRISPR-engineered PcG components, we find that PRC2-dependent H3K27me3 first appears at prospective Polycomb Response Elements in nuclear cycle 14 through a nucleation-and-spreading mechanism and is preceded by PRC1-dependent H2A ubiquitylation. Although the methyltransferase E(z) is present in nuclei throughout early cycles, nucleating factors such as Pho accumulate only after the tenth division. The pioneer factor Zelda promotes H3K27me3 and H2Aub deposition at a subset of loci in a context-dependent manner.

To interpret these dynamics, we developed a mathematical model incorporating nuclear concentrations of E(z) and Pho, replication-driven histone dilution, and allosteric regulation of PRC2 by existing methylation states. Stochastic simulations and in vivo perturbations support a mechanism in which read-write allostery preserves maternal H3K27 methylation at lower-order states, while de novo methylation of the paternal genome is limited by nucleation factor availability. Thus, early embryos functionally decouple maintenance from establishment, relying first on allosteric feedback to copy inherited chromatin states and only later on nucleation to build new PcG domains. This temporal separation provides a unified explanation for how Polycomb states are both inherited and re-established at the onset of development.

#56 · Histone Gene Replacement During the Maternal to Zygotic Transition in Drosophila

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Epigenetic pathways ensure proper development by maintaining gene expression programs that define cell identity. The chromatin states underlying epigenetic gene regulation are often established de novo during early development. In Drosophila, for example, chromatin states in early nuclear cycles are characterized by relatively few histone post-translational modifications (PTMs) and associated regulatory complexes, with canonical chromatin states emerging only upon activation of the zygotic genome. Dissecting the mechanisms of epigenetic regulation at this stage has been challenging due to extensive maternal contributions and limitations deploying conventional loss-of-function genetics. To overcome these barriers, my laboratory has developed an experimental approach that replaces the maternal pool of histones with transgenic histones, enabling direct functional interrogation of individual histone residues during early embryogenesis. By substituting endogenous histones with non-modifiable mutant variants, we can directly assess the role of specific histone residues in early gene regulation. Here, we present our latest findings using this approach to elucidate how epigenetic states are initiated during Drosophila embryogenesis.

EMBO Workshop

SESSION 2: EPIGENETIC REGULATION DURING ZGA

Short RNAs, Long Memories: When Genome Defence Becomes Gene Regulation

Pinelopi Pliota[1], Hana Marvanova[1,2], Alevtina Koreshova[1,2], Yotam Kaufman[3], Polina Tikanova[1,2], Daniel Krogull[1,2], Andreas Hagmüller[1], Sonya A. Widen[1], Dominik Handler[1], Joseph Gokcezade[1], Peter Duchek[1], Julius Brennecke[1], Eyal Ben-David[3,4*], and Alejandro Burga[1,§,*]

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One of the most fascinating examples of epigenetic regulation is the differential expression of genes based on their parent of origin—a phenomenon known as genomic imprinting. Although imprinting is essential for the proper development of a vast number of plant and mammalian species, including humans, we still know surprisingly little about its molecular origins. In this talk, I will address a fundamental question at the core of this problem: how do parent-of-origin effects on gene expression—the substrate for imprinting—evolve in the first place?

In a visionary essay written almost 30 years ago, Denise Barlow hypothesized that imprinting could be evolutionarily related to host defence mechanisms that silence parasitic elements. Unfortunately, a clear mechanistic understanding of this process is still lacking. Toxin–antidote (TA) elements are selfish genetic elements that spread in nature by poisoning non-carriers individuals and have independently evolved in multiple plant, insect, and animal lineages. By performing a genetic tour de force using the nematode Caenorhabditis tropicalis, I will present our recent discovery of how parent-of-origin effects can evolve by co-option of the piRNA pathway. More broadly, I will discuss how this emerging class of selfish elements can offer surprising lessons for developmental biologists about how animals distinguish self from non-self transcripts and non-coding roles of maternal transcripts in zygotic gene expression.

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SESSION 2: EPIGENETIC REGULATION DURING ZGA

Establishing chromatin topology and enhancer function during Zygotic Genome Activation

Eileen Furlong

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Embryonic development is largely driven by complex patterns of gene expression, which in turn is regulated by enhancers. Our previous work1,2 revealed that many embryonic enhancers that are active during cell fate specification (roughly during mid-embryogenesis in Drosophila embryos) are already in close proximity to their target promoters' hours before the gene is expressed. This suggests that pre-formed chromatin topologies in the early blastoderm embryo may prime the system for rapid activation during cell fate specification, when the enhancers presumably come in even closer proximity.

To better understand this relationship between the establishment of enhancer-promoter (E-P) proximity, chromatin topology and the initiation of transcription during early stages of embryogenesis, we have explored topologies at both earlier and later stages of embryogenesis2,3. Looking much earlier, during zygotic genome activation (ZGA), I will discuss how we are combining genetic, opto-genetic, and genomics to understand how chromatin topology is initially established, which has led to the identification of a new potential 'topology bookmarker'.

1. Ghavi-Helm Y., et al. (2014). Enhancer loops appear stable during development and are associated with paused polymerase. Nature Aug 7;512(7512):96-100
2. Pollex T, et al (2024). Enhancer-promoter interactions become more instructive in the transition from cell fate specification to tissue differentiation. Nature Genetics 11th March, 56, 686-696.
3. Cavalheiro GR, et al. (2023). CTCF. BEAF-32, and CP190 are not required for the establishment of TADs in early Drosophila embryos but have locus-specific roles. Science Adv 2023 JFeb 3:9(5): eade1085

Keywords: Developmental enhancers, Chromatin architecture, Genome topology, Enhancer-promoter looping, genetic perturbations, ZGA

SESSION 3 MZT IMPACT ON HUMAN DEVELOPMENT AND FERTILITY

Decoupling principles of mammalian body plan development (Sponsored by ISDB)

Berna Sozen

Yale University, US

#6 • A Human-Specific Mechanism Driven by Retrotransposons Impacts Preimplantation Development in a 3D Model of the Human Blastocyst

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Nearly half of the human genome consists of transposable elements. Unlike protein-coding genes, these sequences vary greatly across species, suggesting that they may contribute to species-specific biological traits. Advances in human embryo models now make it possible to investigate human-specific regulatory mechanisms during early development. A subset of these mechanisms is thought to involve endogenous retroviruses, a class of transposable elements known for their potential to serve as cis-regulatory sequences. Among them, the HERVK LTR5Hs family is unique to hominoids and is strongly expressed in human blastocysts. In this study, we employed human blastoids, a three-dimensional model of the blastocyst, to explore the developmental role of HERVK. Interfering with HERVK activity disrupted blastoid formation and affected lineage decisions, highlighting its critical role in human preimplantation development. We further discovered a single human-specific LTR5Hs insertion that acts as an enhancer for ZNF729, a primate-specific KRAB zinc-finger transcription factor. ZNF729 preferentially recognizes GC-rich motifs and controls genes involved in core cellular processes in naïve pluripotent cells, an unexpected result given the relatively recent evolutionary emergence of both LTR5Hs and ZNF729. Together, these findings demonstrate an essential function for HERVK in early human development and show how endogenous retroviruses have reshaped gene regulatory networks in a species-specific manner over evolutionary time.

EMBO Workshop

SESSION 3: MZT IMPACT ON HUMAN DEVELOPMENT AND FERTILITY

#81 • DGCR8 Haploinsufficiency Impairs Pluripotency Via Primate-Specific RNA Dysregulation

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22q11.2 deletion syndrome (22qDS) is a congenital disorder in which most clinical manifestations originate during embryonic development. The syndrome is caused by a microdeletion on chromosome 22 encompassing approximately 40 genes, including DGCR8, a core component of miRNA biogenesis. Despite its central role in post-transcriptional regulation, the impact of DGCR8 hemizygosity on human development remains poorly characterised.

Here, we generated a human pluripotent stem cell model carrying a single functional DGCR8 allele and interrogated its behaviour across naïve and primed states. At the cellular level, DGCR8+/− cells displayed increased apoptosis together with defects in self-renewal and differentiation in both states. Mechanistically, loss of one DGCR8 allele led to changes in miRNA expression, especially among a large group of primate-specific miRNAs, due to impaired miRNA processing and altered chromatin accessibility. Unexpectedly, we observed a pronounced reduction in the expression of human endogenous retrovirus class H (HERVH), a primate-specific retroelement associated with maintenance of human pluripotency. Reintroduction of primate-specific miRNAs partially restored both cellular and molecular phenotypes of DGCR8+/− cells.

Together, our results identify DGCR8 as haploinsufficient during early human development and uncover a primate-specific regulatory network in which miRNAs and transposable elements cooperate to sustain stem cell identity.

#110 • Beyond Markers: SR-FTIR Microspectroscopy Defines Macromolecular Signatures of Primed-to-Formative Transitions in Oocyte-Informed Human Reprogramming.

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Pluripotency transitions are increasingly recognized as discrete, metastable changes in cellular identity rather than a linear marker-defined progression. Yet, how these transitions are encoded at the macromolecular level—and whether different reprogramming routes traverse equivalent identity states—remains poorly defined in human systems.

Here, we apply synchrotron radiation-based FTIR microspectroscopy (SR-FTIR) as a label-free, single-cell readout of biomolecular identity to map pluripotency transitions during human somatic cell reprogramming. Using menstrual blood-derived stromal cells, we compare canonical OSK reprogramming with an oocyte-enriched factor combination (AOX: ASF1A+OCT4+SOX15) and profile cells across primed and RSET/formative conditions.

SR-FTIR spectral phenotyping reveals that AOX and OSK follow separable early reprogramming trajectories characterized by distinct macromolecular compositions and conformational states, including changes consistent with differential nucleic-acid organization, lipid composition/saturation, and protein secondary-structure signatures.

Importantly, SR-FTIR and flow-cytometry analyses uncover pronounced population heterogeneity in AOX-derived RSET/formative cultures, supporting the existence of mixed or transitional sub-states despite expression of core pluripotency markers.

Integration with orthogonal multi-omics (chromatin accessibility, methylation, and proteomic segregation) supports that these spectral identities reflect underlying regulatory remodeling rather than culture noise.

We propose SR-FTIR as an imaging-compatible, single-cell framework to quantify pluripotency state transitions and heterogeneity beyond classical markers, providing a complementary axis to genome/epigenome-centric definitions of “awakening” during developmental reprogramming.

This spectral-identity approach provides a tractable way to connect reprogramming mechanisms with early-development-like identity states and to benchmark which trajectories best approximate endogenous human pluripotent configurations.

EMBO Workshop

SESSION 3: MZT IMPACT ON HUMAN DEVELOPMENT AND FERTILITY

Synthetic Ex Utero Embryogenesis: from Naive Pluripotent Cells to Complete Embryo Models

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The identity of somatic and pluripotent cells can be epigenetically reprogrammed and forced to adapt a new functional cell state by different methods and distinct combinations of exogenous factors. The aspiration to utilize such in vitro reprogrammed pluripotent and somatic cells for therapeutic purposes necessitates understanding of the mechanisms of reprogramming and differentiation and elucidating the extent of equivalence of the in vitro derived cells to their in vivo counterparts. In my presentation, I will present my group’s recent advances toward understanding these fundamental questions and further detail our ongoing efforts to generate developmentally unrestricted human naive pluripotent cells with embryonic and extra-embryonic developmental potential. I will expand on new avenues for utilizing custom made electronically controlled ex utero platforms and optimized conditions for growing natural mammalian embryos ex utero for extended periods capturing development from pre-gastrulation until advanced organogenesis, for better studying of stem cell transitions during embryogenesis and organogenesis. I will detail how the latter platforms offered an exclusive technical platform to demonstrate and unleash the self-organizing capacity of mouse naïve PSCs to generate post-gastrulation synthetic Bona Fide synthetic whole developmental models with both embryonic and extraembryonic compartment ex utero, as well as our ability to extend these findings with naïve human PSCs and generate complete structured day 14 human developmental models and beyond. Collectively, I will be highlighting prospects for new platforms for advancing human disease and embryogenesis developmental modelling.

SESSION 4 IMAGING MECHANISMS OF MZT

#106 · Molecular Kinetics of Gene Regulation During Embryonic Development

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During embryonic development gene expression patterns progressively emerge as cell fates are determined and the embryo takes form. The emergence of these expression patterns is coincident with changes in the spatial distributions of transcription factors, chromatin remodelers, and transcriptional machinery within nuclei. The mechanisms that drive these changes in spatial distributions and their functional implications are not well understood. I will discuss the application of high-resolution light-sheet microscopy and single-molecule tracking in live pre-implantation mouse embryos and blastoderm stage Drosophila embryos to understand the emergence and functional implications of nuclear protein distribution patterns during development. I will show data and simulations on the molecular diffusion and binding kinetics that underlie the appearance of high-local concentrations of transcription factors, repressive complexes, and transcriptional machinery, and the functional impact of these high-local concentrations on transcription regulation during development. Finally, I will discuss how our new insights on how the molecular kinetics of regulatory nuclear proteins drive changes in their spatial distributions challenge current models of transcription regulation and nuclear organization.

#82 · Chromatin Tracing Reveals Genome Organisation from Sub-Megabase to Chromosome Scales in Early Mouse Embryos

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Understanding how chromatin organisation emerges during early development remains a central question in genome biology. Here, we present a sequence-specific chromatin tracing pipeline that directly visualises chromatin architecture in whole-mount mouse embryos at single-cell resolution, enabling quantitative analysis from whole chromosomes to sub-megabase domains.

Prior to zygotic genome activation, chromosomes adopt an unusually expanded and intermingled configuration and fail to form discrete chromosome territories. As preimplantation development proceeds, chromosome territories are progressively established, coincident with a gradual increase in long-range chromatin folding. At smaller length scales (<1 Mb), chromatin loops and topologically associating domains (TADs) are rare in zygotes and emerge slowly over developmental time. Higher-order features characteristic of somatic cells, including nested looping and fractal globule–like polymer configurations, are robustly established only by the late blastocyst stage.

Strikingly, A/B compartment–associated differences in chromatin compaction are already evident in zygotes and extend to sub-megabase scales, at a stage when chromosome territories and TADs are not yet established. In somatic cells, such compartment-driven differences are largely restricted to megabase scales. This temporal ordering suggests that compartmentalisation is established early and, in the absence of robust loop extrusion, may dominate chromatin organisation even at sub-megabase lengths during early preimplantation development.

Together, this work provides a quantitative framework for understanding how genome architecture is progressively established during the earliest stages of mammalian development.

A role for nuclear organization in transcription regulation

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There is accumulating evidence for a new gene expression paradigm, in which genes and transcription factors cooperate to create specialized transcription bodies. How such bodies form, and how they impact transcription, however, is often unclear. We have taken advantage of two prominent transcription bodies that mark the onset of transcription during zebrafish embryogenesis to address some of these questions. Here, I will talk about our latest results regarding the formation and function of transcription bodies.

EMBO Workshop

SESSION 4: IMAGING MECHANISMS OF MZT

#9 • Nanoscale Imaging Reveals RNA-Dependent Chromatin Compaction During Early Heterochromatin Formation

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University of California, San Francisco

The spatial organization of chromatin is thought to play a central role in regulating gene expression during development, yet how chromatin compaction emerges in vivo and how it relates to transcriptional regulation remain poorly understood. Here, we investigate the mechanisms underlying chromatin organization during zebrafish embryogenesis. We developed an improved implementation of Chromatin Expansion Microscopy (ChromExM) that achieves ~18-fold linear expansion while increasing experimental throughput and reproducibility, overcoming key technical bottlenecks. In parallel, we implemented a machine-learning–based image analysis pipeline to quantitatively assess chromatin organization. This revealed that chromatin undergoes a stepwise transition from a homogeneous organization during ZGA to a heterogeneous state containing discrete nanoscale compaction domains as cells begin differentiation. We next investigated heterochromatin marks as a potential molecular driver of this chromatin compaction. Although we observe that the repressive histone modification H3K27me3 accumulates during the same developmental window, genetic and pharmacological disruption of Polycomb Repressive Complex 2 (PRC2) revealed that H3K27me3 is dispensable for chromatin compaction. Unexpectedly, loss of H3K27me3 failed to decompact the chromatin and was accompanied by transcriptional upregulation of PRC2 target genes, functionally decoupling biochemical repression from physical chromatin condensation. Given this association between transcriptional activity and chromatin compaction, we next tested whether transcription itself contributes to global chromatin organization. We show that transcriptional activity, and specifically nuclear RNA, are key determinants of chromatin compaction. Together, these findings support a model in which nascent RNAs drive chromatin compaction through physical partitioning, suggesting a new mechanism governing the emergence of chromatin organization during development.

EMBO

Spatiotemporal Regulation of Translation during Zygotic Genome Activation

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In all animals, development starts with rapid and large-scale changes in gene expression, occurring during Zygotic Genome Activation (ZGA). While transcriptional dynamics and mRNA turnover have been extensively characterized, translational control remains much less understood during ZGA. We address this gap by quantitatively dissecting translational regulations in Drosophila embryos. Thanks to the SunTag system, we directly visualize translation of individual mRNAs and generate spatial maps of translation efficiency for key transcription factors involved in embryonic patterning. During ZGA, translation is highly spatially organized, with identical mRNAs exhibiting higher translational efficiency in the basal cytoplasm than in the apical region. Correlative ultrastructural mapping generated by scanning electron microscopy shows that the basal cytoplasm is enriched in polysomes and endoplasmic reticulum, defining a translation-permissive microenvironment. We further uncover a global drop in translation efficiency prior to gastrulation. During ZGA, zygotic ribosome biogenesis has barely begun, forcing the embryo to rely on a finite maternal ribosome pool. Our data suggest that the surge of zygotic transcription can titrate this pool, making ribosomes a limiting resource in early embryos. To assess whether this constraint permits selective translation, we quantified the translation kinetics (initiation and elongation rates) of distinct mRNAs using lattice light sheet imaging, single-particle tracking and mathematical modeling. Despite substantial heterogeneity in translation rates across individual mRNAs, we observe little evidence of gene-specific bias. Our work reveals translation as a spatially organized, resource-limited process that fine-tunes protein production during ZGA. We propose that similar principles may operate in other biological contexts characterized by abrupt surges in transcription under limited translational capacity, such as regeneration, viral infection, or oncogenesis.

EMBO

Spatio-temporally regulated maternal RNA decay factor essential for zebrafish development

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Maternal mRNA decay is essential for the Maternal-to-Zygotic Transition (MZT), yet its impact on spatial regulation has remained largely unexplored. We identify Cth1 as the first maternally encoded RNA-decay factor regulated in a spatiotemporal manner outside the germline in zebrafish, driven by conserved 3′UTR cis-elements. RfxCas13d-mediated depletion of maternal cth1 revealed early developmental defects that could not be assessed with SpyCas9 mutants due to infertility from impaired gametogenesis.

Mechanistically, Cth1 acts independently of zygotic genome activation, recognizing AU-rich motifs in maternal 3′UTRs to promote deadenylation and decay; deletion of these motifs or depletion of Cth1 stabilizes both reporter and endogenous transcripts. These findings establish Cth1 as a key component of the maternal program and provide the first direct evidence that spatially regulated mRNA decay, outside the germline, contributes to early vertebrate development.

Keywords: Spatio-temporal maternal RNAs, CRISPR-Cas13d, Zebrafish, MZT .

#36 • Nuclear Speckles Link Nuclear Organization to Gene Regulation in the Early Embryo

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Early embryonic development requires the timely activation of gene expression and subsequent RNA processing programs. How these processes are spatially coordinated within the 3D nuclear space of the developing embryo remains poorly understood. Nuclear speckles are nuclear bodies enriched in RNA processing and splicing factors that are present in most mammalian cells, yet whether they play a role during early developmental transitions has not been addressed.

Using mouse embryos as a model, we show that nuclear speckles form concomitantly with embryonic genome activation and undergo dynamic remodeling across cleavage stages. Perturbation of core speckle components disrupts speckle organization and leads to developmental arrest prior to the first lineage specification. While global transcription remains largely intact in speckle-disrupted embryos, transcriptomic and proteomic analyses reveal selective misregulation of specific gene programs. These changes are accompanied by reduced protein synthesis capacity. Spatial analyses further show that subsets of these developmentally regulated genes localize in proximity to nuclear speckles during early embryogenesis, and that loss of speckle integrity alters this spatial association.

Together, our findings identify nuclear speckles as essential organizers of gene expression programs after fertilization, linking RNA processing to translational output. Importantly, our work reveals how nuclear architecture supports developmental progression during the transition from totipotency toward lineage commitment

EMBO Workshop

SESSION 5: RNA REGULATORY MECHANISMS DURING MZT

#109 • Deciphering Regulation and Functional Conservation of Dormant Ribosomes

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Cellular quiescence is a reversible state of dormancy where energy-intensive processes like transcription and translation are repressed. As translation is one of the most energy-demanding processes, ribosomes play a central role in quiescence: their activity is suppressed, but a stable pool is maintained to allow rapid reactivation. We recently identified a conserved set of four factors that stabilize and repress ribosomes in zebrafish and Xenopus eggs, generating dormant ribosomes (Leesch et al., 2023). While the composition and function of dormant ribosomes in fish and frogs are known, their reactivation mechanisms and relevance in mammals are unclear.

To investigate these open questions, we used FACS-based genome-wide CRISPR screens, genetic and biochemical studies to identify factors able to release the key dormancy factor DAPL1 from the ribosome. In my talk, I will present the results from our studies, highlighting a critical role of the eIF3 complex as a ribosome rescue factor. Our findings suggest that this rescue activity may represent a conserved and so-far largely overlooked mechanism essential for the reactivation of dormant ribosomes. To study mammalian DAPL1's function in a physiologically relevant context, we investigated its role in murine naïve T cells where DAPL1 is endogenously expressed at high levels.

Our studies reveal a conserved role for dormant ribosomes in supporting quiescence from fish to mammals, uncovering a novel layer of translational regulation in quiescent cells.

#94 • Maternal RNA Localization Through Intrinsically Disordered Proteins

Jieon Lee, Andrea Putnam

University of Wisconsin-Madison

Maternal RNAs are transcribed during oogenesis, stored, and drive the earliest stages of embryonic development prior to zygotic genome activation. In many animals, maternal RNAs are enriched within membraneless ribonucleoprotein condensates during oogenesis and early embryogenesis. In C. elegans, a subset of maternal RNAs localizes to germ granules, condensates that contribute to the establishment of germ cell fate. Here, we investigate how intrinsically disordered proteins regulate maternal RNA localization during early embryogenesis. We find that enrichment of maternal RNAs within germ granules correlates with the RNA-binding properties of intrinsically disordered regions. By engineering intrinsically disordered regions with altered RNA-binding affinity, we modulate RNA localization and uncover a direct relationship between RNA enrichment, granule organization, and fertility. Finally, we show that RNA binding is also required for the proper localization of RNA-binding intrinsically disordered proteins themselves, revealing a reciprocal relationship between RNA binding and condensate organization. Together, these findings identify RNA binding by intrinsically disordered proteins as a key determinant of maternal RNA localization and germ granule function during early development.

