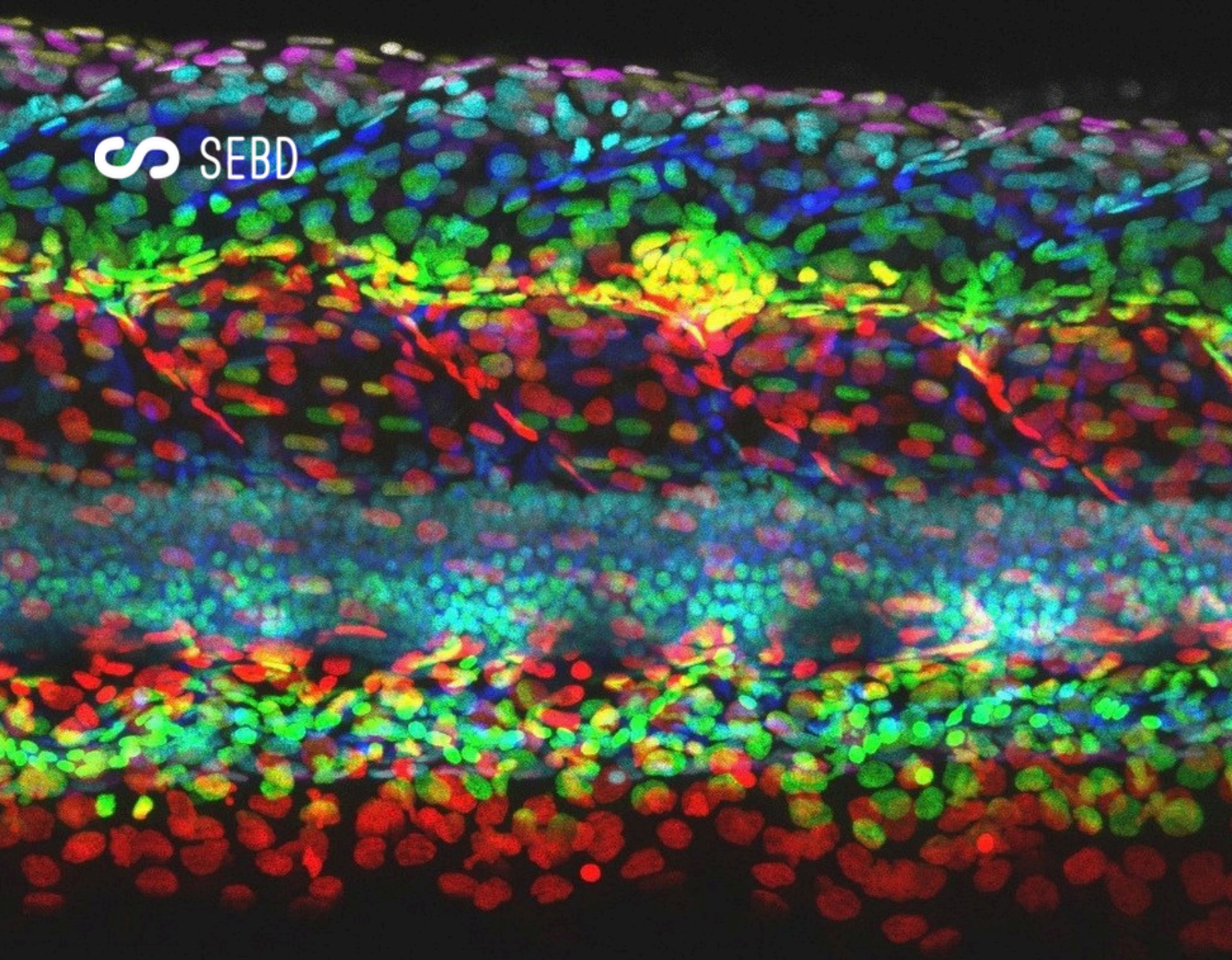


19th Spanish Society for Developmental Biology Meeting

BOOK OF ABSTRACTS

28–30 October 2026 · El Rompido, Huelva, Spain

Cover image: Ariadna Crespo and José María Urbano (Brea-Calvo Group, CABD)