EMBO Workshop

SESSION 5: RNA REGULATORY MECHANISMS DURING MZT

Control of histone biogenesis by a sperm-borne tRNA-fragment

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Over the last decade, it has become increasingly appreciated that epigenetic information carried in gametes at fertilization can impact downstream embryogenesis and even organismal phenotypes. To dissect how preconception environments inform the gamete epigenome, and whether these changes impact early development, we use paternal Low Protein Diet (LPD) paradigm in a mouse model. Our work indicates that male consumption of LPD results in changes in sperm small RNA payload, which, upon delivery to the zygote, regulates the expression of EGA-associated transposable element MERVL. Mechanistically, we show that a single small RNA species, 5'tRNA fragment GCC, plays an important role in histone pre-mRNA processing and by extension, chromatin packaging, and is (variably) sufficient to phenocopy the LPD-induced F1 adult hepatic phenotypes. In my talk, I will be presenting our unpublished work focusing on the role of MERVL expression in the embryo, highlighting comparisons between epigenome editing technologies and the impact of murine strain on downstream molecular and phenotypic readouts.

SESSION 6 ENHANCER REGULATION DURING GENOME ACTIVATION

#14 · Functional Characterization of DUX-Induced Transcriptional Clusters During the Exit from Totipotency

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The transcription factor DUX is expressed in the mouse 2-cell (2C) stage embryo and participates in the induction of the totipotent-associated transcriptional program. The silencing of this program following further cleavage is required to exit from totipotency. DUX expression in mouse embryonic stem cells (ESC) triggers transcriptional and chromatin-associated features of 2C embryos that correlate with the formation of nuclear transcriptional clusters. Biomolecular clusters play a fundamental role in regulating transcription and cell fate determination. However, the role of DUX-induced transcriptional clusters is unclear. We first examined the role of the histone acetyltransferases (HATs) EP300/CBP in cluster formation, as DUX directly recruits them to mediate gene activation. dTAG-mediated degradation of CBP in EP300-knockout ESC demonstrated the relevance of HATs in the formation of DUX-dependent clusters. Next, we used a TurboID-based proximity labeling method to identify the proteins recruited to these clusters and found enrichment for 2C-associated proteins. We hypothesized that progressive accumulation of proteins at these clusters could induce phase separation, promoting transcriptional silencing over time. By using fluorescence recovery after photobleaching (FRAP) to examine the biophysical properties of clusters, we observed progressive phase separation over the life of the clusters. Furthermore, we used the MS2/MCP system in ESC to visualize live transcription of the DUX-induced genes from the ZSCAN4 cluster. We determined that, despite a quick activation following DUX expression and cluster formation, ZSCAN4 transcription is quickly silenced. In summary, our data suggest that initial transcriptional activation mediated by DUX-induced clusters is followed by phase separation and gene silencing.

#39 · Maternal Huluwa Regulates Postfertilization Microtubule Array Organization for Asymmetrical Transport of Dorsal Determinants in Zebrafish

Xin Liu, Anming Meng

School of Life Sciences, Tsinghua University

The dorsal organizer, essential for vertebrate embryonic axis formation, is induced by dorsal determinants via postfertilization microtubule-mediated transport. Maternal Huluwa (Hwa) has been identified as an essential organizer inducer in zebrafish and frogs, functioning at midblastula stages to activate β-catenin signaling in the preorganizer. It remains unknown if maternal Hwa functions at or before fertilization. Here, we report that maternal Hwa protein is critical for organizing the vegetal parallel microtubule array immediately after fertilization in zebrafish. Hwa protein and mRNA are enriched at the vegetal pole and facilitate microtubule network formation, enabling asymmetrical transport of dorsal determinants. Loss of maternal Hwa disrupts this microtubule architecture and abrogates mRNA transport, revealing a self-reinforcing mechanism where Hwa regulates its own asymmetrical distribution. Our findings establish a dual-phase model of dorsal specification: Hwa initially governs symmetry breaking through postfertilization microtubule organization and later on activates β-catenin signaling at blastula stages. This work provides fundamental insights into how the key maternal factor regulates the organizer and body axis formation at different developmental stages.

#59 · Precision vs Plasticity in Enhancer-Driven Cell Fate Specification

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Early embryogenesis requires precise regulation of cell fate specification immediately before gastrulation, yet the molecular mechanisms guiding this transition remain incompletely understood. Using Drosophila embryos, we uncover a previously uncharacterized regulatory mechanism that provides both stability and precision to early gene expression, highlighting how embryonic enhancers act as “mentors of the genome” to guide cell specification and differentiation. We focus on a conserved network centered on the pioneer factors (PFs) Odd-paired (Opa/ZIC3), GAGA factor (GAF) together with Suppressor of Hairless (Su(H)/RBPJ), integrating chromatin accessibility, histone modifications, transcription factor binding, and single-cell multiomics datasets to dissect early enhancer function.

We identify two mechanistically distinct classes of early enhancers. The first comprises distal, Opa-dependent elements that are initially inaccessible and require Opa to overcome Su(H)-mediated repression and H3K27me3 silencing. The second class consists of proximal, feedback-primed accessible enhancers that remain accessible independently of pioneer factors due to RNAPII preloading and promoter proximity. These enhancers function as autonomous regulatory hubs, maintaining accessibility and reinforcing cell identity within H3K27me3 and Polycomb-enriched chromatin.

Our results redefine pioneer factors as stabilizers of chromatin accessibility rather than initiators and reveal a dual-mode enhancer mechanism that balances transcriptional precision with developmental plasticity. This decoupling of PF binding from chromatin accessibility suggests a regulatory strategy that leverages pre-established chromatin states, inherited accessibility, and context-specific cofactors.

This mechanism represents a conserved strategy for early developmental gene regulation, offering a framework for understanding how enhancers integrate multiple regulatory inputs to stabilize cell fate decisions and guide embryonic differentiation.

EMBO Workshop

SESSION 6: ENHANCER REGULATION DURING GENOME ACTIVATION

A Single Molecule View of Zygotic Genome Activation

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I will present the results of recent live imaging assays and single molecule whole genome methods (Fiber-Seq and DAF-Seq) aimed at understanding long-range enhancerpromoter interactions and regulatory switches in nc14 Drosophila embryos. Acute depletion of Nipped-B (NIPBL) results in diminished expression of Charybde transcription by a distal enhancer located nearly 250 kb upstream of the gene. A combination of genetic epistasis and polymer simulations suggest a “scan and snag” model, whereby directional cohesin-driven enhancer scanning promotes diffusion-mediated tethering interactions to produce successful enhancer-promoter contacts and transcriptional activation.

Most genome annotations depend on short DNA sequence reads of mixed cell populations. We have begun to use single molecule methods and long DNA sequence reads to link different states of transcription with nucleosome free regions of regulatory DNAs on the same chromatin fibers. Machine learning methods establish a predictive correspondence between the two. I will present evidence that developmental enhancers are often composed of separate pioneer and activator modules. In some cases, pioneer modules exhibit a temporal switch from Zelda to GAF as transcriptional bursts give way to more uniform patterns of gene activity.

EMBO

#107 · Establishing the Epigenome when Life Begins

Wei Xie

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Drastic transcription and epigenetic reprogramming occur during mammalian early embryogenesis. Deciphering the molecular events underlying these processes is crucial for understanding how life really begins. Probing these questions was previously hindered by the scarce experimental materials that are available from early embryos. By developing a set of ultra-sensitive chromatin analysis technologies, we investigated epigenetic reprogramming during early mouse development for chromatin accessibility, histone modifications, and 3D chromatin architecture. These studies unveiled highly dynamic and non-canonical chromatin regulation during the maternal-to-zygotic transition. Recently, we also identified a number of key transcription factors (TFs) that govern mammalian zygotic genome activation (ZGA) and the first cell fate commitment. However, how the embryonic transcription program is established amid the non-canonical, immature epigenome, and how the embryonic epigenomes are correctly restored still remain enigmatic. In this talk, I will present data on how the embryonic epigenomes including DNA methylation, histone modifications, and the 3D chromatin organization are properly restored in early mammalian development.

EMBO Workshop

EMBO

#78, #120 · Three-Dimensional Genome Reorganization Foreshadows Zygotic Genome Activation in Drosophila

Noura Maziak[1, 2], Yuchen Zhang[3, 4], Fabian Groll[1, 2], Haley E. Brown[5], Alla Madich[6], Yadwinder Kaur[5], Melissa M. Harrison[5], Jian Zhou[3, 4], Juan M. Vaquerizas[1, 2]

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How chromatin conformation relates to chromatin state remains a central challenge in genome regulation. Here we present Pico-C, a low-input Micro-C approach that enables high-resolution, temporally resolved three-dimensional genome mapping during early Drosophila embryogenesis. Contrary to a prevailing view of a disorganized genome before zygotic genome activation (ZGA), we uncover a dynamic and ordered emergence of chromatin loops during pre-ZGA nuclear cycles. Spatial autocorrelation analysis points to context-dependent regulatory influences on chromatin.

Notably, inhibition of transcriptional elongation has site-specific effects, retaining some early loops while weakening insulation at active promoters, suggesting distinct regulatory dependencies. Machine learning models trained on sequence features identify orthogonal, motif-specific contributions to architecture. Co-depletion of the pioneer factors Zelda and GAF leads to factor-specific perturbations in chromatin architecture, further highlighting a modular regulatory logic in genome establishment. Together, our findings reveal that early genome organization is orchestrated by an interplay of overlapping yet separable regulatory inputs.

#103 • Chromatin Gold Electron Microscopy (ChromGEM) Reveals Ultrastructural Hallmarks of Transcribing and Silent Chromatin During Mammalian Genome Activation

Alice Sherrard[1], Caroline Hoppe[1], Liangwen Zhong[1], Scott Youlten[1], Srikar Krishna[1], Fiona Sievers[1], Curtis Boswell[1], Stephen Cross[2], Berna Sozen[1], Antonio Giraldez[1]

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Chromatin architecture underlies genome regulation, yet how chromatin ultrastructure influences function remains poorly understood. Here we developed ChromGEM, a nanogold-enabled electron tomography method to visualize chromatin and associated proteins or RNA, along with ChromAtlas, a multiscale machine-learning framework to quantify chromatin architecture in situ. Using these approaches, we analyzed nucleosome organization during mouse embryo genome activation, a key window of epigenetic remodeling. We identified six distinct chromatin subtypes defined by their packing, topology, and nucleosome arrangement, revealing previously unresolvable structural diversity during early mammalian development. Contrary to the classical view that loosely packed euchromatin is transcriptionally active, nanogold labeling of nascent transcription revealed that low-density regions were rarely engaged in transcription. Instead, active transcription occurred within dense, disorganized filament networks, revealing an unexpected relationship between chromatin compaction and transcriptional activity. Strikingly, these active transcription sites were consistently bordered by highly organized heterochromatin-like domains, composed of tightly packed chromatin chains and dense nucleosome aggregates labeled by the heterochromatin-associated protein Hp1a. With this nanoscale resolution, we reveal diverse chromatin states and structural interplay between active and repressive chromatin, which may help coordinate gene regulation by constraining interactions during early transcriptional activation. Together, our approaches enable integrated, multiscale analysis of chromatin structure in situ to visualize heterogeneous chromatin states beyond binary compartment models.

EMBO Workshop

SESSION 7: STRUCTURAL REMODELING IN RNA AND CHROMATIN DURING MZT

#5 • Partitioning of Transposon-Rich Maternal DNA Via Chromatin Compaction Is a Prerequisite for Embryonic Gene Activation

Duy Nguyen[2], Tara McDonnell[3], Pakinee Welte[2], Michael Welte[2], Andrea Pauli[4], Vince Tropepe[3], Patrick Murphy[1, 2]

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During the maternal-to-zygotic transition (MZT), control of growth and development shifts from maternally supplied factors to zygotic transcription, accompanied by extensive genome-wide chromatin reorganization. Although several factors involved in activation of zygotic genes have been identified, mechanisms underlying genome-wide partitioning of chromatin landscapes remain poorly defined. We therefore profiled chromatin accessibility changes across early and late MZT stages in zebrafish, and uncovered two concurrent, yet functionally distinct reprogramming processes. Developmental gene promoters become progressively more open, as anticipated – gaining competence for RNA polymerase II (Pol II) recruitment, but over this same period vast intergenic domains – enriched for repetitive elements and transposons, underwent marked chromatin compaction. Surprisingly, numerous loci within these repetitive domains were initially bound by RNA Pol II early in MZT, prior to activation of genic loci, but as s compaction advanced, Pol II was released from repetitive elements and redistributed to newly accessible gene promoters. This re-localization coincided with the onset of robust zygotic transcription. We also find that the histone methyltransferase G9a is essential for this chromatin-based transition to occur. In G9a-deficient embryos, repetitive genomic regions failed to become compact, Pol II remained aberrantly retained at these intergenic domains, and proper gene promoter loading of Pol was impaired. Together, our findings reveal that selective compaction of transposon-rich maternal DNA is a critical prerequisite for directing Pol II to developmental genes during MZT.

#26 • Uncovering the Role of Chromatin Material Properties in Zygotic Genome Activation

Joana M N Vidigueira[1,2], Jan Brugués[1,2], Hiroshi Kimura[3]

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Zygotic genome activation (ZGA) is a key developmental event in the early embryos of sexually-reproducing species during which transcription of zygotic genes first occurs. This process involves large-scale chromatin reorganisation, which is determined by biochemical modifications of chromatin. Recent studies in a range of systems have started to highlight how such biochemical events are tightly linked to the material properties of chromatin, an interplay which is important for dictating biological function. Although ZGA has been thoroughly characterised biochemically, the role of chromatin mechanics in this process remains unexplored. I hypothesise that chromatin material properties play a role in ZGA. To characterise changes in chromatin material properties, I am using optical tweezers to perform active microrheology in live zebrafish embryos. These force measurements are done simultaneously with spinning disk confocal microscopy of chromatin (GFP-labelled histones) and active transcription sites (antibody fragments targeting active RNA polymerase II), allowing a spatiotemporal description of transcription activation and chromatin material properties. My results thus far indicate there might be chromatin stiffening during genome activation. Going forward, I plan to combine the microscale force measurements and imaging with perturbations of the physiological properties of chromatin to systematically examine how changes in chromatin material properties might enable transcription initiation. By dissecting ZGA from a materials perspective, I aim to uncover the physical mechanisms regulating transcription activation in early development. These findings will lay out an important foundation for our understanding of ZGA.

EMBO Workshop

SESSION 7: STRUCTURAL REMODELING IN RNA AND CHROMATIN DURING MZT

#105 • P-Bodies During MZT: Functional Compartments or Incidental Condensates?

Geraldine Seydoux, Brennan Danlasky

Johns Hopkins University

P-bodies are condensates that enrich components of the cytoplasmic mRNA decay machinery. I will describe our characterization of P-bodies during the MZT in C. elegans. P-bodies assemble and accumulate deadenylated transcripts coincident with the onset of maternal mRNA decay. smFISH reveals that certain maternal mRNAs concentrate in P-bodies during decay, but the extent of P-body enrichment varies greatly between mRNA species. Mutants that block or delay the enrichment of key P-body proteins in P-bodies delay mRNA decay only for a small subset of maternal mRNAs. Our findings so far suggest that wild-type P-bodies are dispensable for the turnover of most maternal mRNAs during MZT. I will discuss the possibility that P-bodies at this stage are incidental, non-functional condensates that arise when decay mRNPs exceed their solubility limit during MZT.

SESSION 8 EVO DEVO AND NOVEL MODEL SYSTEMS IN THE MATERNAL TO ZYGOTIC TRANSITION

Non-canonical histone modification during the maternal-to-zygotic transition

Miler Lee

University of Pittsburgh, US

#13 • A Balancing Act Between a Transcription Factor and Its Binding Site Facilitates Chromatin Organization

Haley Brown, Melissa Harrison

University of Wisconsin School of Medicine & Public Health, Department of Biomolecular Chemistry

Following fertilization, the chromatin architecture of the zygotic genome is established de novo, partitioning the nucleus into active and silent regions. Failure to properly organize the genome results in developmental defects or death. To provide fundamental insights into how chromatin is organized, we focus on the early stages of Drosophila embryogenesis. The transcription factor GAGA Factor (GAF) promotes transcription of genes in active compartments. Simultaneously, GAF binds and silences satellite repeats. Reflecting this dual role, GAF is non-uniformly distributed in the nucleus – present in diffuse populations in active compartments and concentrated foci in inactive compartments. Yet the mechanism enabling this single factor to partition the nucleus remains poorly understood. GAF may repress at high local concentration and activate at lower concentrations, leading us to propose that at the genome scale GAF levels are precisely balanced with the number of GAF-binding sites. To test this, we altered the ratio between the levels of GAF and the highly repetitive regions it binds. To do so, we leveraged the evolutionary divergence between two Drosophilids that differ dramatically in the abundance of the GAF-bound satellite repeat. We used high-throughput sequencing on hybrid embryos to determine the effect of reducing repeat abundance on chromatin accessibility (ATAC-seq) and gene expression (total RNA-seq). We found that perturbing the ratio of GAF to its binding site results in increased chromatin accessibility over transposable elements that are normally silenced. Our data supports a model in which creating a surplus of GAF disrupts chromatin structure and transcriptional dynamics.

#91 • A Comparative Epigenomic Annotation for Regulatory Elements in the Allotetraploid Genome: Mapping of the Non-Coding Functional Elements During Cyprinus Carpio Embryo Development

Ada Jimenez-Gonzalez[1,2], Alba Madrero Pardo[3], Damir Baranašić[3,4,5], Ferenc Mueller[1], et al.

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Common carp (Cyprinus carpio) is an allotetraploid cyprinid formed by hybridisation ~13 Myr ago, retaining two chromosome-resolved subgenomes (A and B) with distinct gene-expression profiles. To dissect how duplicated cis-regulatory landscapes shape embryogenesis, we built a developmental regulatory atlas by integrating ATAC-seq, CAGE-seq, and ChIP-seq (H3K27ac, H3K4me3, H3K27me3) across key stages, together with a dense RNA-seq time course from unfertilised egg to pre-hatch.

Across 1:1 homologous regulatory regions, we find a strong developmental shift in activity constraint. Immediately after zygotic genome activation, homologous regions frequently show divergent activity between subgenomes. In contrast, as development approaches the phylotypic stage, homologous pairs display markedly higher functional equivalence, with increasingly constrained and concordant activity between A and B homologous regulatory regions, consistent with stronger selection to maintain both regulatory copies during the most canalised period of embryogenesis. This shift is more pronounced for enhancers than for promoters, indicating higher functional conservation of promoters and greater enhancer flexibility early in development. Moreover, homologous regulatory elements in subgenome B consistently show weaker coupling to their A counterparts, supporting subgenome B dominance.

Comparative projection to zebrafish provides an external benchmark for conserved activity: ~69–70% of carp accessible regions can be projected to zebrafish, and ~23–25% map to regions supported by both carp subgenomes and zebrafish. Together, these results reveal progressive tightening of homeologous cis-regulation through development and identify enhancers as key substrates for early developmental divergence after genome duplication.

EMBO Workshop

SESSION 8: EVO DEVO AND NOVEL MODEL SYSTEMS IN THE MATERNAL TO ZYGOTIC TRANSITION

#68 • Beyond the CpG: Dissecting the Functions of Atypical DNA Methylation During Early Vertebrate Development

Thirsa Brethouwer[1], Alvaro Perez Martos[1], Laura Romero Montañez[1], Daniel Summerer[3], Maria Almuedo Castillo[1], Ozren Bogdanovic[1,2]

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Cytosine DNA methylation is widespread in animal genomes and occurs predominantly at CG dinucleotides (mCG). While the roles of mCG, such as in genomic imprinting and genome stability, are well established, non-CG DNA methylation (mCH) remains poorly understood. In most vertebrate tissues, roughly 80% of CGs are methylated, whereas mCH levels are generally low. mCH is most prevalent in neural tissue, oocytes, and embryonic stem cells, and has been linked to neurodevelopmental disorders. Using CRISPR/Cas9 genome editing in zebrafish, we are functionally interrogating the contribution of mCH and its writer, Dnmt3ba, to early development in order to define the developmental requirements for proper mCH patterning. Building on these findings, we have generated stable dnmt3ba knockout lines which, in zebrafish, display redundancy in the maintenance of mCG patterns. These unique genetic contexts enable us to selectively perturb mCH deposition without globally disrupting canonical CG methylation. Strikingly, dnmt3ba knockouts exhibit widespread transcriptional dysregulation, significantly reduced cell size, and defects in the execution of zygotic genome activation (ZGA), suggestive of a critical role for mCH in coordinating early embryonic transcriptional programs. In addition, we observe a strong anticorrelation between mCH and the repressive histone mark H3K9me3, pointing to a potential complementary and stage-specific relationship between these epigenetic mechanisms during early development. Together, these findings support a model in which mCH represents a distinct and developmentally essential epigenetic layer that contributes to embryonic genome regulation independently of canonical CG methylation.

EMBO

Evolutionary landscapes of zygotic genome activation across animals

Manuel Irimia

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During early animal embryogenesis, control over gene expression transitions from maternally deposited products to newly transcribed zygotic RNA. This process, termed zygotic genome activation (ZGA), is universal and essential but remains poorly characterized beyond a handful of model species. Here, we generated a comprehensive transcriptomic atlas of early embryogenesis from 61 animal species, spanning 13 phyla. By applying a unified computational framework, we systematically inferred the timing of ZGA across species. We uncover a large variation in ZGA timing, but find that a proxy for nuclear-to-cytoplasmic ratio, the log of genome size / egg volume, robustly predicts the onset of genome activation. Comparative analyses of the properties of zygotic genes showed that they are shorter and with fewer introns, enriched in functions related to RNA processing and gene expression, and phylogenetically younger than maternal genes. Altogether, our findings suggest that ZGA is universally timed by the stoichiometry between DNA content and specific maternally deposited factors, and this activation involves a highly flexible transcriptomic program that follows a deeply conserved molecular logic.

EMBO Workshop

EMBO

Bridging the gap between oocyte development and MZT

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Over 100,000 babies are born each year in the United States using IVF. However, in 2024 there were 449,000 IVF cycles performed, indicating that most IVF cycles fail. Although embryo aneuploidy shows an age-associated increase, another major cause of IVF cycle failure is cleavage-stage embryo arrest concomitant with failure to activate the embryonic genome. Although it is known that high doses of FSH during ovarian stimulation can lead to poor embryo development during IVF, the molecular mechanism underlying this effect was unclear. To investigate the effect of ovarian stimulation on resultant embryo arrest, we developed a mouse follicle culture system in which we can carefully tune the dosage of follicle stimulating hormone (FSH). We found that low but adequate doses of FSH during in vitro follicle culture (IVFC) led to high rates of follicle survival, antral structure formation, oocyte maturation, egg activation, and embryo development. However, higher doses of FSH in IVFC result in no difference in follicle survival or antrum formation but severely compromise oocyte maturation, egg activation, and embryo development. Surprisingly, high doses of FSH led to mid-zygotic genome activation (ZGA) failure in embryos derived from IVFC eggs. To investigate the molecular mechanism governing high-FSH induced embryo development impairment, we first tested whether the somatic cells surrounding the oocyte are affected during IVFC. Optimal IVFC leads to a subtle change in granulosa cell subtype while high FSH dose IVFC results in a precocious muralization of granulosa cells at the expense of mitotic/atretic/pre-antral cumulus granulosa cell subtypes, indicating that high FSH levels accelerate granulosa cell differentiation during follicle growth. Next to test how granulosa cell identity changes affect oocyte energy levels, we quantified mitochondrial membrane potential—a surrogate measure of intrinsic oocyte energy production. Higher FSH levels leads to lower oocyte mitochondrial membrane potential compared to low dose FSH IVFC, indicating that one effect of accelerated granulosa cell differentiation is an anergic oocyte phenotype. We speculate that the higher doses of FSH experienced in ovarian stimulation can decrease oocyte energy levels and cause pleiotropic effects on oocyte processes such as maturation and egg activation and subsequently impact embryo development through incomplete genome activation. Our mechanistic insights into how follicle development affects oocytes and the resultant embryo have implications for human clinical practice of ovarian stimulation during IVF.

#2 • A ZGA Checkpoint Revealed by Mammalian Interspecific Hybrids

Yang Yu

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Zygotic genome activation (ZGA) marks the transition of early embryos from maternal control to embryonic development programs. Yet, how ZGA timing is controlled remains elusive. Here we created hybrid embryos from diverse mammalian species to address this question. These hybrids can develop up to the 8-cell stage, in which major ZGA occurs in a "first come first serve" manner. Both parental genomes are activated simultaneously, with significant transcription of transposons and housekeeping genes. Through comparative analysis of mouse gene activation in various hybrid contexts, we identified Eif1a-like family proteins as key players in ZGA, influencing a large portion of ZGA genes and potentially acting as licensing factors for zygotic genome activation. Detailed mechanistic investigation will be presented.

EMBO Workshop

SESSION 9: GENE REGULATION DURING GENOME ACTIVATION IN MAMMALS

DNA damage response in the early mammalian embryo: friend or foe?

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Following fertilization, the mammalian embryo undergoes a dramatic epigenetic and transcriptomic reprograming that is likely to put pressure on genome integrity. Preserving the stability of the genome through the coordinated action of cell cycle checkpoints, DNA repair and cell death pathways is critical during these early stages, when the progeny of any given cell is likely to form a large proportion of the final organism and may contribute to the germline. However, we have found the level of activation of critical DNA damage response and cell death pathways is particularly low during the first stages after fertilization. I will present our latest efforts to identify critical changes in embryo sensitivity to DNA damage occurring during the unique cellular reprograming that follows fertilization and how they are coordinated with the milestones of early embryo development and differentiation to ensure optimal embryo fitness.

#74 • A Maternal CHEK1 Variant Delays Mitotic Entry and Impairs Embryonic Genome Activation

Xiao Xu[1], Andrei Rybouchkin[1], Margot Bracke[1], Hongbei Mu[1], Eva Decroos[1], Krishna Chaitanya Pavani[1], Hannes Syryn[2], Björn Menten[2], Dominic Stoop[1], Björn Heindryckx[1]

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A heterozygous missense variant in CHEK1 (c.1325G>A, p.Arg442Gln) was identified by whole-exome sequencing in a patient undergoing ICSI treatment with recurrent zygote arrest in our clinic. To elucidate how this maternal-effect variant disrupts early embryonic development during the maternal-to-embryo transition, we integrated public multi-omics datasets from human and mouse oocytes and embryos to define CHK1 regulation in early development. Functional consequences were assessed using a mouse model in which in vitro transcribed wild-type or mutant CHEK1 cRNA was microinjected into metaphase II oocytes followed by ICSI. Embryo development was evaluated by cleavage kinetics and blastocyst formation, while time-lapse imaging was used to examine pronuclear dynamics and first mitotic entry. Transcriptomic profiling was performed at early cleavage stages, and selected embryos were treated with a CHEK1 inhibitor to assess phenotypic rescue. Integrated translatome–proteome analyses identified CHEK1 as a delayed-pattern protein, with protein accumulation lagging behind translational activation prior to embryonic genome activation (EGA) in both human and mouse embryos. Injection of CHEK1 R442Q caused a severe reduction in cleavage (3% vs. 80%) and blastocyst formation (15% vs. 74%) compared with wild-type embryos (both P=0.0012). Time-lapse imaging revealed normal pronuclear formation but delayed pronuclear breakdown and first mitotic entry. Transcriptomic analysis demonstrated partial downregulation of EGA-associated genes in mutant 2-cell embryos. Importantly, pharmacological CHEK1 inhibition significantly rescued blastocyst development (65%). Together, these findings show that a maternal CHEK1 variant compromises early embryonic reprogramming by delaying cell-cycle progression and impairing EGA, highlighting a critical role for maternal checkpoint control in genome activation.

EMBO Workshop

SESSION 9: GENE REGULATION DURING GENOME ACTIVATION IN MAMMALS

Rise and SINE: Mechanisms of zygotic genome activation

Anastasiia Bondarieva,[1*] Maria Wahle,[2*] Laura Gomez Hernandez,[1*] Alina Ivanova,[1] David Depierre,[1] Siwat Ruengroengkulrith,[1] Johanna Berger,[1] Michael Wierer,[2] Johanna Gassler,[1] Matthias Mann,[2] Kikuë Tachibana[1]

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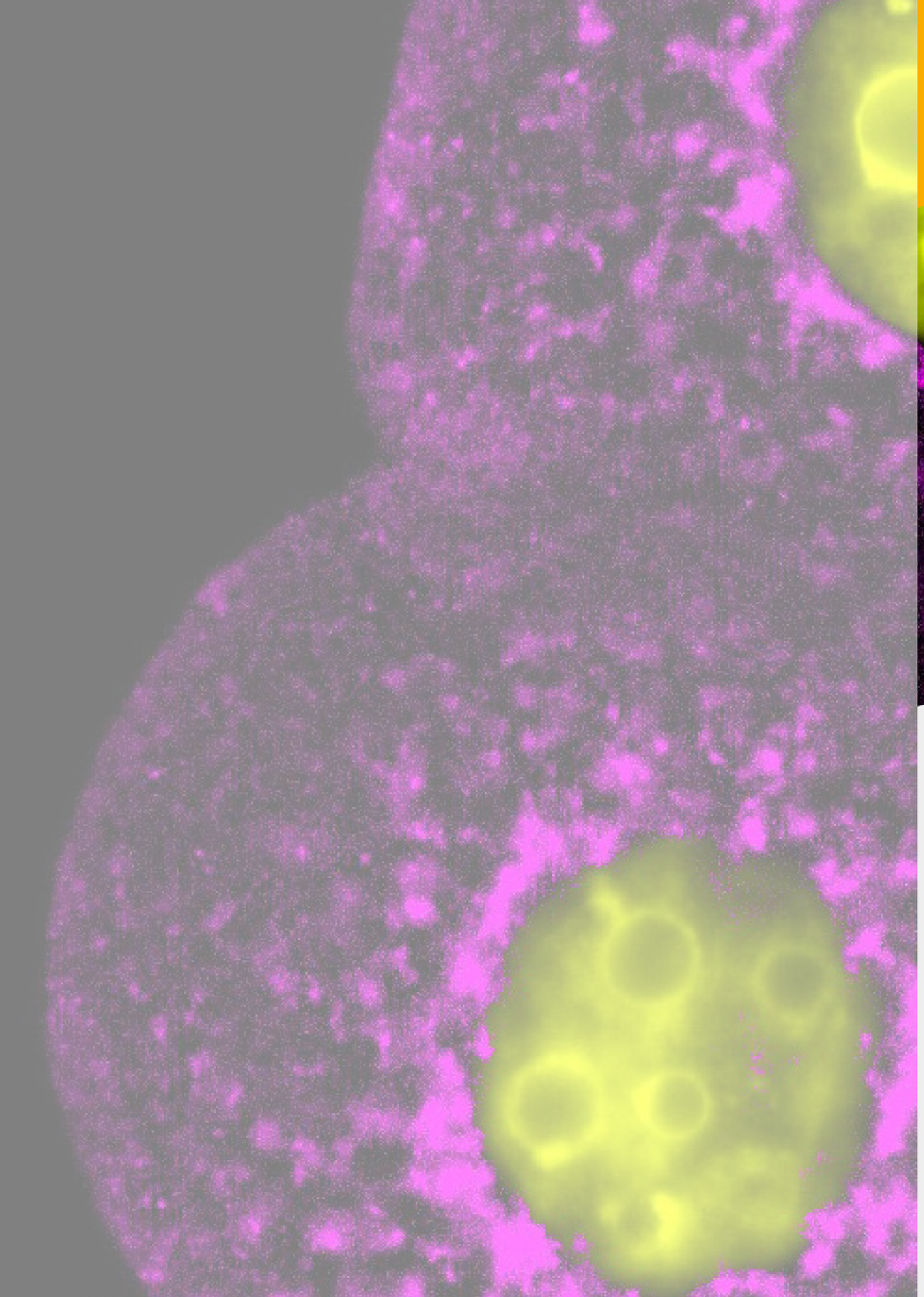
Zygotic genome activation (ZGA) is regulated by maternally provided factors in totipotent embryos. In recent years, several transcription factors including NFYA, NR5A2, OBOX proteins and KLF17 were identified that regulate murine ZGA in 2-cell embryos (Lu et al., Cell 2016; Gassler et al., Science 2022; see also Festuccia et al., Science 2024; Ji et al., Nature 2023; Hu et al., Dev Cell 2024). NR5A2 was first shown to bind to its motif in SINE B1 retrotransposable elements, which are enriched upstream of 72% of ZGA genes. SINE B1 also contain a series of other transcription factor binding motifs and are bound by OBOX proteins and KLF17. Obox1/2/3/4/5/7 genetic knockout and NR5A2 Cleave-Degrade-Away embryos showed downregulation of shared ZGA genes, suggesting that both are needed for proper transcriptional activation. NR5A2 binds to nucleosomes in vitro and partially unwraps nucleosomal DNA according to our cryo-EM structure and single-molecule FRET assays (Kobayashi et al., NSMB 2024), consistent with having pioneer factor activity. Based on this and other findings, we proposed a “Rise and SINE” model that makes testable predictions for how ZGA is regulated in embryos (Kravchenko et al., Nat Rev Mol Cell Biol 2025). Whilst many open questions remain, one of particular interest is how maternally provided reprogramming factors such as NR5A2, OBOX1/2/5/7 and KLF17 are kept inactive until the right moment. We are conducting a ZGA screen in embryos and will present our newest findings that shed light on this fascinating question.



POSTERS

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POSTER COMMUNICATION

#3 • Polyamine-Orchestrated Genome Reprogramming and Epigenetic Regulation During Plant Stress Adaptation: Conceptual Parallels to Developmental Transcriptional Awakening

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Polyamines are increasingly recognized as critical regulators of genome dynamics, integrating metabolic and epigenetic cues that influence transcriptional reprogramming during stress adaptation. Building on our recent work on polyamine polymorphism and precision polyamine intervention strategies in plants, we propose a mechanistic model wherein polyamine flux modulates methylation-demethylation balance, thereby orchestrating chromatin accessibility and transcriptional responsiveness. Using Alternanthera spp. and Parthenium hysterophorus as experimental models, our findings reveal that increased levels of spermidine and spermine likely alter histone methyltransferase activity, affect DNA methylation patterns, and activate defense-related gene networks under stress.

These likely stress-induced genome reprogramming events closely resemble the developmental transcriptional awakening seen in embryos and organoids, where metabolic shifts occur before chromatin restructuring and cell fate determination. The shared principle, metabolic control of epigenetic plasticity, highlights a unifying evolutionary logic underpinning cellular reprogramming across kingdoms.

By linking plant polyamine biology to developmental epigenetics, this work positions plant stress adaptation as a natural paradigm for understanding the metabolic-epigenetic interface driving genome activation. Insights from this comparative framework could inform both sustainable crop improvement and fundamental mechanisms of developmental reprogramming.

EMBO Workshop

POSTER COMMUNICATION

#4 • Human Anencephaly Caused by a Novel Mutation in TRIM36: Dissecting the Etiology and the Interventional Strategies Using Zebrafish as a Model System

Krishna Parjanya Pandu, Upendra Nongthomba, Arun Kumar

Indian Institute of Science

Anencephaly is a severe congenital defect characterized by the absence of a significant portion of the forebrain. It occurs due to failed neural tube closure during the third week of gestation leading to miscarriage, stillbirth, or neonatal death. The etiology of anencephaly is multifactorial, involving a plethora of environmental and genetic factors. A previous study from our lab discovered a novel homozygous missense mutation, in the protein-interacting domain of TRIM36, an E3 ubiquitin ligase, in an anencephalic fetus from a consanguineous marriage. Overexpression of the mutant TRIM36 in cultured cells disrupts the cytoskeletal machinery, and, hence, the normal cell division. Thus, based on the hypothesis that TRIM36 is crucial for neural tube closure by regulating cell division, our objective is to elucidate the role of TRIM36 in early neurulation and determine the tissue-specific implications of the reported mutation. Bioinformatic analysis shows that the human and zebrafish TRIM36 have high sequence and domain similarity. Moreover, there is a sharp increase in the endogenous levels of zebrafish trim36 at RNA and protein levels during the induction of neurulation. Morpholino-mediated knockdown of trim36 in zebrafish shows early developmental defects and a high mortality rate reminiscent of human anencephaly. Thus, we have established TRIM36 as a potential candidate essential for early neurulation in zebrafish. This model system will allow us to dissect the etiology of TRIM36-associated anencephaly, at the cellular and physiological levels. This will also provide avenues for screening therapeutic interventions.

POSTER COMMUNICATION

#7 · Phosphoregulation of RNA-Binding Proteins During Egg Activation

Emily Rivard, Mariana Wolfner

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Egg activation marks the major developmental milestone in which a mature oocyte becomes competent to support embryogenesis. This transition involves conserved signaling events, beginning with a rise in intracellular calcium levels and eventually leading to extensive protein phosphoregulation. However, how these post-translational modifications influence early embryogenesis, including the regulation of maternally deposited mRNAs, remains poorly understood.

To address this gap, I am investigating how RNA-binding protein phosphoregulation coordinate mRNA regulation and translation upon egg activation using Drosophila melanogaster, a highly tractable system due to its large and accessible eggs. First, I am assessing the evolutionary conservation and structural features of phosphosites on oocyte RNA-binding proteins, using comparative genomics and structural modeling. Second, I am generating phosphomimetic and non-phosphorylatable alleles of conserved phosphoregulated RNA-binding proteins using CRISPR/Cas9-based genome editing. I will analyze the consequences of these mutations on protein function, global mRNA regulation, and developmental progression. Finally, I will manipulate oocyte kinases using genetic and optogenetic tools to establish how kinase activity directs RNA-binding protein phosphorylation and regulates subsequent developmental processes.

My findings will uncover how phosphoregulation of RNA-binding proteins during egg activation contributes to the precise post-transcriptional control required for early development. More broadly, they will advance our understanding of how dynamic protein modifications regulate RNA biology, a mechanism that occurs across diverse cell types. Because defects in egg activation can impair fertility—even for couples using assisted reproductive technologies—this research may provide necessary mechanistic insight into a cause of infertility.

POSTER COMMUNICATION

#10 · The Roles of Pou5f3 and Nanog in Germ Layer Specification in Zebrafish

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Zygotic genome activation (ZGA) is a crucial step in embryogenesis, regulated by the pioneer transcription factors Pou5f3, Sox19b, and Nanog (PSN) in Danio rerio. We aim to investigate if and how PSN are involved in early differentiation events immediately following ZGA in zebrafish. For this purpose, we simultaneously profiled transcription and chromatin accessibility in single cells of wild-type (WT) embryos, as well as PS- and N-deficient mutants (MZps, MZnanog), at the early gastrula stage using 10X Multiome sequencing.

We compared the major differentiating embryonic tissues - mesendoderm (ME), enveloping layer (EVL), and the most undifferentiated cell population, which we called stem cells (STEM) across genotypes. While the differentiated tissues were comparable, both MZps and MZnanog mutants formed genotype-specific STEM cell types. This suggested that the formation of early progenitors is affected in the absence of PSN.

Motif enrichment analysis of tissue-specific differentially accessible regions revealed that PSN were required to establish open chromatin specifically in the STEM cell population. In contrast, lineage-defining motifs for mesendoderm (TBX factors) and EVL (IRF, GRHL, KLF) remained similarly accessible across genotypes, indicating that these tissues rely on other pioneer factors' activity already at the early gastrula stage.

Our results demonstrate that already at the early gastrula stage, pioneer activity of PSN is restricted to STEM cell population. Loss of PSN does not entirely block all developmental decisions, yet highlights "hidden" stem-like states and possible compensatory mechanisms during early embryogenesis in zebrafish.

FLASH TALKS

#11 · Investigating Mechanisms Behind Zygotic Genome Activation and Regulatory Networks Coherence by Interspecies Hybridization Within the Oryzias Genus

Cielo Lucía Centola[1], Luis Hernández-Huertas[1,2], Javier Vázquez-Marín[3], Lázaro Centanin[3,4], Miguel A. Moreno-Mateos[1,2,4], Juan R. Martinez-Morales[1,4]

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The maternal-to-zygotic transition (MZT) is a critical developmental window in which control of embryogenesis shifts from maternally deposited factors to the zygotic genome. In hybrids between related species, incompatibilities between parental regulatory programs frequently lead to developmental arrest, providing a powerful system to dissect the mechanisms underlying zygotic genome activation (ZGA), heterochrony, and regulatory coherence during early development.

To investigate ZGA and subsequent gastrulation programs, we focused on hybrids within the Oryzias genus: O. latipes females crossed to O. dancena males. RNA-seq revealed the hybrid transcriptome closely resembles that of O. latipes at ZGA. Orthologs of known O. latipes zygotically-expressed genes were not activated from the O. dancena genome, while a small subset of genes was ectopically or uniquely activated in hybrids suggesting either species-specific ZGA programs or inefficient recognition of the paternal genome by the maternal machinery.

At 70% epiboly, although both genomes contribute nearly equally to the total transcriptome, germ layer specification programs were driven almost exclusively by the O. latipes genome. In contrast, O. dancena-derived transcripts show reduced or delayed activation of gastrulation-associated genes, correlating with severe defects in morphogenetic movements.

Specific lineages, including non-neural ectoderm and EVL, appear particularly affected.

We are now conducting ATAC-seq analysis to determine how genome accessibility is affected in hybrid embryos and its relation to transcriptomic data

Together, these results support a model in which maternal control efficiently activates the maternal genome but fails to timely engage the paternal program even in close-related species, leading to loss of developmental coherence.

EMBO Workshop

FLASH TALKS

#12 · Increased Mitotic Retention of GAGA Factor Does Not Alter Target-Gene Expression in the Early Drosophila Embryo

Annemarie Branks[1], Sadia Dima[2], Hope Hawthorne[1], Marissa Gaskill[1], Kerstin Hurd[1], Bethany Snyder[1], Gregory Reeves[2], Melissa Harrison[1]

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Development requires the faithful propagation of gene-expression programs across cell divisions. Mitosis is a barrier to the maintenance of cell identity as chromatin condenses, transcription arrests, and most transcription factors dissociate from the genome. During early embryonic development cells rapidly proliferate, raising the question of how the transcriptional profile is maintained. A subset of transcription factors remains associated with mitotic chromosomes, and it has been proposed that they drive the rapid reactivation of gene expression. Because most studies have been performed in culture and rely on protein depletion, the functional significance of mitotic retention remains unclear. To test the impact, we leverage the rapid and synchronous mitotic divisions of the early Drosophila embryo where the transcription factor GAGA factor (GAF) is mitotically retained on active euchromatin and silent heterochromatin. We investigated the protein features that promote mitotic retention and showed that these domains are those required for stable chromatin occupancy during interphase. We identified two GAF mutants with increased mitotic retention. Unexpectedly, these mutations do not impair embryonic development. Quantitative imaging revealed that increased mitotic retention results in higher nuclear concentration and altered binding kinetics during interphase compared to wildtype. However, ChIP-seq showed that static chromatin occupancy was unaffected by these changes. We used our gain-of-function mutants to test if increased mitotic retention promoted GAF-target gene expression. Contrary to the current model, our data do not support a role for GAF mitotic retention in increasing gene expression and challenge the prevailing model that mitotic retention impacts gene expression after division.

POSTER COMMUNICATION

#15 · Zygotic Genome Activation by Combinatorial Transcription Factor Dynamics at SINE B1 Elements

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Max Planck Institute for Biochemistry

Zygotic genome activation (ZGA) is a fundamental and tightly regulated developmental process guided by maternal factors that orchestrate wide reorganization of the embryonic chromatin landscape to prime and induce transcriptional activity. Despite the essential role of ZGA, the timing and regulatory factors are poorly conserved across vertebrate and mammalian species. Recent bioinformatic studies have identified enrichment of retrotransposon-derived SINE B1 elements upstream of mouse ZGA genes harbouring a super motif of multiple transcription factor (TF) binding sites. To elucidate the mechanism of SINE B1-directed ZGA activation, we aim to apply in silico modelling of TF perturbation effects on preimplantation development and test the importance of selected candidates on ZGA using in vitro and in vivo assays. The combinatorial dynamics of SINE B1 TFs will be further tested by genomic binding and biochemical interaction studies.

POSTER COMMUNICATION

#16 · Investigating the Regulation of H3.3 Incorporation During Zygotic Genome Activation

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Early embryos often have unique chromatin states prior to zygotic genome activation (ZGA). In Drosophila, ZGA occurs after 13 reductive nuclear divisions during which the nuclear to cytoplasmic (N/C) ratio grows exponentially. Previous work found that histone H3 chromatin incorporation decreases while its variant H3.3 increases, in the cycles leading up to ZGA. In other cell types, H3.3 is associated with sites of active transcription and heterochromatin, suggesting a link between H3.3 and ZGA. We tested what factors regulate H3.3 incorporation at ZGA. We found that H3 nuclear availability falls more rapidly than H3.3 leading up to ZGA. We generated H3/H3.3 chimeric proteins at the endogenous H3.3A locus and observed that chaperone binding, but not gene structure, regulates H3.3 behavior. We identified the N/C ratio as a major determinant of H3.3 incorporation. To isolate how the N/C ratio regulates H3.3 incorporation, we tested the roles of genomic content, zygotic transcription, and cell cycle state. We determine that cell cycle regulation, but not H3 availability or transcription, controls H3.3 incorporation. Overall, we propose that local N/C ratios control histone variant usage via cell cycle state during ZGA. Ongoing work using H3.3 gene replacement flies tests the role of the H3.3 protein in regulating transcription during ZGA.

POSTER COMMUNICATION

#17 • Epigenetic Reprogramming by TET3 Modulates 3D Chromatin Reorganization and Controls Gene Expression in Totipotent Embryos

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Upon fertilization, the totipotent embryo undergoes extensive epigenetic reprogramming of the parental genomes, 3D chromatin reorganization and zygotic genome activation (ZGA). To what extent these are causally linked and how they lead to a totipotent chromatin state are important unanswered questions. DNA methylation (5mC) is a key regulator of gene expression and widely participates in developmental cell fate decisions. Mouse zygotes undergo a genome-wide decrease of DNA methylation. DNA demethylation can occur passively, via DNA replication, or actively, as a result of TET3 enzymatic activity, which acts predominantly on the paternal genome. Here, we investigated whether reprogramming of DNA modifications modulates spatial chromatin organization in mouse embryos. Using a genetically engineered TET3 degron mouse line and single-nucleus Hi-C (snHi-C), we found that TET3 influences multiple aspects of 3D chromatin organization including distal loops, TAD insulation and formation of compartments in early embryos. Remarkably, TET3 is required for expression of 21% of ZGA genes and also maintains transcriptional silencing of genes that will be expressed later in development. Although TET3 is dispensable for development of diploid embryos, which is presumably due to the presence of the maternal genome, we found that it is required for the development of androgenetic embryos containing only a paternal genome. Our work provides evidence that TET3 promotes spatial chromatin reorganization and influences allele-specific gene expression during ZGA.