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List of attendees 141

PEOPLE OF THE CONGRESS

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Antonio Giraldez

Yale University



Marco Milán

IRB Barcelona



Anna Bigas

Hospital del Mar / Josep Carreras Institute

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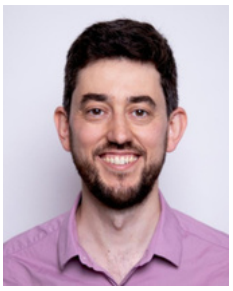
Invited speakers



Manuel Irimia
UPF · CRG



Esteban Hoijman
IBMB · CSIC



Sergio Acebrón
University of the Basque Country (EHU)



Elena Camacho Aguilar
Universidad de Sevilla



Katerina Karkali
CBM Severo Ochoa · CSIC



Hannah Stuart
EMBL Barcelona



Noel Blanco Touriñán
IBMCP · CSIC–UPV



Fernando García-Moreno
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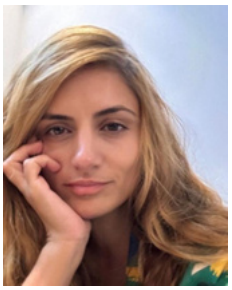
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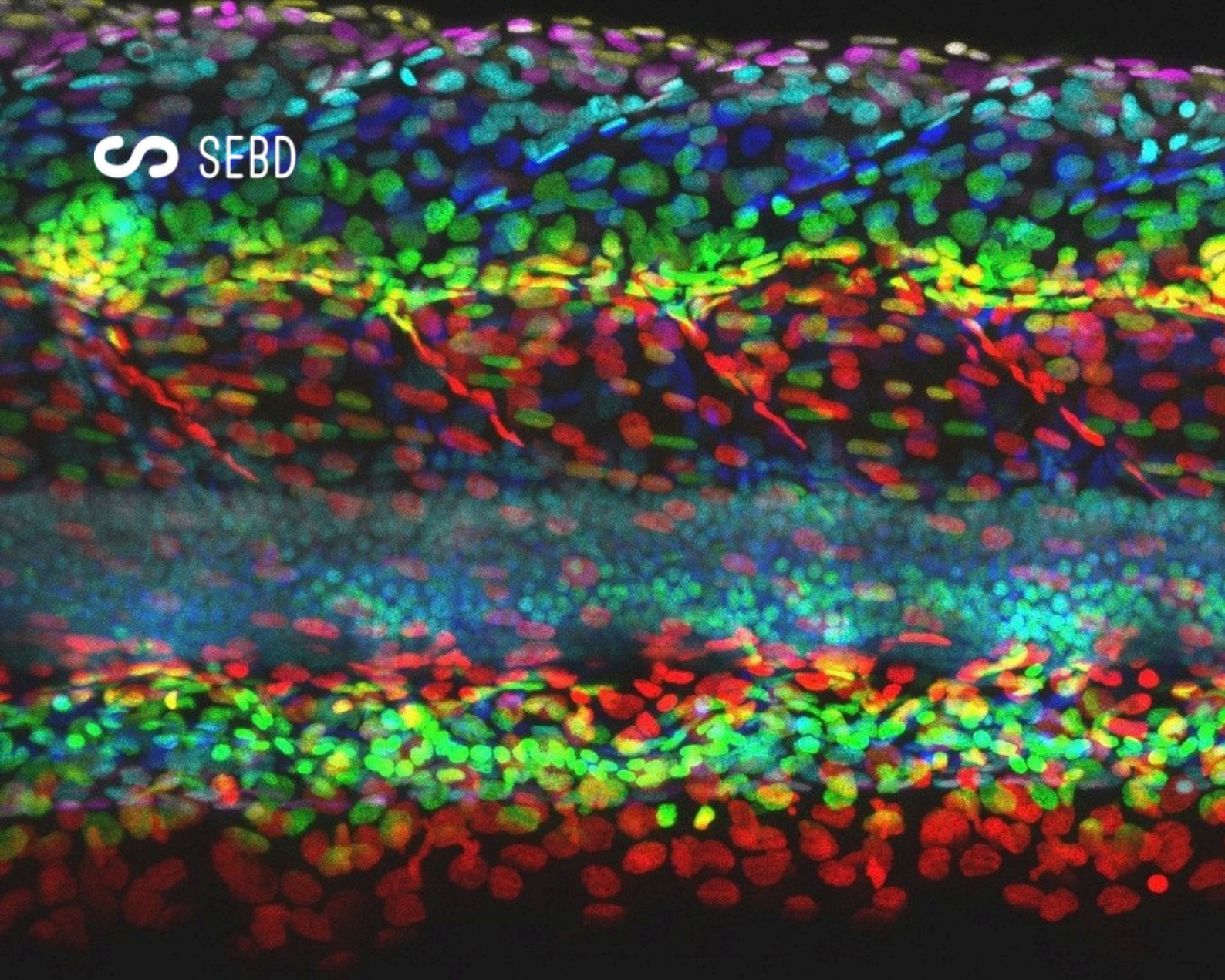
Jorge Hernández García
CBGP