FLASH TALKS

#18 • A Novel TIP-3C Method that Integrates Enhancer–Promoter Contacts with Transcriptional Regulation During Early Embryonic Development

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A central problem in developmental biology is how a genome that consists of a defined DNA sequence is repeatedly reprogrammed to support distinct cellular states. This challenge is acute during early embryogenesis, when zygotic genome activation (ZGA) occurs and totipotency is established. How enhancers and 3D chromatin organization contribute to the first waves of ZGA in mammalian embryos has remained unclear. This lack of knowledge is because existing enhancer-promoter (EP) methods require hundreds of thousands of cells and are thus largely inapplicable to extremely limited embryonic material. Here, we present a new method that we term TIP–3C (Targeted Insertion of Promoters for Chromosome Conformation Capture), which is an ultralow-input, chromatin conformation assay that captures protein-anchored chromatin interactions. TIP–3C accurately reflects target occupancy on genomic sites and sensitively reports changes in genome folding following cohesin depletion. Using H3K27ac as a target, we performed TIP-3C from oocytes to 8-cell-stage mouse embryos and detected developmental remodelling of enhancer-associated genome architecture. TIP-3C reveals a progressive establishment of an active enhancer “landscape” including the emergence of enhancer–promoter loops. During ZGA in 2-cell embryos, TIP–3C maps enhancer–promoter connectivity genome-wide. While global EP contacts correlate weakly with transcription, ZGA genes display increased enhancer connectivity and dense chromatin interaction networks, implying a close link between spatial proximity and transcriptional activation. Our data provides new insights into the EP networks underlying ZGA and establish TIP–3C as a powerful method to integrate genome architecture, regulatory sequences and transcription in early development.

POSTER COMMUNICATION

#19 · DNA Replication Is Required for Allelic Reprogramming in Early Mouse Embryos

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Fertilisation initiates a highly dynamic developmental period in which the maternal and paternal genomes are epigenetically reprogrammed and transcriptionally activated during zygotic genome activation (ZGA). Interestingly, DNA replication correlates temporally with ZGA in Drosophila melanogaster, Xenopus laevis, Danio reiro and Mus musculus which has raised the question of whether these processes are causally linked. Previous studies that tested the dependency of murine ZGA on DNA replication have often relied on chemical inhibition against DNA polymerase activity, which can be accompanied by pleiotropic effects such as DNA damage and checkpoint activation. Using an alternative approach that exploits the licensing mechanism of the MCM replicative helicase, we tested whether minor and major ZGA depend on DNA replication. Specifically, we prevented DNA replication by expressing a non-degradable version of geminin, which in turn prevents recruitment of MCM complexes to chromatin. We found that minor ZGA occurs independently of DNA replication in zygotes. In contrast, proper transcription during major ZGA depends on DNA replication in 2-cell embryos. While major ZGA genes were largely unaffected, later developmental genes were precociously transcribed in an allele-specific manner in the absence of DNA replication. Allelic CUT&RUN showed that DNA replication facilitates reprogramming of repressive chromatin modifications, especially on paternal chromatin. Our work reveals that DNA replication is required for allele-specific epigenetic reprogramming in mouse 2-cell embryos.

EMBO Workshop

POSTER COMMUNICATION

#20 · Direct Visualization of the Molecular Organization of Replication and Transcription During ZGA

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Zygotic genome activation (ZGA) often coincides with rapid cell cycling, requiring simultaneous binding of replication and transcription factors to the genome. Despite their fundamental importance, it remains unclear how transcription and replication are coordinated during this time. To elucidate how these factors organize during ZGA, we leverage Chromatin Expansion Microscopy (ChromExM) in zebrafish embryos to directly visualize replication (marked by PCNA) and transcription (marked by RNA Polymerase II) at single-molecule resolution. Comparing early and late times of development, we find that ZGA stage embryos exhibit unique PCNA organization, which takes on a markedly different spatial distribution as cells begin to differentiate. Our analysis of ZGA embryos revealed ~33,000 PCNA molecules per nucleus located at variable distances from transcription, with 2% of Pol II molecules found within 30 nm of PCNA, while the overall average distance between molecules was ~180 nm. Surprisingly, PCNA appears equidistant from elongating and non-elongating Pol II, suggesting its organization is not influenced by transcription at this time. Consistent with a high maternal load of replication factors, PCNA organization during ZGA can be recapitulated by simulations of randomly distributed molecules, indicating that PCNA organization is largely density-driven. Supporting this model, PCNA organization is also largely insensitive to transcription inhibition or acute replication inhibition. We are currently investigating different developmental stages and additional replication factors to further characterize how replication and transcription interplay during early development. Together, this work provides a quantitative, molecular view of the nanoscale organization of replication and transcription during the earliest stages of development.

POSTER COMMUNICATION

#21 · Cleave-Degrade-Away Reveals that NR5A2 Is Required for Zygotic Genome Activation and 2-Cell Embryo Development

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Zygotic genome activation (ZGA) is the defining moment at the start of life when the embryonic genome takes control of development. Identifying the transcription factors (TFs) that are essential for ZGA is critical for understanding the mechanism of totipotency reprogramming. These factors are maternally deposited as proteins and RNA in oocytes to function after fertilization. Previous inhibition experiments indicated that the pioneer TF NR5A2 is a key regulator of ZGA, but mouse knockout embryos derived from Nr5a2 conditional knockout oocytes have little defects in ZGA and early development. It is therefore unknown whether NR5A2 is required for ZGA, and, if required, how it cooperates with other ZGA TFs, such as OBOX proteins. Here, we show using a direct protein inactivation approach termed Cleave-Degrade-Away that NR5A2 is required for ZGA and 2-cell embryo development. Cleave-Degrade-Away inactivates NR5A2's function as a TF by TEV protease cleavage and auxin-mediated degradation of its DNA-binding domain. NR5A2 depletion caused downregulation of >2000 genes including 36% of ZGA genes and a 2-cell arrest or delay of Nr5a2AID-TEV/AID-TEV embryos. NR5A2 and OBOX TFs are required for expression of a shared set of ZGA genes. Mechanistically, NR5A2 promotes binding of OBOX proteins to SINE B1/Alu elements to regulate ZGA. Our findings provide evidence for a hierarchical "Rise and SINE" ZGA model where pioneer factor NR5A2 initiates chromatin opening and facilitates binding of other ZGA TFs. Our results also highlight the importance of protein inactivation approaches for maternally deposited proteins that cannot be fully depleted by conditional gene deletions.

EMBO Workshop

POSTER COMMUNICATION

#23 · Chemical Induction of Human 8-Cell-like Cells from Naïve Pluripotent Stem Cells

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8-cell-like cells (8CLCs) represent a rare and transcriptionally distinct subpopulation of naïve human pluripotent stem cells (hPSCs) that recapitulate key molecular features of the human 8-cell embryonic stage. This stage coincides with zygotic genome activation (ZGA), a pivotal developmental milestone during which the embryonic genome takes control of transcription for the first time. 8CLCs are defined by the upregulation of ZGA markers such as TPRX1, LEUTX, and ZSCAN4, activation of endogenous retrotransposons, and dynamic epigenetic remodeling. To identify regulators of this transient and rare state, we generated a TPRX1-mCherry knock-in reporter hiPSC line that enables direct visualization and quantification of 8CLC emergence. Using this system, we conducted a focused screen of 96 small molecules enriched for epigenetic modulators. This screen uncovered three distinct compounds that significantly increased the mCherry^{+} 8CLC population. Each compound targeted a different chromatin-associated pathway, yet all converged on a shared transcriptional outcome. Marker validation by qPCR and transcriptome-wide profiling via RNA sequencing confirmed strong induction of the 8CLC gene signature across treatments to a similar extent to DUX4 overexpression. Gene ontology analysis highlighted activation of pathways involved in genome regulation, chromatin remodeling, and transcriptional initiation. Notably, these compounds also acted additively with retinoic acid—a previously known enhancer of the 2-cell-like (2CLC) state in mouse—suggesting conserved synergy between epigenetic and signaling inputs in the induction of ZGA-like states. Taken together, our results provide new insight into the epigenetic control of 8CLCs and establish a scalable chemical framework for studying early human developmental transitions.

POSTER COMMUNICATION

#25 · Decoding Diverse Histone Modification States in the Zebrafish Neural Crest

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Histone modifications are an integral element of the epigenetic landscape, representing chromatin states and regulating gene expression. Although these diverse modifications have been studied extensively, it is still poorly understood how they are first established in a cell type–specific manner during embryonic development. In particular, the extent to which their initial deposition causally contributes to cell fate decisions remains largely unresolved.

To address this, we aim to systematically characterize the establishment of the histone modification landscape in the neural crest of zebrafish embryos. The neural crest is a transient, multipotent population whose specification and diversification are tightly ordered in time and space. This makes it an ideal system to measure epigenetic heterogeneity and determine how variation in histone modification states relates to fate specification. Moreover, the rapidly developing zebrafish embryo provides unique opportunities to manipulate the epigenetic landscape in vivo.

We perform joint single-cell profiling of various histone modifications with transcription by implementing the recent T-ChIC technology. This will uncover epigenetic heterogeneity between cells with unprecedented resolution, and enables direct linkage with the underlying cell type and state. We profiled whole embryos as proof-of-concept, which yielded high quality data that can readily be integrated with other epigenetic measurements. Additionally, by depleting histone modifications either globally or specifically in the neural crest, we can assess the functional role of these marks in lineage specification. By integrating single-cell profiling with in vivo perturbations, this study provides a mechanistic framework for understanding how epigenetic information is established and interpreted during cell fate specification.

EMBO Workshop

FLASH TALKS

#27 · Modulation of Mammalian Embryonic Growth by Intracellular Glycosylation

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The main form of intracellular glycosylation in animals is O-GlcNAcylation, the reversible linkage of a monosaccharide (O-GlcNAc) to serine and threonine protein residues. The donor substrate for O-GlcNAc, UDP-GlcNAc, is the end product of a nutrient-responsive metabolic pathway. O-GlcNAc is present on thousands of mammalian proteins in all cellular compartments, including RNA Polymerase II and developmentally relevant transcription factors such as OCT4 and SOX2. In spite of its pleiotropy, only one enzyme is responsible for O-GlcNAcylation, called O-GlcNAc transferase (OGT). The mammalian Ogt gene is essential for both cellular proliferation and embryonic development. Specifically, a functional maternal Ogt copy is required for the mouse embryo to pass the blastocyst stage. Because of this obstacle for genetics studies, the molecular function of O-GlcNAc in early mammalian development remains poorly understood and certainly never addressed in vivo.

We profiled for the first time the subcellular localization of the O-GlcNAc modification across preimplantation development in the mouse embryo, and discovered O-GlcNAc's enrichment in the nucleus concomitantly with embryonic genome activation (EGA). Then, we addressed O-GlcNAc's role in the mouse preimplantation embryo by enzymatically depleting the O-GlcNAc modification itself from the embryonic nuclei, thus overcoming cellular and embryonic lethality. By analyzing the transcriptome of single embryos at key pre- and postimplantation stages upon the depletion of O-GlcNAc, we discovered that nuclear O-GlcNAc is dispensable for EGA and blastocyst differentiation, but that reducing O-GlcNAc slows down embryonic growth. Our studies established a novel link between a maternally-transmitted intracellular protein glycosylation and developmental pace.

POSTER COMMUNICATION

#28 • Chromatin Landscape at Cis-Regulatory Elements Orchestrates Cell Fate Decisions in Early Embryogenesis

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The establishment of germ layers during early development is crucial for body formation. The Drosophila zygote serves as a model for investigating these transitions in relation to the chromatin landscape. However, the cellular heterogeneity of the blastoderm embryo poses a challenge for gaining mechanistic insights. Using 10× Multiome, we simultaneously analyzed the in vivo epigenomic and transcriptomic states of wild-type, E(z)-, and CBP-depleted embryos during zygotic genome activation at single-cell resolution. We found that pre-zygotic H3K27me3 safeguards tissue-specific gene expression by modulating cis-regulatory elements. Furthermore, we demonstrate that CBP is essential for cell fate specification functioning as a transcriptional activator by stabilizing transcriptional factors binding at key developmental genes. Surprisingly, while CBP depletion leads to transcriptional arrest, chromatin accessibility continues to progress independently through the retention of stalled RNA Polymerase II. Our study reveals fundamental principles of chromatin-mediated gene regulation essential for establishing and maintaining cellular identities during early embryogenesis.

EMBO Workshop

POSTER COMMUNICATION

#29 • A CRISPR-RfxCas13d Maternal Screening Uncovers Two Novel Post-Transcriptional Regulators of the MZT.

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Early development across the animal kingdom is driven by maternally provided proteins and mRNAs that are essential to activate the zygotic genome. By using our recently optimised CRISPR-RfxCas13d technology, an RNA-guided RNA nuclease system, we have completed a maternal screening where we knocked down 52 maternally provided transcripts. These genes are coding proteins with a potential role in post-transcriptional regulation during the maternal-to-zygotic transition in zebrafish. Upon this screening, we identified two potential novel regulators of this process: atxn2l (Ataxin 2-like), an RNA binding protein predicted to be involved in the formation of stress granules and P bodies, and ddx3xa (DEAD-box helicase 3 X-linked a) an RNA helicase. Depletion of the maternal mRNA from these genes generated epiboly defects, a deregulation of ZGA, and an alteration in some of the degradation pathways for maternally provided mRNAs. Currently we are working towards the characterisation of these candidates by performing different genomics, transcriptomics and molecular biology approaches to ultimately analyse the role that these two genes play in the post-transcriptional regulation of the MZT. In summary, by using RfxCas13d, an already stablished tool to study the early embryogenesis, we were able to uncover two new potential post-transcriptional regulators of this process.

FLASH TALKS

#30 • High-Resolution Mapping of Embryonic Genome Activation Unveils a Functional Decoupling of Transcriptional Activation from Precocious H3K4me3 Remodeling

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The lack of temporal resolution in transcriptomic data during mammalian embryonic genome activation (EGA) has precluded the comprehensive understanding of the functional relationships between the various gene-regulatory mechanisms governing this process. Here, we finely dissect the transcriptional dynamics of mouse EGA using precision in vitro fertilization (IVF) coupled with single-embryo RNA sequencing. Our highly temporally resolved dataset uncovers an extensive, step-wise remodeling of the embryonic mRNA landscape, impacting ~30% of the total transcriptome over a 9-hour timeframe. We capture the gradual shift from maternal to embryonic mRNAs, identify ribosome biogenesis and translation as hallmarks of EGA, and discover complex gene expression patterns previously undetected in embryonic mRNA datasets. We further uncover a set of eight histone demethylating enzymes among the earliest upregulated EGA genes, and leverage our precision transcriptome mapping to dissect the transcriptional versus developmental impact of H3K4me3 remodeling during EGA. Our results indicate that precocious removal of H3K4me3 from embryonic chromatin only modestly impacts embryonic transcription without perturbing EGA timing, suggesting that remodeling of maternally-inherited H3K4me3 after fertilization is, in itself, not instructive for genome reactivation. Importantly, high-resolution transcriptome mapping coupled with functional perturbations allows us to distinguish direct gene expression effects from general impact on developmental timing, opening up new avenues for further quantitative characterization of the impact of epigenome remodeling on embryonic genome output.

EMBO Workshop

FLASH TALKS

#31 • The Role of Transcription Factor Complex NFY in Genome Activation and Epigenetic Control During Early Mouse Development

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Zygotic genome activation (ZGA) marks the onset of embryonic transcription and is essential for successful embryonic development. The regulatory principles governing this transition remain incompletely understood. By integrating exonic and intronic transcriptional information, we identify that most genes activated during ZGA are also maternally provided, are driven by CpG island (CGI) promoters, and predominantly encode housekeeping functions required for early embryogenesis. CGI promoters of ZGA genes are highly enriched for binding motifs of multiple transcription factors (TFs), including GABPA, NRF1, YY1, and NFY. Functional perturbation by siRNA-mediated knockdown demonstrates that these TFs are critical for early development. Using dominant-negative approaches, we identify the NFY complex as the earliest ZGA activator described to date and show that it is essential for development beyond the 2-cell stage. Single-embryo expression analyses reveal that NFY also activates a broad transcriptional program later in pre-implantation development, including histone genes, chromatin regulators, and KRAB zinc finger proteins. Failure to properly induce this program results in aberrant reactivation of oocyte- and early embryo–specific genes and retrotransposons at the morula stage, likely due to impaired TRIM28-dependent H3K9me3 silencing and reactivation of transposable element–associated TFs such as DUX and OBOX. Together, our findings identify NFY as an indispensable activator of early ZGA and founder of transcriptional and epigenetic stability during pre-implantation development.

POSTER COMMUNICATION

#32 · CRISPR-Cas13d Maternal Screenings to Uncover Ubiquitination Regulatory Factors Involved in Early Development.

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The maternal-to-zygotic transition (MZT) is a universal process present in all animals that drives early development. Here, we have applied our optimized CRISPR-RfxCas13d system to perform a screening and target maternally provided mRNAs involved in protein ubiquitination, a poorly studied post-translational regulatory mode in the context of MZT in zebrafish. Upon knockdown, nine out of twenty-five initial ubiquitination-related candidates showed epiboly defects, a phenotype observed when the zygotic genome activation (ZGA) is altered. After independent gRNAs validation and a phenotypic rescue, we selected rfwd3, a ubiquitin ligase involved in DNA repair in mammalian cells, as our main candidate, as rfwd3 showed the most severe developmental phenotype upon knock-down in our screening. However, after transcriptomic analysis we concluded that there is a lack of ZGA alteration. Furthermore, we have studied the role of this gene in DNA repair during zebrafish early development and concluded that similarly to what happens in other non-mammalian organisms this gene is not involved in the reparation of collapsed replication forks in zebrafish nor in replication. We also performed proteomics to further understand the regulatory landscape in the knock-down condition of this gene, finding altered levels of a subset of proteins during early zebrafish embryogenesis. Currently, we are exploring the role of rfwd3 during MZT and early development through ubiquitin-proteomics, with the idea of finding the direct targets of this ubiquitin-ligase in the context of MZT. Altogether, our results will ultimately help to better understand the post-translational mechanisms controlling early embryogenesis mediated by protein ubiquitination.

POSTER COMMUNICATION

#33 · Pol II Ser5 Macroclusters Associated with Genomic Activation Form Independently of Pioneer Transcription Factors

Cesar Melendez-Ramirez, Antonio Giraldez

Yale

After fertilization, the zygotic genome is transcriptionally silent and undergoes extensive reprogramming during zygotic genome activation (ZGA). In zebrafish ZGA is orchestrated the pioneer transcription factors Nanog, Pou5f3, and Sox19b (NPS), which open the chromatin, recruit histone acetylation machinery and ultimately RNA polymerase II (Pol II) to enhancers and promoters. Chromatin Expansion Microscopy showed that during ZGA, the transcriptional initiation form of Pol II (Pol II pSer5) adopts distinct spatial organizations within the nucleus, including strings associated with transcription, as well as compact assemblies of particles termed macroclusters. Unlike elongating Pol II strings, these macroclusters are unaffected by transcription inhibition, suggesting that they represent a distinct organizational state where Pol II is transcribing weekly or its paused; however, the genomic locations of these clusters and their functional roles in transcriptional regulation during ZGA remain unknown. To understand the molecular features of Pol II clusters, we investigated potential mechanisms of regulation of transcription. First, we investigated CDK9, a kinase that promotes phosphorylation of Pol II at Ser2 and facilitates the transition into elongation. CDK9 overexpression had no effect on the number of Pol II pSer5 macroclusters. Next, to assess whether macroclusters depends on NPS, we used the GEARS system to deplete NPS from the nucleus in zebrafish embryos. Pol II pSer5 macroclusters persisted in NPS-depleted nuclei, demonstrating that their formation is independent of NPS. We also found a stronger association between macroclusters and transcription in NPS-depleted nuclei compared to wild-type embryos. Future studies will investigate the genomic location of these macroclusters.

POSTER COMMUNICATION

#34 · NIPBL Disruption in Cornelia De Lange Syndrome Alters Cohesin/NIPBL Chromatin Association

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Cornelia de Lange syndrome (CdLS) is a rare disease affecting multiple organs and systems during development. Mutations in the cohesin loader, NIPBL/Scc2, were first described and are the most frequent in clinically diagnosed CdLS patients. The molecular mechanisms driving CdLS phenotypes are not understood. In addition to its canonical role in sister chromatid cohesion, cohesin is implicated in the spatial organization of the genome. We investigated the transcriptome of CdLS patient-derived primary fibroblasts and observed the downregulation of genes involved in development and system skeletal organization, providing a link to the developmental alterations and limb abnormalities characteristic of CdLS patients. Genome-wide distribution studies demonstrate a global reduction of NIPBL at the NIPBL-associated high GC content regions in CdLS-derived cells. In addition, cohesin accumulates at NIPBL-occupied sites at CpG islands potentially due to reduced cohesin translocation along chromosomes, and fewer cohesin peaks colocalize with CTCF. To investigate whether mutated-NIPBL or its interaction with cohesin affects long-range interactions in the genome causing the transcriptional alterations, we are currently studying by using 3D DNA organization techniques to demonstrate how mutated-NIPBL affects loop extrusion and chromatin organization around the developmental genes deregulated in CdLS.

POSTER COMMUNICATION

#35 · Expression of an Alternative Zelda Isoform in the Fly'S Embryonic Central Nervous System

Goncalo Fernandes, Christine A Rushlow

New York University

The pioneer transcription factor Zelda (Zld) develops key roles in Drosophila development. Containing 6 zinc fingers, Zld's DNA binding specificity is dependent on a C-terminal cluster of 4 zinc fingers that recognize CAGGTAG and related sequences. However, multiple RNA/protein isoforms are possible due to differential transcription start and stop sites and alternative splicing. Interestingly, two of the possible isoforms lack the 3 terminal zinc fingers that are responsible for the DNA recognition and CAGGTAG-binding functions, but not much is known about them. Isoform F RNA is expressed at low levels in the larval wing disc and isoform D RNA is expressed in the embryonic central nervous system (CNS) (Pearson et al., 2012). Isoform D appears to be the major isoform expressed in the CNS and we are further exploring two main aspects: how it is transcribed and what is its function? Transcription of this longer isoform requires the bypass of the transcription termination signaling used to produce the other isoforms. Interestingly, in the larval CNS, long 3'UTR extensions are driven by members of the ELAV/Hu family of RNA binding proteins (Wei et al., 2021). Initial results indicate that isoform D transcription-termination bypass is mediated by ELAV in the embryo. Using antibodies and fluorescently tagged-zld embryos (Hamm et al., 2017), we found that both isoforms are translated into protein, raising the possibility that isoform D plays a functional role in the developing CNS, and we are currently manipulating the system in an isoform specific fashion.

POSTER COMMUNICATION

#37 · Examining the Role of Nanog Phosphorylation During the Maternal-to Zygotic Transition.

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A set of maternal transcriptional factors and chromatin modifiers regulating zygotic genome activation (ZGA) during the maternal-to-zygotic transition (MZT) have been identified in zebrafish and other vertebrate models. However, the role at post translational modifications regulating this process remains largely unexplored. Recently, using a CRISPR-RNA targeting screening we identified Bckdk as a previously unrecognized kinase that governs the MZT in teleosts. We performed a phospho-proteome and revealed that many crucial factors regulating MZT such as Nanog, Dicer, Brd4 among others, are phosphorylated at the onset of ZGA. Here, we aim to investigate whether phosphorylation of Nanog is important to regulate the MZT. We found that the overexpression of Nanog WT and a non-phosphorylatable version of this protein overexpression induced epiboly defects, whereas a constitutively phosphorylated version did not. Further, neither the constitutively phosphorylated nor the non-phosphorylated versions of Nanog were able to rescue the nanog loss-of-function phenotype that also triggers epiboly defects. In contrast, the co-injection of both versions in a stochiometric balanced manner resulted in rescue levels comparable to those observed with WT Nanog rescue, suggesting that a precise Nanog phosphorylation level plays an important role in MZT. We are currently investigating the role of Nanog phosphorylation in its cellular localization and stability, as well as the impact of its overexpression on the embryonic transcriptomic profile. Altogether, our initial results suggest a crucial role of Nanog phosphorylation as a post-translational mechanism to control MZT in zebrafish.

POSTER COMMUNICATION

#38 · Chromatin Organization Around Nuclear Speckles Is Crucial for Early Mammalian Development

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Recent studies deciphering establishment and function of 3D genome organization during early mammalian development have emphasized the importance of nuclear compartments in orchestrating gene regulation. For example, inactive hubs like lamina associated domains and nucleolar associated domains have been shown to form before Embryonic Genome Activation (EGA) and play essential roles in regulating gene expression. However, the role of active hubs like Speckle Associated Domains (SPADs) which form around Nuclear Speckles is unclear. Nuclear Speckles are condensates of mRNA splicing and processing factors that can increase the splicing efficiency of surrounding DNA loci or SPADs. SPADs correlate with a high density of RNApol2 around them and are associated with active chromatin. Yet, whether nuclear speckles are essential during early mammalian development is not known.

We observe that in mice, nuclear speckles appear at mid-to-late 2-cell stage along with EGA. Knockdown of core speckle components, SON and SRRM2, leads to developmental arrest and change in gene expression of a subset of genes. DNA-FISH confirms that these genes localize close to nuclear speckles and delocalize upon their perturbation.

Thus, our results suggest that proper positioning of specific loci around active nuclear hubs like nuclear speckles may be crucial for their regulation and proper developmental progression. Further, we aim to use RNA-DNA SPRITE for a genome-wide readout of 3D chromatin organization in early embryos to better understand SPAD regulated processes during early development.

POSTER COMMUNICATION

#40 · Critical Role of Glis3 in Zygotic Genome Activation in Mice

Toshinobu Nakamura

Nagahama Institute of Bio-Science and Technology

Sperm and oocyte are terminally differentiated cells that transfer genetic information to the next generation, yet they reacquire totipotency through reprogramming soon after fertilization. Similar reprogramming occurs during the induction of induced pluripotent stem (iPS) cells, but whether genes involved in iPS reprogramming contribute to post fertilization reprogramming remains unclear. Here, we examined the roles of Glis family genes, which promote reprogramming during iPS induction, in the reprogramming process after fertilization.

Gene expression analysis of preimplantation embryos showed that among the Glis family genes, Glis1 and Glis3 are expressed in unfertilized oocytes, zygotes, and 2 cell embryos, whereas Glis2 is expressed only after the 2 cell stage. Although Glis1 knockout mice are viable and grossly normal, Glis3 knockout mice die soon after birth due to kidney dysplasia, leaving the function of Glis3 in early embryos unclear.

To investigate Glis3 function, we generated dominant negative (DN) Glis3 constructs consisting of the DNA binding domains of Glis3 fused to EGFP NLS and lacking transactivation domains. cRNAs encoding Glis1-DN (negative control) or Glis3-DN were microinjected into zygotes and cultured for 4 days. Almost all DN Glis1–expressing embryos developed to the blastocyst stage, whereas most Glis3-DN–expressing embryos arrested at the 2 cell stage.

RNA seq analysis of Glis3-DN–expressing zygotes and 2 cell embryos revealed that Glis3-DN did not affect genes activated during minor zygotic genome activation (ZGA), but severely impaired the activation of genes during major ZGA. These findings demonstrate that Glis3 is essential for proper ZGA during post fertilization reprogramming.

POSTER COMMUNICATION

#41 · Identifying Upstream Activators of Pioneer Factors in Mouse Early Embryos

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Transposable elements (TEs) have emerged as important regulators of host gene networks in a variety of different processes. Among these processes is the involvement of TEs in achieving the highest degree of cell potency: totipotency. Totipotency is the ability of a cell to generate all cell types and a complete organism, including extra-embryonic tissues. In mouse, the zygote and the two-cell embryo are totipotent. The two-cell stage is an essential checkpoint in embryonic development as it marks zygotic genome activation (ZGA), which is when the embryo becomes transcriptionally active. A hallmark of ZGA is the transient upregulation of TEs and 2-cell-specific clustered-genes driven by TE-derived sequences. Recently, our laboratory and others discovered key pioneer factors that activate TEs and clustered genes in preimplantation embryos. However, which factors lie upstream of these pioneers remains largely unknown. To uncover the mechanism by which cells enter ZGA and how this transcriptional program is initiated, we utilized translation inhibition to identify candidate initiator proteins. We are currently in the process of screening these candidates.

FLASH TALKS

#42 · Separate CBP Functions in Drosophila Zygotic Genome Activation

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The acetyltransferases CBP and p300 are well-established transcriptional co-activators responsible for depositing H3K27ac, a histone modification widely associated with active enhancers and promoters. This mark is known to accumulate during zygotic genome activation (ZGA) across multiple species. In this study, we investigate the role of dCBP (the sole Drosophila melanogaster ortholog, encoded by nejire) in ZGA. Using genome-edited embryos lacking dCBP or expressing a catalytically inactive version, we show that dCBP recruitment depends on pioneer transcription factors but is not required for chromatin opening. Nonetheless, dCBP is essential for the transcription of many zygotic genes. PRO-seq and CUT&Tag profiling reveal that dCBP supports transcription initiation through a non-catalytic mechanism, while its acetyltransferase activity is specifically required for efficient RNA polymerase II pause release. Using mutant embryos, we demonstrate that these functions can be uncoupled across embryonic germlayers, suggesting spatial regulation of dCBP activity. Our data suggest that distinct transcription factors may differentially modulate dCBP activity at specific genomic loci, highlighting a potential mechanism of TF-specific regulation of dCBP during early development. Finally, we provide evidence that, beyond its co-activator function, dCBP also contributes to Polycomb-mediated repression during ZGA, highlighting a dual role of CBP regulated by its activity state.

POSTER COMMUNICATION

#43 · Unraveling the DNA Damage Response During Early Embryogenesis

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Zygotic Genome Activation (ZGA) represents the first major transition during preimplantation development and occurs at the 2-cell stage in mice. During ZGA, developmental control shifts from maternally inherited RNAs to embryonicly produced ones. This is acompanied by epigenetic remodelling and metabolic changes essential for proper development. Recently a conserved and programmed splicing failure has been found to occur during ZGA which disproportionately affects genes involved in the DNA Damage Response (DDR). Consequentially, this protective system appears downregulated in the early embryo at a time when there is strong requirement to preserve genome integrity. Very little is known about how the the DDR functions in the first stages of embryo development and how it interacts with other biological processes that occur at this time.

Our group aims to fully characterize the DDR during early embryogenesis from two complementary perspectives. First, we present a characterization of preimplantation embryos at different stages exposed to single-strand or double-strand DNA break inducers (MMS, etoposide) to assess basal and induced DDR activity across embryonic development. In parallel, we present an established reprogramming system to generate an in vitro totipotent-like model using a reporter cell line, which allows straightforward tracking of 2-cell-like cells while applying the same DNA damaging treatments for comparison with embryos. Through this work, we aim to uncover key aspects of the complex DDR in early embryos and to generate a stable, tractable in vitro tool that will facilitate further exploration of this transient yet critical developmental event.

FLASH TALKS

#44 • Regulation of Zygotic Genome Activation by Nuclear Envelope Plasticity

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After fertilization, the embryo is transcriptionally silent and relies on maternal factors until zygotic genome activation (ZGA) occurs. In the mouse, the major wave of ZGA takes place at the 2-cell stage and involves activation of several thousand genes and transposons. While this is a fundamental step in development, the molecular mechanisms allowing the transcription of thousands of genes in such a coordinated manner are not fully understood. Several transcription factors have been implicated in this process, however, whether and how environmental cues such as mechanical stimuli regulate this process is unknown. The nucleus can act as a mechanosensory organelle, but whether its mechanobiological properties contribute to the awakening of the embryonic genome remains unknown. Here, we have investigated nuclear envelope plasticity in mouse pre-implantation embryos during the time of ZGA. We provide the temporal dynamics of such plasticity and have identified the molecular regulators behind. We further demonstrate that nuclear envelope remodeling is required for a correct execution of ZGA. Our work highlights the functional interplay of nuclear mechanics in the regulation of transcription at the beginning of mammalian development.

POSTER COMMUNICATION

#45 • Direct Measurement of DNA Damage Across Preimplantation Mouse Development Using Single-Cell Comet Assays

Guillermo Rodriguez-Real[1,2], Ana Alcaina-Caro[1,2], Iris Guerrero-Jordano[1,2], Ana Maria Sevilla-Jiménez[1,2], Thomas Spruce[1], Barbara Pernaute-Lomba[1,2]

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Early mammalian embryos undergo rapid cleavage divisions while exhibiting an attenuated DNA damage response (DDR), limiting the use of DDR signaling markers as reliable readouts of genomic integrity. As a result, DNA damage occurring during preimplantation development may remain undetected when inferred indirectly from signaling outputs. Here, we establish alkaline and neutral single-cell comet assays as direct approaches to measure DNA damage across preimplantation mouse development.

As an initial validation step, comet assay conditions were optimized in mouse embryonic stem cells (mESCs), which provide an accessible and abundant system for calibrating assay sensitivity. Using established protocols with adaptations, robust induction of DNA damage was achieved using standard positive controls for alkaline comet assays (hydrogen peroxide, H_2O_2; methyl methanesulfonate, MMS) and for neutral comet assays (ionizing radiation, IR; etoposide, ETOP; neocarzinostatin, NCS).

To enable accurate analysis in embryos, we developed a workflow combining zona pellucida removal and controlled dissociation into single cells, enabling nucleus-resolved comet measurements. This step is particularly important at later developmental stages, such as morulae and blastocysts, where cell dissociation is technically challenging and comet assays have frequently been applied to intact embryos, resulting in limited cellular resolution. Using single-cell comet assays, DNA damage was directly quantified at the level of individual nuclei across cleavage stages (1-cell to 8-cell embryos), morulae, and blastocysts.

Together, our work establishes single-cell alkaline and neutral comet assays as robust tools for the direct measurement of DNA damage during early mouse development and provides a framework to study genome integrity during preimplantation development.

FLASH TALKS

#46 · Histone Lactylation Links Glucose Metabolism with Mesoderm Morphogenesis

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During gastrulation, the first major cell fate decisions in the embryo proper derive the three primary germ layers. This is a critical developmental window when metabolic shifts coincide with large-scale epigenetic remodeling to drive lineage specification. How specific metabolites influence the chromatin landscape to coordinate morphogenesis remains poorly understood. Following up on our previous work showing a role of late-stage glycolysis in mesoderm development (Cao, Bergmann et al., Nature, 2024), we now investigate how the glycolytic product lactate may function during this critical period in development.

We identify H3K27 lactylation (H3K27la) as a stage-specific epigenetic mark that emerges simultaneously with gastrulation onset. Using mouse embryos and 3D gastruloids, we demonstrate that H3K27la is enriched in mesodermal domains. Functional inhibition of lactate production impairs gastruloid elongation and disrupts the mesodermal transcriptomic program. Our findings suggest that lactate's role is not limited to bioenergetics, instead coupling glycolytic activity to the epigenetic control of mesoderm development. This work provides a new framework for understanding how the metabolic environment of the embryo shapes developmental gene expression.

FLASH TALKS

#48 · Mitotic Chromosome Scaling in Cleavage-Stage Mouse Embryos.

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Early embryonic development in metazoans coincides with cleavage divisions, during which the total embryo volume remains constant while cell, nuclear, and spindle sizes progressively decrease. To accommodate these changing size constraints, cells implement scaling mechanisms, including mitotic chromosome scaling, in which mitotic chromosome length adjusts to cell size during cleavage-stage development. This study provides the first in situ evidence of mitotic chromosome scaling in early mammalian embryos, demonstrating that chromosomes progressively shorten and widen during preimplantation development.

While several factors—such as Condensin I abundance, condensin isoform ratios, nuclear-to-cytoplasmic volume ratios, and DNA density—have been implicated in mitotic chromosome scaling, how these parameters mechanistically regulate chromosome scaling remains unclear. To address this, we quantified condensin abundance in early mouse embryos using fluorescence correlation spectroscopy (FCS)-calibrated imaging, revealing developmental changes in condensin occupancy on mitotic chromosomes. Results show that neither the chromatin abundance of each Condensin isoform nor their relative ratio directly correlated with the progressive reduction in mitotic chromosome length. Condensin isoform-specific depletions in zygotes indicate an inhibitory relationship between the two complexes. Furthermore, nanoscale 3D DNA tracing revealed that G2-phase chromosomes in zygotic embryos are highly extended and progressively occupy less volume across subsequent stages, affecting compaction dynamics during mitosis. Integration of these experimental findings into a unified in silico polymer model demonstrates that mitotic chromosome scaling in cleavage-stage embryos is a multistep process shaped by the interplay of multiple factors: pre-mitotic chromosome configuration, Condensin isoform abundance, timing of NEBD, and inhibitory effects between Condensin isoforms.

POSTER COMMUNICATION

#49 · Vitamin D Receptor-Mediated Endosomal Regulation Is Essential for Gut Adaptation During Metabolic Transitions

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National Institute of Immunology

Although the intestine is one of the tissues with the highest expression of the vitamin D receptor (VDR), the functional understanding of VDR in the gut remains largely restricted to its classical role in mineral homeostasis, particularly calcium and phosphorus absorption. Beyond its established role, the contribution of VDR to intestinal adaptation under dynamic metabolic and physiological conditions remains poorly understood. Given the gut's continuous remodeling in response to diet, microbiota, and development, elucidating VDR-dependent regulatory mechanisms is essential.

In this study, we examined the role of VDR in gut adaptation using an integrated multi-omics approach that combined chromatin immunoprecipitation sequencing (ChIP-seq), bulk RNA sequencing, and quantitative proteomics in intestinal epithelial cells. Integrated transcriptomic and proteomic analyses revealed vesicular trafficking as one of the most consistently downregulated pathways in the intestinal epithelium of VDR-deficient mice. In particular, early endosomal proteins such as APPL1 and EEA1, which are the key regulators of endosome maturation, signal transduction, and cellular differentiation, were significantly reduced at both the transcriptional and protein levels in the absence of VDR. Further genomic interrogation demonstrated the presence of VDR binding sites within the promoter regions of multiple genes involved in vesicular trafficking, suggesting direct transcriptional regulation by VDR.

Collectively, our findings uncover endosomal trafficking as a novel VDR-dependent regulatory pathway that is crucial for intestinal epithelial integrity. This study reveals how disrupted vitamin D signaling contributes to intestinal dysfunction and identifies endosomal regulation as a potential therapeutic target in vitamin D-associated gastrointestinal disorders.

EMBO Workshop

POSTER COMMUNICATION

#51 · Chromatin Tethering by the Trithorax Homolog, MLL2, During the ESC Naive to Primed Transition

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The histone 3 lysine 4 (H3K4) methyltransferase, MLL2 is the major H3K4 methyltransferase in oocytes and is required for global transcriptional silencing (1), possibly because the greatly elevated H3K4me3 levels disperse and dilute promoter complexes (2). We also found that MLL2 is the major H3K4 methyltransferase for bivalent promoters in primed mouse ES cells (3). However Mll2 is not required for ESCs or until E6.5 (4). Differentiation of Mll2-/- ESCs towards neural stem cells stops before the onset of Nestin positive neural rosettes. Using time courses of conditional mutagenesis, the requirement for Mll2 in neuroectodermal differentiation is exerted during ESC exit from naïve to primed pluripotency about 10 cell cycles earlier than the onset of neural rosettes. Single-cell transcriptomic analysis revealed that Mll2 is not required for the transition from naïve to primed pluripotency. However Micro-C/Hi-C indicates early loss of Mll2-dependent 3D chromatin looping. Using live-cell 3D single-molecule localisation microscopy we found that global chromatin movement is increased in primed pluripotent cells lacking MLL2. Consequently, we propose that MLL2 is a multivalent chromatin tethering factor that secures long-range interactions during priming before the transition to neuroectoderm. Notably H3K4 methylation by MLL2 is not required for this tethering. These observations have implications for all four MLLs, altering the previous focus on H3K4 methylation.

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POSTER COMMUNICATION

#54 · Skin Organoids Generated from Dual Reporter iPSC Lines

Xianyin HUANG, Hoi Hung CHEUNG

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The human skin consists of three layers: the epidermis, dermis, and subcutis, and skin organoids derived from human induced pluripotent stem cells (iPSCs) provide a powerful platform to model skin development and disease in vitro. However, current protocols generate highly heterogeneous organoids containing multiple cutaneous and non-cutaneous lineages, highlighting the need for improved methods to track and enrich defined skin cell populations. Here, we engineered two CRISPR/Cas9 knock-in iPSC reporter lines, DSP-3XmCherry to trace epidermal lineages and PDGFRα-3XGFP to trace dermal lineages. Correct targeting was confirmed by Sanger sequencing and RT-qPCR, and both lines were differentiated into skin organoids for up to 45 days. By day 14, organoids adopted a “head-and-tail” morphology, with a cystic epithelial “head” and a mesenchymal “tail” enriched in migrating PDGFRα+ cells. RT-qPCR at day 33 demonstrated significant upregulation of epidermal and dermal genes, confirming lineage induction. Live imaging revealed robust mCherry (DSP) expression by day 14 that persisted through day 45 and outlined the cystic epithelial layer, whereas GFP (PDGFRα) appeared weakly at day 14, intensified from day 17 onward, and remained largely confined to the mesenchymal tail. RNA-seq of sorted mCherry+ and GFP+ cells at day 30 indicated incomplete transcriptional segregation, suggesting ongoing lineage intermixing. These dual reporter lines enable real-time tracking of epidermal and mesenchymal differentiation and allow purification of enriched subpopulations that can be reassembled into skin-like grafts or used to screen compounds that accelerate lineage maturation and improve the homogeneity of iPSC-derived skin organoids.

EMBO Workshop

POSTER COMMUNICATION

#55 · ERK Inhibition Preserves a naïve-like ICM with Extended Lineage Competence in Ruminant Embryos

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Understanding how signaling pathways regulate cell fate decisions during early embryonic development remains a central question in developmental biology. In ruminant species, the prolonged pre-implantation period and the formation of a large extra-embryonic compartment provide a unique model to investigate lineage specification and metabolic regulation during blastocyst development.

In this study, we investigated the role of ERK signaling during the morula-to-blastocyst transition in sheep and bovine embryos, focusing on its contribution to lineage specification and developmental competence.

During defined two developmental windows to assess its impact on blastocyst development, lineage allocation, and inner cell mass (ICM) identity.

ERK inhibition with a highly selective inhibitor Ulixertinib (BVD-523; VRT752271) resulted in a complete absence of SOX17-positive hypoblast cells in blastocysts. Strikingly, the loss of the hypoblast was associated with the stabilization of SOX2 and KLF4 in epiblast cells, indicating that ERK inhibition maintains the ICM in a developmentally immature, naïve-like state.

Functional assays demonstrated that, unlike control ICMs, ERK-inhibited ICMs retained the ability to differentiate toward extra-embryonic lineages, highlighting an increased developmental plasticity.

Together, these findings suggest that ERK signaling is not only essential for hypoblast formatio but also acts as a key driver of ICM maturation and lineage restriction. By preventing hypoblas specification, ERK inhibition preserves a naïve-like ICM state with extended lineage competence, providing new insights into the regulation of pluripotency and early cell fate decisions in mammals with prolonged preimplantation development.

POSTER COMMUNICATION

#57 · Mechanisms of Self-Organization in Vivo: Insights from Killifish Embryos

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Biozentrum, University of Basel

Establishing body axes during early development requires symmetry breaking in initially homogeneous cell populations, a process that is inherently unstable and must be robustly controlled. While self-organization has been extensively studied in vitro, how organizer formation and symmetry breaking emerges and stabilizes in vivo remains poorly understood due to the presence of pre-existing molecular pre-patterns in most model systems. In particular, how organizer formation and symmetry breaking can arise in the absence of such pre-patterns remains unknown.

Uniquely, the killifish Nothobranchius furzeri lacks maternal dorsal determinants, making it an ideal model to explore self-organization in vivo. Strikingly, during killifish early development, embryonic blastula cells disperse and subsequently re-aggregate to form and pattern the body axes. Given the correlation between cell aggregation and the expression of organizer genes, a mechanotransduction-based mechanism may drive organizer formation in this system. However, the mechanisms that drive cell re-aggregation and initiate axis formation remain unknown.

Here, we combine single-cell omics and imaging approaches to investigate the role of mechanotransduction pathways in symmetry breaking. This work aims to reveal how killifish embryos self-organize and robustly pattern their body axes after dispersion, providing new insights into in vivo self-organization during early vertebrate development.

FLASH TALKS

#58 · FLASH-PAINT Resolves Nanoscale Nuclear Architecture During Zygotic Genome Activation in Zebrafish.

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Zygotic genome activation marks a major transition in early embryogenesis accompanied by extensive nuclear reorganization. Yet how chromatin and transcriptional regulators are organized at the nanoscale during this process remains poorly understood. Here, we apply FLASH-PAINT, a super-resolution DNA-PAINT technique with ~10 nm spatial resolution, to visualize nuclear architecture in zebrafish embryo cells during zygotic genome activation. This method enables simultaneous imaging of up to 12 molecular targets within the same sample, providing a comprehensive nanoscale view of nuclear organization during early embryonic development.

Our preliminary data resolve histone clutches and fine-scale chromatin organization at nanoscale resolution. While RNA Polymerase II Ser5P–positive macroclusters have been reported shortly after ZGA onset, we further reveal their internal subcluster architecture and characterize their spatial relationships with surrounding chromatin and active transcription sites. These observations suggest a highly organized nanoscale coupling between chromatin structure and the transcriptional machinery during genome activation.

We are currently expanding this work to generate a highly multiplexed dataset encompassing histone H3, RNA polymerase II, pioneer transcription factors, epigenetic modifications, architectural proteins, and active transcription sites. Together, these datasets aim to uncover coordinated nanoscale organization of chromatin and transcriptional regulators during developmental reprogramming. Our study establishes FLASH-PAINT as a powerful platform for interrogating the structural and functional dynamics of genome activation in zebrafish embryos.