Ethel Queralt
IBV · CSIC



Murielle Saade
IBMB · CSIC



01 / 09

Programme

Day 1

Wednesday, 28th October

15:00–16:00	Opening Keynote: Antonio Giraldez. Yale University (USA). ChromGEM reveals chromatin ultrastructural transitions underlying the first lineage decision in mammalian embryos.
16:00–17:30	**Session 1. Early Embryonic Development: Gastrulation and MZT**
16:00–16:30	Invited talk 1: Manuel Irimia. UPF–CRG. Evolutionary landscapes of zygotic genome activation across animals.
16:30–16:45	Selected talk 1: Blanca Pijuan-Sala. EMBL. Visualising the subnuclear distribution of open chromatin in situ during early Drosophila embryogenesis.
16:45–17:00	Selected talk 2: Marion Thompson. CABD. Uncovering the role of post-translational modifications in regulating the maternal-to-zygotic transition.
17:00–17:30	Invited talk 2: Esteban Hoijman. IBMB, Barcelona. How the microenvironment shapes early development.
17:30–18:00	Coffee Break.
18:00–19:30	**Session 2. Early Embryonic Development: Stem Cells**
18:00–18:30	Invited talk 3: Sergio Acebrón. University of the Basque Country (EHU). How to build a mosaic: a role for embryo patterning signals in genome maintenance.
18:30–18:45	Selected talk 3: Thomas Spruce. CABD. YAP1, its isoforms and interactors as critical regulators of ES cell pluripotency.
18:45–19:00	Selected talk 4: María Olmedo. Universidad de Sevilla. HLH-30/TFEB orchestrates chromatin reorganization and maintains stem cell quiescence during developmental arrest in C. elegans.
19:00–19:30	Invited talk 4: Elena Camacho Aguilar. Universidad de Sevilla. Dynamic signalling and cell fate transitions during early human development: insights from quantitative stem-cell-based models.
19:30–20:00	Developmental Dynamics Poster Flash Talks.
20:00–21:30	Dinner.
21:30–23:00	Developmental Dynamics Poster session 1 (odd numbers) with refreshments.