FLASH TALKS

#60 • Decoding Cis-Regulatory Grammar of the Maternal-to-Zygotic Transition in Zebrafish Using Synthetic STARR-Seq

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Yale University School of Medicine

The maternal-to-zygotic transition (MZT) marks a critical developmental window in which control of gene expression shifts from maternally deposited products to activation of the zygotic genome. Although key transcription factors (TFs) such as Pou5f3, Nanog, and SoxB1 have been identified as central regulators of zebrafish zygotic genome activation, the cis-regulatory grammar that encodes their combinatorial and temporal control remains poorly defined. In particular, how short sequence motifs integrate transcription factor binding to drive stage-specific enhancer activity is not well understood. Here, we employ Self-Transcribing Active Regulatory Region sequencing (STARR-seq) using a fully synthetic library composed of defined hexamer regulatory sequences to interrogate cis-regulatory logic during the zebrafish MZT. By assaying enhancer activity of synthetic elements in embryos collected around genome activation, we generate a high-resolution, quantitative map of sequence features that drive temporal transcriptional output. Our analysis identifies distinct classes of synthetic regulatory elements with preferential activity at early-zygotic, or late-zygotic stages. Hexamers enriched for Pou5f3, Nanog, and SoxB1 binding motifs exhibit strong activation coincident with zygotic genome activation, while other sequences and their cognate TFs show maternal-stage repression or delayed activation. We interrogate these motifs using TF-specific degron strategies and reveal how short regulatory motifs encode developmental timing and transcriptional potency during early embryogenesis. Together, this work demonstrates the power of synthetic STARR-seq to uncover fundamental cis-regulatory principles governing the zebrafish MZT and provides a framework for systematically decoding enhancer grammar underlying developmental transitions.

EMBO Workshop

POSTER COMMUNICATION

#61 • The Role of Endogenous Retroviruses in Mouse-Specific Placenta Development

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Placenta is an essential organ in fetus development, mainly functioning as a fetal-maternal interface and secreting hormones. While its importance, its gross morphology and internal structure display large heterogeneity across eutherians. Placenta structure is determined by th variation and number of internal cells, and this suggest that some species-specific mechanisms regulate cell proliferation and cell fate decision in placenta development. Recent studies showe that endogenous retroviruses (ERVs), which potentially can drive host genome evolution, become active specifically in early embryos and developing placenta due to genome-wide DNA hypomethylation. While the increasing number of evidence suggest that ERVs plays an important role for cis-regulatory element, functionality of ERVs transcription itself in placenta development is still unknown.

We identified MMERVK10C that are highly expressed in extra-embryonic ectoderm. To find out the role of MMERVK10C, we utilized trophoblast stem cells (TSCs), which is the in vitro model of mouse placenta development, and established the experimental system to knockdown the MMERVK10C RNA in TSCs. Antisense-Oligonucleotides (ASOs) mediated MMERVK10C knockdown compromised cell proliferation capacity and repressed the expression of some transcription factors which are dispensable for undifferentiated maintenance in TSCs. In vitro differentiation assay revealed that the knockdown of MMERVK10C causes the differentiation bias into trophoblast giant cells, and this suggests that MMERVK10C is involved in cell fate choice in TSCs differentiation. These data suggest that MMERVK10C, which evolved only in Mu genome, is critical for normal mouse placenta development and acts as a factor that regulates the mouse-specific mechanism in placenta development.

POSTER COMMUNICATION

#62 · Functional Characterization of an Alternative Human TFAP2C Promoter

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TFAP2C is among the earliest transcription factors activated during human embryonic development. Despite its essential role in human development and in cell models of human embryos, the mechanisms by which TFAP2C regulates stem cell lineage specification remains incompletely understood. RNA-sequencing data of human embryos suggest a previously poorly annotated alternative promoter for TFAP2C (promoter 2), located upstream of the previously known promoter (promoter 1). We activated both TFAP2C promoters in wild-type and promoter knockout human embryonic stem cells using CRISPRa. In addition, we overexpressed TFAP2C variants that are produced by each promoter in human embryonic stem cells and studied their protein-protein interactions. Our results demonstrate that TFAP2C can be expressed from an alternative promoter, resulting in an N-terminal variant that alters its transactivation potential and protein-protein interactions. Promoter usage and N-terminal variation also change the efficiency of TFAP2C-induced trophoblast-like differentiation of human embryonic stem cells. A deeper understanding of TFAP2C promoter usage and protein function can advance understanding of its role in early human embryonic development and in stem cell models that recapitulate this process.

POSTER COMMUNICATION

#63 · NR5A2 Orchestrates the Totipotency-to-Pluripotency Transition with Lineage-Determining Transcription Factors

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Pioneer transcription factors are crucial for regulating transcriptional programs throughout preimplantation development. In mice, the orphan nuclear receptor NR5A2 was identified as a transcriptional regulator during both zygotic genome activation and the transitional stages to the blastocyst. However, how NR5A2 facilitates these developmental transitions remains unclear. Here, we determined the chromatin binding profiles of NR5A2 from the 2-cell to morula stages. We identified the 8-cell stage as a key transitional stage where NR5A2 binding shifts towards a pluripotency profile, coinciding with strong binding of KLF5 and GATA6. In vitro assays revealed that NR5A2, KLF5, and GATA6 can bind to the same nucleosome, suggesting that they can simultaneously function at the same chromatin context. While zygotic knockdown of Nr5a2 affected both the transcriptional program and active CHROMATIN in 8-cell embryos, double knockdown of Nr5a2 and Klf5 resulted in stronger effects and caused a morula-stage developmental arrest, suggesting co-regulatory roles of these factors. Furthermore, NR5A2 regulates transcription of both Klf5 and Gata6. We therefore provide evidence for a feed-forward regulatory mechanism in which NR5A2 promotes expression of lineage determination factors and these together co-regulate transcriptional reprogramming during the totipotency-to-pluripotency transition.

POSTER COMMUNICATION

#65 · Genome Folding by Cohesin Loop Extrusion Influences Minor Zygotic Genome Activation

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Genome folding by cohesin-mediated loop extrusion has been proposed to regulate gene expression. Although cohesin loss has merely moderate effects on transcription in cells under steady-state conditions, stronger transcriptional changes were observed during differentiation, implying that loop extrusion is more important for regulating gene expression during development. We hypothesized that cohesin loop extrusion promotes minor zygotic genome activation (ZGA) in the one-cell embryo since this transcriptional “awakening” reflects an extreme developmental transition. Single-nucleus Hi-C studies previously revealed that zygotic genome architecture consists of loops and weakly insulated topologically associated domains (TADs) that depend on SCC1-cohesin and CTCF (Flyamer, Nature 2017; Gassler, EMBO J 2017; Dequeker, Nature 2022). Zygotic loops and TADs are strengthened by loss of the cohesin release factor WAPL and further strengthened by loss of additional barriers mediated by MCM complexes (Dequeker, Nature 2022). In contrast, chromatin-bound SMC3 cohesin subunit is challenging to detect in zygotes and increases from 2- to 8-cell embryos (Yu, Nature 2025), raising the question whether genome architecture has gene regulatory functions in zygotes. To test whether loop extrusion influences minor ZGA, we examined gene expression in maternal conditional knockout zygotes lacking Scc1 or Wapl. Loss of SCC1 and WAPL resulted in ~600 and >1000 differentially regulated genes, respectively. The majority of downregulated genes are normally activated during the maternal-to-zygotic transition. These results suggest that loop extrusion and cohesin dynamics promote proper expression of a subset of minor ZGA genes. We conclude that zygotic genome architecture influences gene expression at the start of life.

EMBO Workshop

POSTER COMMUNICATION

#66 · Pronuclear Transfer Restores Epigenetic Reprogramming and Genome Activation in Padi6-Deficient Mouse Embryos

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Padi6 is a maternal effect factor essential for preimplantation embryo development. Loss of PADI6 results in severe developmental defects, characterized by embryo developmental arrest (EDA), failure of embryo genome activation (EGA) and widespread epigenetic dysregulation. In this study, we applied pronuclear transfer (PNT), a technique transferring nuclear DNA from a zygote with defective cytoplasm into healthy recipient cytoplasm, to restore the developmental competence and epigenetic landscape of Padi6-deficient mouse embryos.

Our results demonstrate that PNT efficiently rescues EDA, increasing the blastocyst formation rate (40/44, 90.91%) compared to Padi6-KO embryos (2/52, 3.85%; $P < 0.0001$), reaching levels comparable to wild-type controls (30/35, 85.71%). Single embryo proteomic analyses revealed that PNT-reconstructed embryos display a normal proteomic profile at the 2-cell stage unlike Padi6-deficient embryos. Notably, the increased abundance of multiple EGA and post-EGA proteins in the PNT group suggests a restoration of the EGA program, which is impaired in Padi6-deficient embryos. Previous studies have shown that Padi6 deficiency induces a hypermethylated state at the 2-cell stage, potentially due to mis-localization of DNA methylation regulators. We found that PNT corrected the aberrant nuclear localization of a DNA methylation maintenance factor, UHRF1. Furthermore, bisulfite sequencing confirmed that PNT embryos achieved a global DNA methylation level at the blastocyst stage comparable to those of wild-type controls.

Together, these findings provide strong evidence that PNT can bypass cytoplasmic defect-induced EDA while restoring the epigenetic and proteomic landscapes required for early embryonic development, highlighting its potential as a strategy to rescue maternal effect gene-associated infertility.

POSTER COMMUNICATION

#67 · Germinal Vesicle Transfer Uncovers Cytoplasmic Control of Age-Dependent Oocyte Competence

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Maternal aging is associated with reduced oocyte developmental competence, yet whether age-related defects originate from the nucleus, or the cytoplasmic environment remains unclear. To distinct nuclear versus cytoplasmic contributions to age-dependent oocyte developmental competence, we performed germinal vesicle transfer (GVT), transferring nuclei from advanced maternal age (AMA) (56-week-old; approximately equivalent to 36-year-old humans) and very AMA (70-week-old; 45-year-old humans) mouse oocytes into young cytoplasm (8-week-old; 25–30-year-old humans).

Very-AMA oocytes displayed a significantly reduced meiotic maturation rate (48.07%) compared with young oocytes (77.22%), with aged oocytes showing an intermediate phenotype. Strikingly, transfer of GV nuclei from very-AMA oocytes into young cytoplasm restored meiotic maturation to levels comparable to young controls, demonstrating that age-associated defects at the GV stage are predominantly imposed by the cytoplasmic environment rather than the nucleus.

To define the molecular basis of this cytoplasmic control, we performed single-oocyte proteomic profiling of GV and metaphase II oocytes across the three age groups and in GVT-reconstructed conditions. In parallel, copy number variation sequencing young and AMA oocytes and embryos across key developmental stages, including MII, 2-cell, and blastocyst, was used was used to capture age-associated changes in ploidy and assess whether GVT restores these defects.

Together, these findings indicate that the oocyte cytoplasm plays a dominant role in age-dependent competence and suggest that cytoplasmic aging represents a key barrier to successful embryonic development. Ongoing proteomic and genomic analyses aim to define the molecular features underlying this cytoplasmic control and to assess the extent to which GVT can restore age-associated defects.

FLASH TALKS

#69 · The Relationship Between Chromatin Dynamics and Gene Mobility During Zebrafish ZGA.

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During embryonic development, chromatin transitions from a dynamic and loosely organized state to a more structured state within the nuclear environment. This transition accompanies the onset of transcription during zygotic genome activation (ZGA). How the reorganization of chromatin structure impacts, or is impacted by, individual gene motion remains unclear.

To address this, we developed a live-imaging approach to measure both global chromatin dynamics and individual gene mobility during ZGA in zebrafish. Using spinning-disk confocal-microscopy combined with optical flow-based analysis, we generate spatially-resolved maps of chromatin mobility across developmental stages. In parallel, we track individual gene loci to directly probe their positioning and motion within the evolving nuclear environment. This dual approach allows us to determine whether individual genes follow the movement of surrounding chromatin or instead exhibit distinct, locus-specific dynamics.

We find that chromatin mobility is higher in the nuclear interior than at the periphery at all stages. This mobility decreases during ZGA, with the largest change at the oblong stage. When comparing global chromatin mobility with individual gene dynamics, we find that inactive genomic loci display a mobility pattern similar to bulk chromatin, whereas genes activated during ZGA exhibit increased mobility upon transcriptional onset. This suggests that gene activation can facilitate escape from chromatin constraints.

Ongoing work includes comparison of the gene dynamics and the chromatin immediately surrounding them, as well as local and global perturbations of transcription and chromatin organization. With these, we aim to disentangle local from global chromatin-driven dynamics and uncover the mechanisms governing gene mobility.

POSTER COMMUNICATION

#70 · Transcription Initiation Base Choice During Genome Activation and Early Development

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Transcription start site (TSS) selection represents a fundamental but underexplored layer of gene regulation with the potential to shape mRNA stability, translation and cellular phenotype. While alternative TSS usage has been observed in cancer and development, the biological significance of TSS choice, particularly pyrimidine (Y/C) initiating transcription, remains poorly understood. Our work shows that C initiation is dynamically regulated during the maternal to zygotic transition and during subsequent differentiation stages in zebrafish development. Using single-cell CAGE sequencing, we capture promoter activity at a previously unachieved cellular resolution and show that single-cell TSS usage recapitulates bulk trends while revealing substantial promoter level heterogeneity within cell types and states. We identify distinct populations of cells characterised by high C initiation in genes which drive expression of metabolic and growth-associated genes, suggesting that C-initiation marks metabolic adaptability. Together, our results establish TSS selection as a conserved and previously underappreciated source of transcriptomic diversity in development. Our work also demonstrate how C-initiation diversifies mRNAs and selectively channel them towards mTOR-dependent translation control, similarly to 5'TOP mRNAs and thus providing a biological function to initiation base switching.

EMBO Workshop

POSTER COMMUNICATION

#71 · Competition for Resources in Transcription

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Transcription is essential for normal development. Simply speaking, it is regulated by gene-specific transcription factors (TFs) that bind to DNA and recruit general transcriptional machinery (GTFs). Recent studies by others and us have shown that GTFs can be limiting (Ugolini et al., 2024; Ji et al., 2023). This raises the possibility that TFs compete for this machinery and determine where the machinery goes.

To investigate this possibility, we focused on Nanog, a major regulator of zebrafish ZGA. We analyzed transcriptome changes in Nanog mutants and found that, in addition to the expected downregulated genes, many genes were upregulated. These genes are not bound by Nanog, but are enriched for other TF binding-sites. This suggests that the presence of Nanog limits the activity of genes regulated by these other TFs, and that Nanog is better at recruiting GTFs than other TFs.

Because intrinsically disordered regions (IDRs) mediate interactions with the GTFs, we tested whether IDRs of Nanog and other TFs differ in recruitment strength using a reporter assay. Excitingly, Nanog IDRs indeed drive stronger transcriptional activation than IDRs of other TFs when hooked up to the same DNA-binding domain.

Next, to investigate which component of transcriptional machinery is limiting in the presence of Nanog, we analyzed their Pol II occupancy. Pol II profiles indicated constraints on initiation and elongation steps, suggesting Nanog limits the recruitment of different machinery components.

Together, our results provide further evidence for competition in transcription regulation and highlight the role of TF strength in shaping this competition.

POSTER COMMUNICATION

#72 · Selective HDAC6 and HDAC8 Control of Early Zebrafish Development

Sabrina Carbone, Gaia Galassi, Loredana Brioschi, Anna Marozzi, Paola Viani, Alex Pezzotta, Anna Pistocchi

University of Milan, Dept. Biometra

Early embryonic development requires extensive epigenetic reprogramming to enable genome activation and the establishment of developmental gene regulatory networks. Histone deacetylases (HDACs) are central regulators of chromatin and protein acetylation dynamics, yet how specific HDACs coordinate developmental reprogramming in vivo remains poorly understood.

Here, we use zebrafish embryos as a vertebrate model to investigate how selective modulation of HDAC6 and HDAC8 reshapes developmental programs during early embryogenesis. Combining genetic and pharmacological perturbations with in vivo proteome and acetylome profiling, we dissect the contribution of acetylation-dependent regulation to early developmental transitions. Untargeted proteomic analysis identified over 3,000 proteins expressed during early development, while acetylome mapping revealed a restricted set of acetyl-lysine sites whose regulation is largely independent of changes in total protein abundance. Normalization of acetylation levels to corresponding protein expression uncovered site-specific acetylation events directly associated with developmental regulation rather than global proteome remodeling.

Functional enrichment analyses highlighted acetylation-dependent modulation of pathways involved in cytoskeletal organization, sarcoplasmic structure, metabolism, and developmental signaling, including WNT- and Hedgehog-associated processes. Perturbation of HDAC6 and HDAC8 activity during defined developmental windows resulted in altered lineage specification, tissue organization, and morphogenetic outcomes, indicating a strong temporal dependency of acetylation-based control during genome awakening.

Together, our results demonstrate that selective HDAC-mediated control of the embryonic acetylome represents a key regulatory layer of developmental reprogramming. By integrating zebrafish embryo models with quantitative proteome and acetylome analyses, we provide a systems-level view of how epigenetic regulators fine-tune genome activation and developmental trajectories in vivo.

POSTER COMMUNICATION

#73 · Gene-Regulatory Mechanisms Governing the Timing of Mammalian Embryonic Genome Activation

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The elucidation of gene-regulatory mechanisms that kickstart transcription of the embryonic genome during the maternal-to-zygotic transition (MZT) in mice has been obscured by the uncertainty in developmental timing associated to embryos obtained through natural mating. Our group has established a framework that combines in vitro fertilization with low-input sequencing technologies, providing novel insights into the dynamics of chromatin accessibility and gene expression underlying embryonic genome activation (EGA) in precisely staged embryos. This has revealed the sequential progression of changes in the transcriptome across short time intervals within the 2-cell stage, revealing the expression kinetics of known key players in major EGA and hightling novel factors as putative regulators of this developmental transition. Here, we functionally dissect if and how these factors play a role in the timing of transcription elongation during the MZT.

POSTER COMMUNICATION

#75 · Visualizing En-Pr Contact Using Chromatin Expansion Microscopy

Ayushi Hegde, Caroline Hoppe, Antonio Giraldez

Yale University

Zygotic genome activation (ZGA) requires interactions between enhancers and promoters, yet the physical and molecular mechanisms by which these activate transcription are not fully understood. Understanding how cis-regulatory elements and the transcriptional machinery converge at individual gene loci requires advanced spatial genomics tools. We are currently adapting DNA fluorescence in situ hybridization (FISH) to visualize enhancers and promoters by ChromExM. In order to benchmark this approach for labeling of nonrepetitive loci, we have devised three strategies targeting a set of “control” gene regions with DNA FISH probes. First, to determine the minimum genomic length capable of producing FISH signal in ChromExM, we tested labeling of increasingly shorter subregions. Second, to determine the minimum resolvable distance between targets, we labeled subregions separated by an increasingly shorter gap. Third, to determine the minimum detectable density of probes, we tested labeling using 75, 50 or 25 percent of readouts. At this meeting, we will present our progress on this project. Based on the minimum requirements that we determine for FISH signal in ChromExM, it will be possible to resolve enhancer-promoter pairs active in early ZGA, together with their associated transcriptional machinery and nascent RNA. In summary, we leverage a new approach to visualize the chromatin landscape during a key window of transcriptional activation in development, which in the future will allow us to explore relationships between contact, transcription and epigenetic marks.

POSTER COMMUNICATION

#76 · Channel-Dependent and Independent Functions of CFTR Orchestrate Early Developmental Reprogramming

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Embryonic development requires the coordinated awakening of the genome and the integration of signaling cues that guide patterning, lineage specification, and organogenesis. Increasing evidence indicates that proteins classically associated with differentiated cellular functions can acquire non-canonical roles during early developmental reprogramming.

Here, we investigate the role of CFTR as a developmental regulator during early embryogenesis using zebrafish embryos. By combining genetic loss-of-function and gain-of-function approaches with temporal pharmacological modulation, we uncover distinct channel-dependent and channel-independent functions of CFTR that operate during sensitive developmental windows. We show that CFTR modulates key developmental signaling pathways, including WNT signaling, through protein–protein interactions independently of its ion-channel activity. This non-canonical function influences primitive haematopoiesis and myeloid lineage establishment, as reflected by altered neutrophil activation. In contrast, CFTR ion-channel activity at the plasma membrane is specifically required for left–right patterning and correct cardiac looping, revealing a functional uncoupling of CFTR activities during morphogenesis.

To explore how enhanced CFTR activity impacts developmental programs, we used pharmacological activation as a controlled perturbation during early embryogenesis. CFTR activation during epiboly reshapes both signaling-dependent and channel-dependent developmental outputs, highlighting the importance of dosage and timing during early genome activation. These findings indicate that increased activity of a single multifunctional protein can rewire developmental trajectories when applied during critical phases of embryonic reprogramming.

Together, our study identifies CFTR as a dual-function regulator that integrates signaling and morphogenetic processes during early development, illustrating how modulation of protein activity during genome awakening can influence cell fate decisions and organ patterning.

POSTER COMMUNICATION

#77 · Interplay Between DNA-Templated Processes Regulating Embryonic Genome Activation

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Embryonic genome activation (EGA) is a crucial and highly regulated process that occurs during early development, replacing the maternally inherited proteins and transcripts with embryonic genes. Despite being conserved, how timely activation of the embryonic genome is regulated remains uncertain. Epigenetic modulators such as transcription factors, 3D chromatin structure, DNA replication and chromatin assembly shape EGA, yet their molecular regulation is largely elusive. In mouse embryos, DNA replication occurs concurrently with EGA at the 2-cell stage. Replicated DNA must be properly packaged by histones and organized by the chromatin assembly machinery.

We designed two objectives to finely dissect the interdependency (if any) between the different DNA-templated processes of DNA replication, chromatin assembly and EGA. To this end, I am employing low-input genomics in mouse embryos to elucidate if replication timing coincides with gene transcription at the beginning of EGA. Using optimized in-vitro fertilization (IVF), we can achieve precise control over the timing of EGA. Transient chemical inhibition of DNA replication, without impairment of subsequent embryonic development, is used to analyze its impact on EGA. Preliminary single-embryo RNA-seq data from treated embryos suggests genes expressed during EGA that are potentially influenced by DNA replication block. These results indicate that blocking DNA replication in the 2-cell stage mouse embryo can lead to adverse effects on EGA, suggesting a link between the two processes. Future work, through modulation of chemical perturbations, will allow us to identify key regulators and pathways involved in directing the gene expression changes during EGA.

EMBO Workshop

POSTER COMMUNICATION

#79 · Integrated Transcriptomic Analysis Reveals Metabolic Remodeling and Gene Expression Networks Related to Human 8-Cell-Stage Embryo-like Cells

Pauliina Paloviita, Sonja Nykänen, Sandra Harjuhaahto, Heli Grym, Reetta Santaniemi, Henna Tyynismaa, Rubén Torregrosa-Muñumer, Sanna Vuoristo

University of Helsinki

Human early development is challenging to study due to limited samples and cell numbers. The emergence of 8-cell-stage (8C) embryo-like cells (8CLCs) offers new opportunities to understand embryonic genome activation (EGA) in humans. Our research compares and characterizes 8CLCs from various stem cell-based systems to determine how well these models reflect human early embryonic development. Using single-cell RNA sequencing datasets from multiple studies, we integrate data to identify key gene co-expression modules, transposable element expression, and biological processes recapitulated in 8CLCs. We identify both mature and intermediate 8CLCs, with the Yoshihara and Mazid datasets best representing 8C embryos. 8CLCs show remodeling in energy and RNA metabolism, regulation of RNA splicing, and ribosome biogenesis, mirroring human 8C embryos. Our findings underscore the importance of distinguishing mature 8CLCs from partially reprogrammed cell states to improve their use as models for human EGA.

POSTER COMMUNICATION

#83 · The Molecular Mechanisms of Replication and Transcription Coordination

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There is a long-standing assertion that RNA Polymerase II (Pol II) is evicted from chromatin during replication to avoid transcription-replication conflicts that can lead to DNA damage. However, recent observations in both developmental and disease contexts suggest that the relationship between replication and transcription machinery may be more complex than avoidance. We investigated the coordination of replication and transcription in blastoderm stage Drosophila embryos using correlative lattice light-sheet imaging of transcription and replication machinery. Prior to the major wave of Zygotic Genome Activation (ZGA) interphase times in these embryos last on the order of 10 minutes or less, during which both replication and transcription occur. These rapid cycles provide a powerful context to study the temporal and spatial coordination of transcription and replication. Our imaging data shows that Pol II is retained at a subset of active replication sites marked by Proliferating Cellular Nuclear Antigen (PCNA). Furthermore, we find that these sites of retention are early replicating regions and Pol II is excluded from late-replicating regions. This differential retention is more pronounced as the cell cycle elongates after ZGA. To probe the functional significance of this retention, we examined how transcriptional perturbations affect replication timing. We have found inhibiting transcription initiation disrupts the replication timing program. Our data suggest that rapid transcriptional initiation at active developmental genes marks them for early replication by influencing origin selection and may allow for rapid transcriptional reactivation following DNA synthesis.

POSTER COMMUNICATION

#84 · DUX4 Shapes the 3D Genome at TEs Via RNA Polymerase II

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Transcription factor DUX4 is expressed during the human oocyte-to-embryo transition, concomitant with chromatin reorganization and embryo genome activation (EGA). DUX4 is known to induce extensive chromatin opening and activate EGA genes as well as transposable elements (TEs). Here, we show that DUX4 binding is associated with RNA polymerase II accumulation and increased chromatin insulation at specific genomic loci. Notably, these loci overlap TEs, suggesting that RNA polymerase II accumulation at DUX4-bound TEs may contribute to genome reorganization in early human development.

POSTER COMMUNICATION

#85 · An Endogenous Retrovirus Plays an Essential Role in Zebrafish Development

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Endogenous retroviruses (ERVs) have shaped vertebrates' genomes, but the extent of their influence on evolution, functionality and disease remains to be elucidated. Although most ERVs have lost the ability to mobilize they are transcribed at stage- and tissue-specific manners during embryonic development raising important questions about how their expression is regulated and whether they are functionally important during development.

Here, we focus on the ERV1-3 subfamily, a member of the zebrafish ERV1 superfamily that shows specific expression in the axial and paraxial mesoderm during development. We show that this expression is primarily driven by three recently inserted full-length copies that contain only the pol gene and harbour a unique combination of binding sites for specific mesodermal transcription factors, including tbx16, a Nodal-induced transcription factor. Indeed, we demonstrate that ERV1-3 expression is mediated by the Nodal signalling pathway, an essential pathway for early embryonic development. Furthermore, depletion of ERV1-3 RNA using CRISPR-RfxCas13d resulted in early and late developmental phenotypes that strikingly resemble Nodal mutants, like cyclopia, open neural tubes and somite defects. Importantly, Nodal inhibition and ERV1-3 depletion phenotypes were partially rescued overexpressing a predicted transcript derived from ERV1-3 potentially encoding an RNase H domain. Taken together, our findings demonstrate that a relatively young endogenous retrovirus has been co-opted for developmental roles within one of the most evolutionarily conserved signalling pathways.

EMBO Workshop

POSTER COMMUNICATION

#86 · Reconstructing Oocytes Via Haploidization: Donor Cell Type Determines Spindle Organization and Euploidy Outcomes

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Women lacking functional oocytes currently rely on oocyte donation or adoption. Haploidization has emerged as a potential alternative, aiming to reprogram and reduce a diploid somatic genome to support embryonic development and enable genetic parenthood.

Although haploidization has previously yielded live offspring, developmental efficiency remains low, and the mechanisms governing spindle assembly and ploidy remain poorly defined. We hypothesized that embryonic stem cells (ESCs) may better support this process than differentiated somatic cells, as their epigenetic state is already reprogrammed.

In this study, we assessed the developmental competence and euploidy of mouse pre-implantation embryos following haploidization by comparing different donor cell types. Cumulus cells from different genetic backgrounds (B6DF1 n=164, and B6 n=160) and ESCs (n=101) were subjected to haploidization and embryo development was tracked to the blastocyst stage. Spindle-chromosome organisation (n=59) was examined by live confocal imaging, and 45 embryos underwent CN-Vseq for ploidy analysis.

Embryos resulting from haploidization showed 77% 2-cell formation (71% for B6D2F1 cumulus cells; 96% for B6 cumulus cells; 65% for ESCs), yet blastocyst formation was rare. Live imaging revealed striking donor-dependent differences in spindle organization. Reconstructed oocytes derived from ESCs most frequently formed bipolar spindles with aligned chromosomes (55%), yet consistently resulted in aneuploid embryos, whereas cumulus cell–derived reconstructions rarely displayed normal spindle organisation (4%) but yielded euploid embryos in 8% and 14% of cases for B6D2F1 and B6 donors, respectively.

Together, these findings show that donor cell identity shapes spindle organization and euploidy but uncouples spindle morphology from chromosomal integrity.

POSTER COMMUNICATION

#88 · Maternal mRNA Modulates the Severity of the zbtb17 Phenotype

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Cardiovascular diseases (CVDs) remain the leading cause of mortality worldwide, with cardiomyopathies representing a subgroup with a strong genetic basis. Dilated cardiomyopathy, in particular, has been linked to variants in ZBTB17, a gene encoding a transcription factor involved in cell cycle regulation and apoptosis in response to DNA damage. To investigate the role of zbtb17 in cardiac development, a CRISPR/Cas9-generated zbtb17 knockout (KO) zebrafish line was established. Homozygous zbtb17 mutants exhibit pronounced cardiac hypoplasia from 96 hours post-fertilization (hpf) onwards. This phenotype is characterized by reduced cardiomyocyte (CM) proliferation starting at 72 hpf accompanied by increased apoptosis at later developmental stages. Interestingly, the penetrance of this phenotype varies between homozygous mutants,with approximately half exhibiting a pronounced phenotype, while the remainder displayed a milder presentation. Hypothesizing that maternal zbtb17 mRNA could play a role in this genotype-to-phenotype variance, we performed knockdown experiments. Zbtb17 knockdown resulted in a high rate of phenotypic embryos, when using a Start-Mopholino, targeting the TSS of zbtb17 mRNA. Whereas the use of a splice Morpholino targeting the splice donor site between exon 6 and intron 6 showed a penetrance comparable to homozygous mutants. Injection of Start Morpholino in zbtb17 mutants, resulted in a 100% penetrance, suggesting that the variance can in fact be due to maternal mRNA deposition.

POSTER COMMUNICATION

#89 · FISH-Based Visualization of Small Non-Coding RNAs

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Small non-coding RNAs (sncRNAs) are regulatory transcripts shorter than 200 nucleotides that do not encode proteins yet play critical roles in diverse biological processes, including development and stress response. Despite their functional importance, imaging sncRNAs has remained technically challenging due to constraints in probe design.

Here, we adapt signal amplification by exchange reaction (SABER) to enhance fluorescence in situ hybridization (FISH) signals specifically from sncRNAs.

We first validated the specificity of sncRNA detection by transfecting synthetic sncRNA mimics to culture cells, followed by FISH-based visualization. Subsequently, we demonstrated the ability to detect dynamic changes in endogenous sncRNA levels upon cellular perturbations. We are currently investigating the spatial organization of sncRNAs in the context of differentiation and early embryonic development.

Overall, we demonstrate the feasibility of directly visualizing endogenous sncRNAs at subcellular resolution, enabling further investigation of these understudied RNA classes without the need for overexpression systems or fluorescent tagging that may perturb the cellular physiology.

FLASH TALKS

#90 · Spatial Asynchrony of RNA Modifications Orchestrates Maternal-to-Zygotic Transition

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Early embryogenesis depends on the maternal-to-zygotic transition (MZT), which encompasses maternal factor clearance and zygotic genome activation (ZGA), processes essential for early cell fate specification. Despite its importance, the spatiotemporal regulation of the MZT remains incompletely understood. We previously identified a spatially and temporally gradual pattern of ZGA in Xenopus embryos, in which blastomeres at the animal pole (AP) activate transcription earlier than those at the vegetal pole (VP), correlating with sequential germ layer specification. However, the molecular mechanisms underlying this spatial asynchrony are unknown. To determine whether regionally enriched factors regulate ZGA timing, we performed region-specific proteomic analyses of AP and VP tissues from early Xenopus embryos. Gene ontology (GO) analysis revealed a striking enrichment of N6-methyladenosine (m6A) RNA-binding proteins in the AP, including the m6A writer METTL16 and the readers YTHDF1/2. Western blot analyses confirmed that these m6A regulators are consistently enriched in the AP from the egg stage through the mid-blastula transition, with ongoing translation in both regions. Using Nanopore direct RNA sequencing, we found a significantly higher abundance of m6A-modified transcripts in the AP compared to the VP, supporting spatially asynchronous m6A deposition. GO analyses showed that AP-enriched m6A-modified RNAs are associated with RNA splicing and processing, whereas VP-enriched targets are linked to fatty acid metabolism. Pharmacological inhibition of METTL16 or YTHDF1/2 disrupted early embryonic development, a finding currently being validated using Trim-Away–mediated depletion. Together, our results reveal a previously unrecognized spatial role for RNA epigenetic regulation in coordinating the MZT during early embryogenesis.

EMBO Workshop

POSTER COMMUNICATION

#92 · Mapping Embryonic Cranial Neural Crest Fate Decisions Across Development Using Single-Cell Multiomics

Inês Naidenko-Rosa[1], Mona Christensen[1], Nikita Vaulin[1], Igor Adameyko[1,2]

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Cranial neural crest cells (CNCCs) exhibit a unique developmental plasticity, transiently acquiring mesenchymal characteristics that enable their contribution to craniofacial cartilage, bone, and connective tissue, typically derived from mesoderm. How this expanded potential arises, and why it is restricted to the cranial region, remains to be fully understood.

Here, we aim to map embryonic cranial neural crest fate decisions across development using single-cell multiomic profiling. We applied 10x Genomics multiome to simultaneously profile gene expression and chromatin accessibility in mouse CNCCs from embryonic days E8.5–E10.5, and compare their developmental trajectories to mesodermal lineages whose programs are thought to be co-opted by CNCCs. To address axial specificity, we included trunk neural crest, which displays more restricted differentiation potential. Adult dermal populations were included to investigate whether cranial neural crest–derived cells retain lineage-specific epigenetic signatures or instead converge epigenetically with mesoderm-derived cells, enabling us to assess long-term epigenetic fingerprints across distinct embryonic origins.

In parallel, we performed CUT&RUN profiling of TWIST1, a transcription factor known to bias CNCCs toward ectomesenchymal fates and capable of redirecting trunk neural crest identity when activated.

Together, this integrative approach will allow us to define the temporal, axial, and regulatory features that enable CNCCs to generate mesoderm-like derivatives during development.

POSTER COMMUNICATION

#93 · Exploring the Parallels Between Developmental Reprogramming and Regenerative Reprogramming During Axolotl Limb Regeneration

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Salamanders are unique among tetrapods in that they possess the unrestricted capacity to fully regenerate limbs upon amputation. Why is this the only tetrapod taxon harboring such a striking regenerative potential and how did it evolve are two of the biggest outstanding questions in regenerative research. During axolotl limb regeneration, mature connective tissue cells undergo a reprogramming process into a developmental-like, limb-bud-like state which allows them to redeploy the developmental programs required to replace the missing structure. However, how adult axolotl cells derepress these developmental programs remains poorly understood. Here, we investigate whether axolotl limb regeneration involves epigenetic and transcriptional features reminiscent of early embryonic developmental reprogramming. Using transcriptomic and epigenomic analyses of regenerating axolotl limbs, we examine the activation of transposable elements, a hallmark of zygotic genome activation, and assess the expression of early developmental reprogramming factors typically associated with embryogenesis.

POSTER COMMUNICATION

#95 · Analysis of Transcription Initiation in Early Embryonic Development of the Surface and Cave Forms of Astyanax Mexicanus

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Astyanax mexicanus (Mexican tetra) is a powerful model for studying environmental adaptation because it exists as two morphologically distinct varieties: a surface fish phenotype adapted to life in daylight and a cavefish phenotype adapted to permanently dark cave environments. Notably, many of the major phenotypic differences between these forms are thought to arise primarily from changes in gene regulation rather than from protein-coding sequence evolution. However, how early in embryonic development these regulatory differences can be detected-and to what extent they are influenced by inherited (maternal) expression programs-remains largely unexplored.

Here, we used Cap Analysis of Gene Expression (CAGE) to profile promoter activity and transcription start site (TSS) usage across four stages of embryonic development, spanning maternal stages through zygotic gene activation and into pre-hatch development, in both surface and cave forms. These data recapitulate established regulatory differences between the morphs, including upregulation of shhA and shhB in cavefish, as well as pronounced downregulation of the eye-development gene pax6 and the pigmentation-associated gene oca2. Importantly, by including maternal stages, we observe signatures consistent with an inherited expression program that may contribute to early developmental trajectories underlying phenotypic adaptation.

Together, our results provide a developmental timeline for regulatory divergence in A. mexicanus and highlight how early gene regulation-including maternally contributed transcripts-may shape phenotypic outcomes in contrasting environments.

POSTER COMMUNICATION

#96 · Comparative Cryopreservation of Human Ventral Forebrain Organoids Derived from Dravet Syndrome and Reference iPSC Line

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Human brain organoids enable modeling of early neurodevelopment and disease, yet achieving consistent developmental timing across experiments and enabling long-term storage remain key technical challenges for experimental design and reproducibility. Although multiple cryopreservation strategies for neural organoids have been published, their performance across different organoid generation methods and disease-relevant genetic backgrounds remains insufficiently evaluated, particularly for ventral forebrain organoids used in epilepsy-related disease modeling.

We compared published cryopreservation strategies, including CryoStor-based and MEDY-based approaches, in human ventral forebrain organoids derived from induced pluripotent stem cells. The study included both non-disease reference line and patient-derived lines from individuals with Dravet syndrome. Organoids were cryopreserved on day 18 of differentiation and assessed at one and two weeks after thawing to evaluate early recovery. Outcomes were assessed based on morphology and size, immunocytochemical analysis of ventral forebrain progenitor and early neuronal differentiation markers, and functional assays of metabolic activity and viability using AlamarBlue and CellTiter-Glo 3D, relative to matched unfrozen controls.

Preliminary results demonstrate clear method-dependent differences in early recovery. CryoStor-based cryopreservation preserves ventral forebrain organoid architecture and growth consistency following thawing, whereas MEDY-based cryopreservation is associated with reduced structural stability during early recovery. Ongoing analyses examine whether these structural differences are accompanied by changes in developmental marker expression and metabolic activity, particularly in Dravet syndrome patient-derived organoids.

Overall, this study characterizes early recovery following cryopreservation and provides practical guidance for selecting preservation strategies compatible with reproducible developmental and disease-focused ventral forebrain organoid studies.

This study was financed by ABM grant KPOD.07.07-IW.07-0105/25.

POSTER COMMUNICATION

#97 · H3K27me3 Resolves Unique, Spatially Distributed Patterning States in Drosophila Embryogenesis

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Northwestern University

For any organism, defining the body plan during development requires the establishment of positional identity through early patterning systems. During Drosophila zygotic genome activation (ZGA), graded maternal factors position spatially restricted gene patterns through transient patterning networks. Patterning networks ultimately direct Polycomb group (PcG) proteins to establish epigenetic silencing via trimethylation of Histone H3 lysine 27 (H3K27me3) and ubiquitylation of Histone H2A (H2Aub). Although there are clear requirements for PcG silencing in the long-term maintenance of positional information, how patterning networks direct the spatial establishment of these modifications is unclear. A challenge in studying this problem is the embryo is a heterogenous mixture of regulatory states. To overcome this, we utilize mutant embryos that develop uniformly with a single, neuroectodermal identity. Thus, we can resolve the unique epigenetic regulatory state of patterning genes at the onset of patterning through differentiation. We observe that H3K27me3 reflects transcriptionally “off” patterning genes from the onset of ZGA. H3K27me3 dynamics are surprisingly rapid during differentiation; upon initiation of neurogenesis, H3K27me3 is depleted at neural genes. In contrast, H2Aub is retained at PcG targets throughout ZGA and differentiation. We hypothesized that H2Aub deposition reflects a competency to undergo PcG silencing. Consistent with this model, embryos lacking H2A ubiquitin ligase activity fail to rapidly establish H3K27me3 at ZGA. Together, these findings define a regulatory logic for the establishment and maintenance of H3K27me3 as a function of developmental patterning cues.

POSTER COMMUNICATION

#98 · Deciphering the Code of Decay: Codons, Codon-Codon Interactions, Amino Acids, and Peptide Bonds Regulate mRNA Stability

Haejeong Lee, Damir Musaev, Charles Vejnar, Srikar Gopinath, Ethan Strayer, Jean-Denis Beaudoin, Mario Abdelmessih, Antonio Giraldez

Yale University

Translation produces proteins but also influences mRNA stability through codon optimality. The codon optimality is a major determinant of maternal mRNA decay during the maternal-to-zygotic transition (MZT). However, codon optimality has been mainly studied at the level of individual codons. Furthermore, the sequence complexity of endogenous transcripts has prevented us from disentangling codon-specific effects from other features determining mRNA stability. To address this challenge and elucidate how codons, codon pairs, amino acids, and peptide bonds collectively regulate mRNA stability, we designed a massively parallel reporter assay (MPRA) and measured reporter half-lives in zebrafish embryos. Our results showed that the effect of individual codons only partially explains how coding sequence (CDS) regulates mRNA stability. We found that mRNA stability depends on both the identity of neighboring codons and the codon order, as well as amino acids and the peptide bonds between specific amino acids. Moreover, we decoupled translation-dependent and independent RNA stability regulation and characterized them. Translation is a major determinant of mRNA stability; however, some mRNAs are less stable during translation if they contain strong structures or sequence motifs that RNA-binding proteins can bind. Currently, we are investigating how codon pair stability relates to maternal and zygotic transcripts and how codon pair sequence influences mRNA translation. These results will be presented at the meeting. Together, our comprehensive study on the CDS-mediated mRNA stability provides key insights into how codon pair interaction and peptide bond formation influence mRNA stability dependent on translation, expanding our understanding of RNA stability regulation during MZT.

EMBO Workshop

POSTER COMMUNICATION

#99 · Dnd1 Safeguards Post-Specification Germ Cell Fate Via Translational Proteostasis

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Dead end1 (Dnd1) is a vertebrate RNA-binding protein that controls RNA stability and translation and serves as a key regulator of germ-cell development. The role of Dnd1 in zebrafish was extensively studied at the time of primordial germ-cell (PGC) specification. Specifically, the lack of Dnd1 function at this stage leads to the transdifferentiation of PGCs into cells belonging to different somatic lineages. However, the role of the protein at later stages of embryonic development is not well understood. Using temporally controlled knockdown of Dnd1 we revealed a new function of Dnd, which is to control and reduce conflicting differentiation cues that can lead to cell death. Accordingly, PGCs lacking Dnd1 function at these stages maintain germline transcriptional identity but undergo cell-autonomous apoptosis marked by p53-dependent Caspase-3 activation. Single-cell RNA-seq analysis reveals that PGCs lacking Dnd1 at this stage cluster closely with control PGCs and maintain expression of canonical germline transcripts. However, analysis of the proteome revealed extensive post-transcriptional abnormalities observed particularly in trafficking and proteostasis pathways, as well as in metabolic and translational molecular programs.

These findings suggest that Dnd1 functions to prevent incompatible molecular programs from coexisting within the same cell, thereby reducing the need for elimination of cells unable to maintain a stable fate. Together, our results underline the importance of tight regulation over RNA function by RNA-binding proteins in controlling the progress of developmental pathways.

Joint poster with Leon Schmidthuis

POSTER COMMUNICATION

#100 · Differences in Maternal and Zygotic Initiation Grammar After the Whole-Genome Duplication Event in Cyprinus Carpio

Ada Jimenez-Gonzalez[1, 2], Alba Madrero Pardo[3], Yavor Hadzhiev[1], Bojan Zunar[4], Boris Lenhard[5, 6], Damir Baranasic[3, 5, 6], Ferenc Müller[1]

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The maternal-to-zygotic transition (MZT) is a major event in early embryonic development, marked by large-scale reprogramming of transcription initiation and promoter usage. Some genes exhibit different maternal and zygotic initiation grammar on the same promoter known as promoter shifting. However, how large evolutionary events such whole-genome duplication (WGD) affects this regulatory transition during the MZT in allotetraploid species remains largely unknown.

We investigate promoter dynamics during the MZT in the allotetraploid common carp (Cyprinus carpio), which originated from a WGD ~13 million years ago and retained two evolutionarily distinct subgenomes. We hypothesize that singletons, genes which purged one copy after WGD are enriched for housekeeping functions and exhibit more frequent promoter shifting events than ohnologs, genes retaining both copies.

To test this, we analyzed high-resolution CAGE sequencing data from early embryonic stages. We quantified changes in transcription start site (TSS) usage and promoter architecture during the MZT. We observe a promoter shifting enrichment between maternal and zygotic grammar in singleton promoters and homologous regions without evidence of promoter activity, whereas ohnologous promoters show reduced shifts in TSS usage.

These results suggest that singleton promoters are more essential for both maternal and zygotic expression, while ohnolog promoters tend to be expressed either in maternal or zygotic. This pattern supports the idea that singleton genes, enriched for housekeeping functions, present multiple grammars and require adaptable promoter architectures to maintain expression across distinct regulatory stages during early development.

POSTER COMMUNICATION

#101 · Characterization of Chromatin Ultrastructure During Early Pre-Implantation Development

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Following fertilization, chromatin organization is remodeled across genomic and spatial scales as the mouse embryo undergoes epigenetic reprogramming and its genome is transcriptionally activated. However, the underlying nucleosome-level structural basis of this process remains poorly understood. To address this, we use 2-tilt ChromEMT – an electron microscopy technique that visualizes chromatin structure at nucleosome resolution – to characterize changes in chromatin organization during the early stages of mouse pre-implantation development. We developed a novel analysis pipeline to quantify distinct chromatin properties allowing us to link chromatin packaging and topology to nucleosome spacing and spatial organization. We identify embryo stage-specific changes in chromatin organization at the global and local scale, including unique chromatin packing densities. Our results suggest that from the zygote to 8-cell stage, chromatin compacts not only at the scale of domains and compartments, but also at the ultrastructural scale. We also observe the progressive establishment of nucleolar-associated heterochromatin after zygotic genome activation (ZGA). We are currently developing nanogold-based approaches to visualize transcription during ZGA and hope to present the results of these experiments at the meeting. Overall, our results reveal the chromatin structure underlying genome activation and the establishment of totipotency in the mouse embryo, providing a means to define how transcription and histone modifications remodel chromatin structure to ultimately achieve epigenetic reprogramming and ZGA.