Day 2

Thursday, 29th October

	Special session in honour of Antonio García Bellido
9:00–9:15	Introduction by the President of the Spanish Society for Developmental Biology, Eloisa Herrera
9:15–10:15	ISDB Opening Keynote: Marco Milan. Institute for Research in Biomedicine. (Barcelona). Aneuploidy and development.
10:15–11:45	**Session 3. Morphogenesis and organogenesis I**
10:15–10:45	Invited talk 5: Katerina Karkali. CBMSO–CSIC. CNS Structural and Functional Optimization in the Drosophila Embryo.
10:45–11:00	Selected talk 5: Luis María Escudero. IBiS / Universidad de Sevilla. A natural programmable metamaterial controls 3D curvature of compound eyes.
11:00–11:15	Selected talk 6: James C-G. Hombría. CABD. Shade enhancers, a novel class of redundant shadow enhancers uncovered by functional JAK-STAT signalling pathway dissection in Drosophila.
11:15–11:30	Selected talk 7: Lucas Cunha. UPF. Early development of the early otic placode and the morphogenetic balance between the asymetric epithelizing OEPD and migrating neural Crest.
11:30–12:00	Coffee Break.
12:00–13:30	**Session 4. Morphogenesis and organogenesis II**
12:00–12:30	Invited talk 6: Hannah Stuart. EMBL. Self-organisation in neural tube organoids.
12:30–12:45	Selected talk 8: Antonio Jesús Tagua Jañez. Institut Pasteur. Spatio-temporal synchronisation of caspase activation and its impact on cell death pattern.
12:45–13:00	Selected talk 9: Elena Sánchez. CSIC–CABD. Cell type-specific regulation of gene expression and chromatin accessibility by mechanical tension during gastrulation.
13:00–13:30	Invited talk 7: Noel Blanco-Touriñán. IBMCP (UPV–CSIC). Regulation of tissue integration in emerging organs.
13:30–16:00	Group photo, lunch and free time.

Day 2

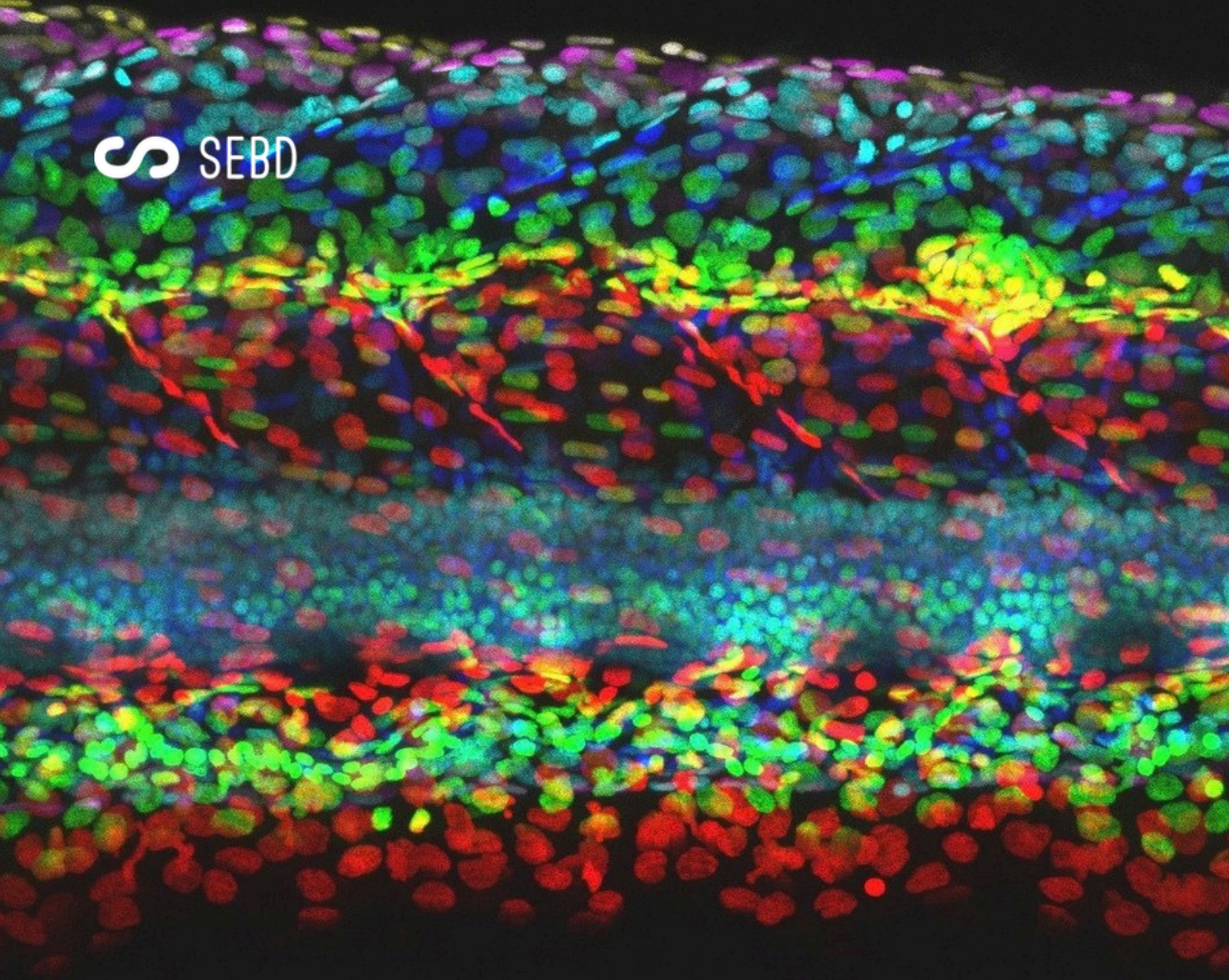
Thursday, 29th October

16:00–17:30	**Session 5. Neurodevelopment.**
16:00–16:30	Invited talk 8: Fernando García-Moreno. Achucarro Basque Center for Neuroscience. How early embryos shaped vertebrate brain evolution.
16:30–16:45	Selected talk 10: Antonio Herrera Camacho. EPFL / UGR. Organizers or disorganizers? A folate-sensitive cortical hem organizer dictates the divergent outcomes of one-carbon-limited brain development.
16:45–17:00	Selected talk 11: Eduardo Fernández Ortuño. Instituto de Neurociencias de Alicante. Early diversification of neural progenitor cells in the dorsal pallium of amniotes.
17:00–17:30	Invited talk 9: Joaquina Delas. LMCB, UCL. Cis-regulatory strategies for cell fate choice: spatial, temporal and synthetic patterning in the vertebrate neural tube.
17:30–18:00	Coffee Break.
18:00–19:30	**Session 6. Evo-Devo.**
18:00–18:30	Invited talk 10: Francisca Martínez Real. CABD. Regulatory mechanisms underlying limb adaptation in moles.
18:30–18:45	Selected talk 12: Isabel Almudí Cabrero. IRBio / UB. Gene duplication and co-option originated a male specific visual system in mayflies.
18:45–19:00	Selected talk 13: Christina Paliou. CABD. Characterizing the small-spotted catshark embryonic fins at single-cell resolution.
19:00–19:30	Invited talk 11: Jorge Hernández García. CBGP (CSIC–UPM). The rise of auxin as a signal during complexification.
19:30–20:00	Developmental Dynamics Poster Flash Talks.
20:00–21:30	Dinner.
21:30–23:00	Developmental Dynamics Poster session 2 (even numbers) with refreshments.