POSTER COMMUNICATION

#102 · Decoding Early Head Development Dynamics Through Single-Cell Multi-Omics and Tissue-Specifi c Chromatin Profiling

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Cellular diversity in the brain is established through fi rmly regulated transcriptional decisionsmade early in embryos development. Our previous research identifi ed the pioneer transcriptionfactor (PTF) Odd-paired (Opa/ZIC3) and Ocelliless (Oc/OTX2) as essential co-regulators of geneexpression in the future Drosophila brain primordium before gastrulation. While bulk RNA-seq,ATAC-seq, and ChIP-seq datasets have defi ned their precise spatiotemporal dynamics underlyininitial lineage specifi cation remain partially understood.

In this study, we apply single-cell multi-omic approaches to simultaneously profi le chromatinaccessibility and transcriptional states in individual head cells across the entire embryo at twodevelopmental stages, before and after gastrulation. To specifi cally interrogate nascent brainspecifi cation, we are developing a targeted strategy to isolate future head cells from the rest ofthe embryo. Using fl uorescent reporters for head-patterning genes, including oc, hunchback(hb), which is broadly expressed in the head, and twin of eyeless (toy), which marks the futurebrain, we employ FACS to selectively capture these early populations. This strategy utilizes oc-dsRed, toy-AausFP1.4, and hb-GFP reporter lines to label nuclei, enabling real-time visualvalidation and image-based sorting of cells at the onset of head and brain identityestablishment. Also, using an ems.MS2.CRISPR-Cas9 reporter, we visualize live Opa–Occoordination in brain gene transcription. Trajectory analyses reveal progressive transitions fromspatially patterned neuroectodermal states to neural progenitor identities, accompanied bydynamic changes in enhancer accessibility at Opa- and Oc-associated regions. Together, thisintegrated sc-multi-omic and tissue-specifi c ChIP-seq approach provides a high-resolutionbackground for dissecting early brain specifi cation at the onset of gastrulation.

EMBO Workshop

POSTER COMMUNICATION

#111 · Novel Male DUX4-Inducible Stem Cell Lines for Studying Embryonic Genome Activation

Katariina Kordemets[1, 3], Lea Mikkola[2], Laura Danti[1], Nina Boskovic[1], Shintaro Katayama[1, 3], Jere Weltner[1, 3], Juha Kere[1, 3]

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We have previously demonstrated that inducible DUX4 expression in the female human embryonic stem cell line H9 induces early embryonic genome activation (EGA) like transcriptional programs. However, in order to study sex-dependent differences, comparable male cell lines have to be established. Here, we generated four doxycycline-inducible DUX4 clonal lines from a male hiPSC background and analyzed their transcriptional responses after induction using scRNA-seq. All clones reproducibly formed an iBM-like cluster expressing EGA marker genes, with pseudotime analysis showing a clear separation of the iBM trajectory from other cell states. This reproduces prior observations in the female H9 hESC line, demonstrating conservation of DUX4-driven programs across pluripotent stem cell origin and genetic sex. These XX and XY lines enable future investigation of sex-dependent regulatory dynamics during DUX4-induced cell state transitions.

FLASH TALKS

#113 • Activation of Replication-Dependent Histone Genes at MZT: The Exception that Proves the Rule

Anna Hakes[1, 2], Geraldine Seydoux[1, 2]

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In embryos with germ plasm, the maternal-to-zygotic transition is delayed in embryonic germ cells compared to somatic cells. In C. elegans and Drosophila, the delay depends in part on germ plasm factors that inhibit positive transcription elongation factor b (P-TEFb) and prevent phosphorylation of the RNAPII C-terminal domain on serine 2 (RNAPII-Ser2P). Because RNAPII-Ser2P is associated with productive elongation, mRNA transcription was thought to be globally repressed in early germ cells.

Unexpectedly, we have identified one class of transcripts that escapes this repression in C. elegans: replication dependent histones (H2A, H2B, H3 and H4). We find that histone genes are transcribed in all cycling embryonic cells including germ cell precursors as early as the 8-cell stage. Depletion of elongation factors PTEF-b and CDK-12 does not block histone transcription, but blocks transcription of other RNAPII transcripts. Interestingly, we find that increasing the length of histone transcripts by inserting GFP is sufficient to create a dependence on cdk-9/12 for transcription and to prevent expression in early germ cells. These findings are consistent with work in human cells reporting PTEF-b-independent transcription of histone H2B (Medlin et al, 2005 EMBO J).

We conclude that, due to their short length, replication-dependent histone genes do not require the elongation form of RNAPII, which permits zygotic expression of histone genes to initiate at the same time in germ and somatic blastomeres during MZT, even while transcription of most mRNAs is blocked.

EMBO Workshop

FLASH TALKS

#114 • Transcription Factor Hub Properties Reflect Enhancer Binding Site Grammar

Samantha Fallacaro[1, 2], Lillian Encarnation[1, 2], Manya Kapoor[1, 2], Apratim Mukherjee[1, 2], Meghan Turner[3], Hernan Garcia[3, 4], Mustafa Mir[1, 2, 5]

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Many transcription factors (TFs) form dynamic, high-concentration clusters, or "hubs". While hubs have been proposed to amplify gene expression, especially during the maternal-to-zygotic transition, it remains unclear how enhancer sequence tunes hub properties, how hubs associate with active genes, and whether these structures drive or reflect TF binding kinetics. We investigated how enhancer grammar dictates properties of hubs formed by the TF, Dorsal, in live Drosophila embryos. To enable these insights, we developed a live imaging-based framework that quantifies the spatiotemporal relationship between TF hubs and active transcription sites in vivo. Using this framework, we measured Dorsal hub properties including local Dorsal enrichment and the persistence of hubs at endogenous and synthetic enhancers during genome activation. We find that Dorsal hubs exhibit gene-specific and tunable biophysical properties: enhancers containing more Dorsal binding sites form more enriched and longer-lived hubs, whereas loci lacking canonical motifs show only transient, incidental interactions. Systematic perturbation of binding-site numbers confirms this relationship. Reducing Dorsal motifs decreases hub enrichment and persistence. Importantly, hub persistence and enrichment show minimal correlation with transcriptional activity beyond what is explained by binding-site number. Thus, both clustering and gene output appear to reflect enhancer-encoded TF occupancy rather than a direct regulatory role for hubs. Together, our results demonstrate that hub enrichment and persistence scale with enhancer binding-site composition within the limits of detection. These findings support a model in which transcription factor hubs are emergent features of TF–DNA interactions encoded by enhancer sequence rather than higher-order regulatory assemblies.

FLASH TALKS

#116 · Transposable Element Activity and RNA Export Form a Feedback Circuit Regulating Embryonic Gene Expression

William Hamilton[1], Yupei You[1], Matthew Ritchie[1], Melanie Eckersley-Maslin[2], Vihandha Wickramasinghe[1, 2]

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[2] *Peter MacCallum Cancer Centre*

Early embryonic transcription is characterised by widespread activation of transposable elements (TEs), normally silenced in somatic cells yet robustly expressed in pre-implantation embryos. In culture, a rare subpopulation of embryonic stem cells transiently adopts a transcriptional state resembling early embryogenesis (2-cell–like cells, 2CLCs), providing a tractable system to investigate how cells accommodate elevated TE activity. Using direct RNA sequencing, we define the gene expression network associated with activation of the stage-specific endogenous retrovirus MuERVL and identify its dominant downstream effect as coordinated induction of paralogous RNA export adaptors related to ALYREF and NELFA.

Concomitant with this state, we observe widespread production of short, misprocessed SINE-derived RNAs that accumulate within nuclear speckles. This phenotype coincides with reduced U6 snRNA abundance, consistent with functional sequestration of RNA polymerase III and perturbation of spliceosomal balance. Cells exhibiting this profile display hallmarks of promoter-proximal RNAPII. We propose that spliceosomal imbalance, driven by limiting U6 snRNA, stabilises RNAPII in a primed yet transcriptionally constrained configuration, thereby maintain a primed transcriptional state.

Functionally, CRISPRi-mediated depletion of ALYREF paralogs collapses the 2CLC state, indicating that amplified RNA export capacity is required for maintenance of this transcriptional program. Together, these findings support a model in which modulation of Pol III activity, spliceosomal composition, RNAPII pausing, and RNA export forms a coordinated adaptive response to elevated SINE transcription. This framework positions TE expression not as transcriptional noise, but as an active driver of regulatory state transitions that remodel nuclear RNA metabolism and gene expression programs.

FLASH TALKS

#117 · Bovine DUXC as a Key Regulator of Embryonic Genome Activation and Cell Reprogramming Toward the 8-Cell like State

Matthias Tisserand Erpeldinger[1], Laurent Boulanger[1], Michelle Halstead[3], Catherine Archilla[1], Sophie Calderari[1], Hervé Acloque[2], Vincent Brochard[1], Elodie Poumerol[1], Luc Jouneau[1], Olivier Dubois[1], Soahary Jean-Rene[1], Cécile Burette[1], Lucas Midoux[1], Amélie Bonnet-Garnier[1], Alice Jouneau[1]

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Background: The group of double homeodomain genes DUX, unique to placental mammals, is expressed in cleavage stage embryos. They are pioneer transcription factors that play a role in orchestrating the transition to totipotency during embryonic genome activation (EGA). In humans, DUX4 is notorious for its link to the disease FSHD (Facioscapulohumeral muscular Dystophy) and its role in EGA. The bovine ortholog DUXC is poorly studied, yet it offers a unique perspective on the evolutionary conservation of mammalian development.

Methods & Results: Using ddPCR, we first defined the precise expression kinetics of DUXC during bovine early development. At the 8-cell stage, we then identified DUXC's target genes through siRNA-mediated knockdown followed by RNA-Seq in in vitro-produced embryos. This approach uncovered over 924 differentially expressed genes and revealed a significant decrease in blastocyst development. Together, these results establish DUXC as a key regulator of bovine EGA.

To assess DUXC's reprogramming potential, we used bovine embryonic stem cells (ESCs) as recipients and optimized conditions for its transient overexpression. This approach successfully reactivated EGA-specific genes upon DUXC induction. To further probe its functional plasticity, we extended our analysis through cross-species overexpression of bovine DUXC in porcine and murine ESCs.

Conclusion: Our findings suggest that bovine DUXC may act as a key regulator of the 8-cell stage transcriptional program, while also exhibiting the capacity to reactivate a totipotent-like signature upon overexpression. These results point to a potential dual role for DUXC in both embryonic genome activation and cellular reprogramming, warranting further investigation.

POSTER COMMUNICATION

#119 · Spatially Patterned Zygotic Genome Activation Fulfills Embryo Quality Control

Wenchao Qian[1], Hui Chen[2], Hongju Lee[1], Matthew Good[1]

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Early embryo development features autonomous, maternally driven cell divisions that self-organize the multicellular blastula or blastocyst tissue. Maternal control cedes to the zygote starting with the onset of widespread zygotic genome activation (ZGA), which is essential for subsequent cell fate determination and morphogenesis. Intriguingly, ZGA onset is often assumed to be uniform at the level of an entire embryo, we find instead that it can be non-homogenous and precisely patterned at the single-cell level. We previously demonstrated a stereotyped spatial and temporal ordering of ZGA in a model vertebrate embryo. Unknown, however, was whether this precise ZGA patterning was required for development. To address this fundamental question, we devised a strategy to spatially control cell divisions that perturb blastula embryo organization. We demonstrate the feasibility of spatially inverting the cell size pattern of embryos and find that these inverted embryos exhibit a re-oriented pattern of ZGA. Mispatterned ZGA along the animal-vegetal axis triggers embryo apoptosis, revealing that gastrula embryos have a built-in quality control system to sense inappropriate ZGA patterning, including regionalized defects in transcription onset. The quality control response is non-autonomous, dependent on an anti-apoptotic signal that suppresses cell death outside the animal hemisphere. These results reveal the requirement of properly patterned ZGA for normal development and the existence of a surveillance system for embryo quality control exquisitely tuned to the spatial and temporal ordering of genome activation and zygotic gene expression. The paper describing these results in under revision and a preprint has been posted to BioRXIV.

POSTER COMMUNICATION

#122 · Identifying Fates of Aneuploid Cells in a Human Blastocyst Model

Rina Sakata[1,2,3], Viviane Rosa[1,2], Sarah Cooper[3], Mi Trinh[3], Sam Behjati[3,4], Roser Vento-Tormo[2,3], Marta Shahbazi[1,2]

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Aneuploidy is frequent in early human embryos but rare in live births, suggesting active quality-control mechanisms taking place. How the fate of individual aneuploid cells is linked to the developmental potential of embryos remains unknown. Using blastoids, a stem cell-based model of the human blastocyst, we have developed a time-course, chromosome-resolved, single-cell, single-blastoid RNA sequencing screen to track aneuploid cell states and developmental outcomes. We have shown that cells carrying specific complex (multi-chromosome) aneuploidies are selectively eliminated, whereas those that persist exhibit disrupted cell-adhesion programs and fail to execute appropriate lineage specification. At the blastoid level, these defects are associated with failed development in a proportion-dependent and chromosome-specific manner. These findings are also validated in human embryos. Together, this work establishes a scalable platform to connect karyotype to cell fate and supports a model in which distinct aneuploidies drive divergent trajectories—selective elimination and impaired lineage specification—to shape human blastocyst development.

POSTER COMMUNICATION

#123 · Coordinated Histone Acetylation and Methylation Establish Cell Type-Specific Transcriptional Programs in the Airway Epithelium.

Marcos Olivera-Gómez, Jose Maria Carvajal-Gonzalez

Universidad de Extremadura

Airway epithelial differentiation depends on tightly coordinated transcriptional programs, but the epigenetic mechanisms linking histone modifications to cell type-specific transcription remain unclear. Here, we show in mice that H3K27 acetylation, together with its recognition by Brd3, and H3K79 trimethylation catalyzed by Dot1l, promote recruitment of the transcription factor Pitx1 to promoters of Notch pathway genes, enabling their activation. Conversely, H3K27ac recognition by Brd4 at transcription start sites of multiciliated cell-specific genes drives their expression and the acquisition of the multiciliated cell fate. These results reveal that distinct epigenetic writers and readers selectively control gene programs in the airway epithelium, providing a mechanistic framework for understanding how basal stem cells integrate chromatin cues to execute precise differentiation programs during early development and regeneration.

EMBO Workshop

POSTER COMMUNICATION

#124 · Exploring whether Adult Tumorigenesis Hijacks Developmental Trophectoderm Programs

Benedetta Mannelli[1,2], Christina Fissoun[1], Eleonora Russo[1], Vincenzo Costanzo[1,3], Beatrice Zitti[1], Marta Kovatcheva[1]

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Cancer is a heterogenous group of diseases that share core traits like uncontrolled growth, invasiveness, and immune evasion. How diverse cancers converge on shared biological hallmarks remains poorly understood. A growing hypothesis suggests that early transformation may reactivate embryonic programs resembling trophectoderm (TE), which also exhibits invasion, immune tolerance, and angiogenesis. ‘Trophectodermization’ in pre-malignant cells offers a promising lens on cancer development, but its study is limited by a lack of suitable models. This project proposes to use Yamanaka factor (OSKM)-mediated reprogramming as a model to investigate this link. Transient OSKM expression induces epigenetic changes that can either lead to pluripotency or, if interrupted, may result in tumorigenesis. Notably, single-cell analyses reveal TE-like cells as an alternative fate during reprogramming, mutually exclusive from full pluripotency. Considering transformation as a ‘trophectodermization’ could help unify and explain the common characteristics shared by various cancer. I hypothesize that the TE-like branch of OSKM reprogramming is responsible for tumorigenesis, and reflects the endogenous process of carcinogenesis. Thus, limiting the emergence of the TE-like state can limit tumorigenesis. To test this hypothesis, we use a multi-pronged approach, merging computational analyses, in vitro and in vivo OSKM reprogramming, and pharmacologic and immunologic manipulation to determine the mechanistic control and tumorigenic contribution of specific cell populations. This poster will present our latest advances on the project.

POSTER COMMUNICATION

#125 · The Mechanism of Embryonic Failure Induced by DNA-Damaged Gametes

Ying Cai[1, 2], Tommaso Cavazza[1, 2]

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[2] *Department of Molecular Life Sciences, University of Zurich, Zurich, Switzerland*

Most mammalian embryos that fail in development are aneuploid, which features an incorrect chromosome number. Aneuploidy arises from chromosome missegregation during meiotic/mitotic events or unrepaired DNA damage. While fertilization with DNA-damaged gametes reduces embryo competence, how embryos respond to such inherited damage remains unknown. Using a live reporter for DNA damage, we found that the two parental pronuclei in the zygote can display an asynchronous entry into mitosis. We showed that the pronucleus positive for DNA repair foci has a delayed progression into mitosis in comparison to the other pronucleus. To elaborate, when the healthy pronucleus enters mitosis with highly condensed chromosomes and undergoes nuclear envelope breakdown, the delayed pronucleus has still uncondensed chromosomes and an intact nuclear envelope. Notably, the damaged and delayed pronucleus is most frequently of paternal origin, fitting with the knowledge that sperm cells often deliver damaged DNA. This asynchronous mitotic entry results in chaotic chromosome segregation, severe aneuploidy, and embryonic failure. We proved that DNA damage can cause pronuclear asynchronous mitotic entry by using ionizing radiation on either sperm or egg. Mechanistically, we found that the local activation of the DNA damage checkpoint in the pronucleus containing damaged DNA is responsible for the asynchronous mitotic entry.

EMBO Workshop

POSTER COMMUNICATION

#126 · A maternal thyroid hormone–pax6a–VEGFAA axis coordinates neurovascular coupling during hindbrain development

Marlene Trindade, Joana Rodrigues, Marco António Campinho

Universidade do Algarve, Algarve Biomedical Center – Research Institute, Faro, Portugal.

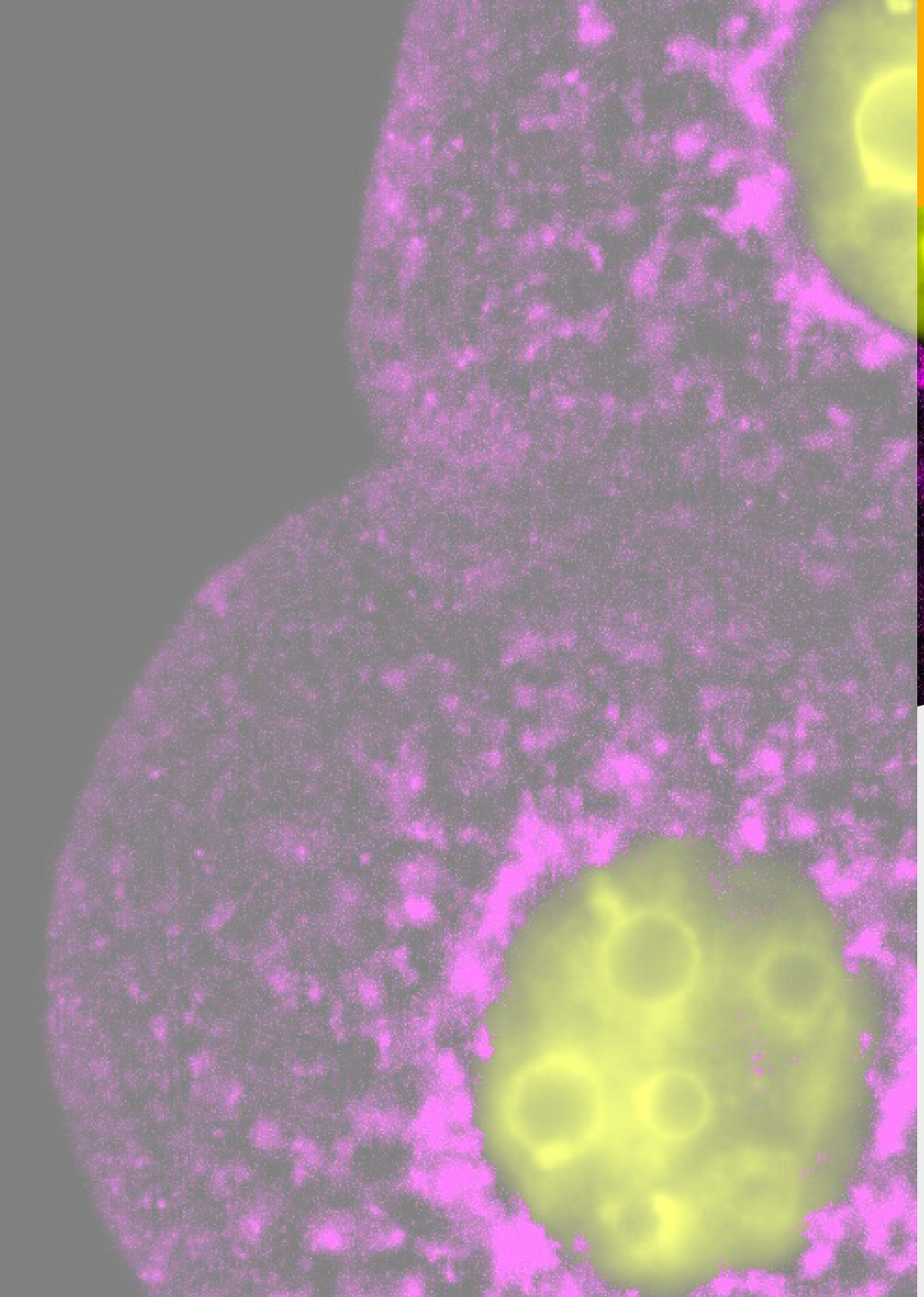
Hindbrain vascularization in zebrafish occurs in a highly regulated, stereotypic pattern, established through coordinated interactions between the developing vasculature and hindbrain cells. Primordial hindbrain channel (PHBC)-derived central arteries (Cts) invade the hindbrain in a precisely controlled manner, such that exactly one Ct pair per side ingresses into each rhombomere.

In zebrafish models of Allan–Herndon–Dudley syndrome (AHDS) — characterized by loss-of-function of the intracellular thyroid hormone transporter MCT8 (mct8/slc16a2), which abolishes intracellular maternal thyroid hormone (MT3) availability — hindbrain vascularization is severely impaired. Using complementary zebrafish knockdown and knockout approaches, we demonstrate that the selective loss of specific Cts ingressing the hindbrain in AHDS models reflects the loss of discrete pax6a^{+} hindbrain cell populations.

In the *mct8* morphant model, only a subset of Cts successfully ingressed into the hindbrain. To determine whether reduced *vegfaa* signaling underlies this defect, we overexpressed vegfaa in MCT8 morphants. This rescued ingression of Ct1, 2, 3, and 7, but not Ct4, 5 and 6, indicating that ingression of individual Cts is governed by distinct — or combinatorially acting — chemoattractive cues rather than a single, uniform signal.

To identify the cellular source of *vegfaa* in the hindbrain, we next determined that *vegfaa* is produced by a subset of *pax6a*$^{+}$ hindbrain progenitor cells. Consistent with their responsiveness to T3, these cells express T3 cognate receptors (TRs) during zebrafish neurodevelopment, establishing them as direct targets of MT3 signaling.

We further validated these findings using a *pax6a* loss-of-function mutant, in which hindbrain vascular development was similarly impaired, phenocopying the defects observed in both mct8 knockdown and knockout models. Our data indicate that MT3 is required for normal hindbrain vascular development by promoting the differentiation of a *pax6a*$^{+}$ progenitor cell subset that provides chemoattractive cues for vessels. Collectively, our data shows that MT3 acts as a maternal signal that orchestrates the integration of the brain into the developing organism.



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