Day 3

Friday, 30th October

9:00–10:00	ISDB Opening Keynote: Anna Bigas. Hospital del Mar Research Institute/Josep Carreras Leukemia Research Institute. The complexity of making Blood Stem Cells: from the embryo to the dish
10:00–11:30	**Session 7. Developmental Disorders.**
10:00–10:20	Invited talk 12: Ethel Queralt. IBV–CSIC. Nipbl disruption in Cornelia de Lange Syndrome alters chromatin organisation.
10:30–10:45	Selected talk 14: Isabel del Pino. Instituto de Neurociencias CSIC–UMH. A Neurodevelopmental Syndrome Arising from Enhancer of Polycomb Homologue 1 Haploinsufficiency.
10:45–11:00	Selected talk 15: Peter Askjaer. CABD. A progeria mutation in baf-1 confers neuroprotection against polyQ-based aggregates.
11:00–11:30	Invited talk 13: Murielle Saade. IBMB–CSIC. From building to breaking: Human-specific cellular mechanisms in neurodevelopment.
11:30–12:30	Coffee Break and SEBD Assembly.
12:30–13:30	SEBD award talks.
12:30–13:00	José Luis Gómez Skarmeta award for Scientific Excellence in Developmental Biology 2025.
13:00–13:30	Best thesis awards (2024, 2025).
13:30–14:00	Developmental Dynamics Posters Awards, Closing remarks and Departure



02 / 09

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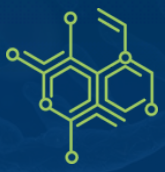
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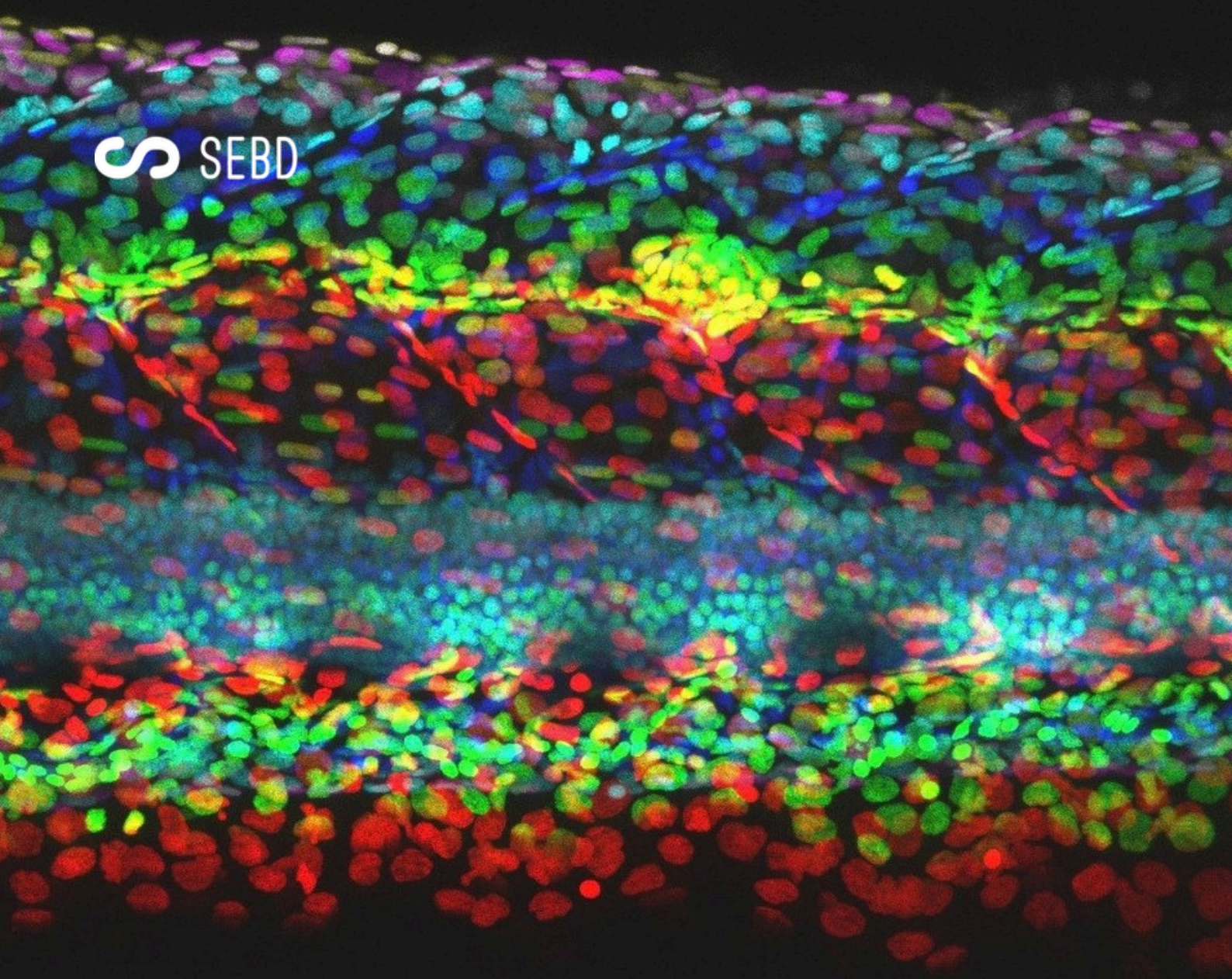
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03 / 09

Keynote lectures

KEYNOTES · DAY 1

ChromGEM reveals chromatin ultrastructural transitions underlying the first lineage decision in mammalian embryos

Antonio J. Giraldez (1); Alice Sherrard (2)

(1) Yale University; (2) Cancer Research UK

Chromatin organization underlies gene regulation and cell fate specification, yet how nucleosome-scale chromatin architecture is established during the first lineage decision remains largely unknown. Here, we developed ChromGEM (Chromatin Gold Electron Microscopy), a multimodal imaging platform that integrates endogenous nanogold protein labeling, nascent RNA detection, and electron tomography to visualize chromatin architecture, associated proteins, and transcription at nucleosome-scale resolution in situ. Applying these approaches to mouse embryos and human blastoids, we resolved chromatin remodeling during lineage specification and identified several chromatin ultrastructural subtypes defined by nucleosome density and spacing that emerge as developmental potential is progressively restricted, revealing that chromatin compaction and accessibility are not strictly coupled.

We show that pluripotent and trophectoderm cells adopt fundamentally distinct chromatin architectures. During trophectoderm specification, dense chromatin progressively relocates from the nucleolus to the nuclear lamina, with tightly packed nucleosome clusters accumulating at the nuclear periphery in both mouse embryos and human blastoids, revealing a conserved reorganization of nuclear architecture during differentiation. Mechanistically, Lamin A/C is specifically upregulated in trophectoderm across mouse, human, and opossum embryos, while loss of Lamin A/C decompacts peripheral heterochromatin, reactivates pluripotency genes, disrupts chromatin organization, and impairs human blastoid formation, establishing Lamin A/C-mediated chromatin compaction as a conserved regulator of trophectoderm identity and exit from pluripotency. Together, these findings define the ultrastructural principles by which chromatin architecture and nuclear organization regulate developmental potential and drive the first cell lineage decision in mammalian embryos.

KEYNOTES · DAY 2

Aneuploidy in Development

Marco Milán(1,2)

(1) Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Baldiri Reixac, 10, 08028 Barcelona, Spain. (2) Institució Catalana de Recerca i Estudis Avançats (ICREA) Pg. Lluís Companys 23, 08010 Barcelona, Spain

Aneuploidy, an imbalance in the number of chromosomes, is highly detrimental at both the cellular and the organismal levels and is the major cause of miscarriages in humans. Despite its deleterious effects, recent data have revealed a high prevalence of aneuploidy in early human embryos. On one hand, organismal aneuploidy is strikingly common due to the high frequency of aneuploid gametes, particularly aged oocytes. On the other hand, mosaic aneuploidy has been recently shown to be also widespread in early embryos, due to a high rate of chromosome segregation errors during the first post-zygotic divisions. Of note, aneuploid mosaic embryos can result in healthy individuals. Here, I will present our efforts in the use of Drosophila to address how aneuploidy impacts development, and how cell competition may serve as a crucial mechanism by which both developing and somatic tissues deal with the emergence of aneuploidy. If time allows, I will also present new data on the impact of metabolism on development.

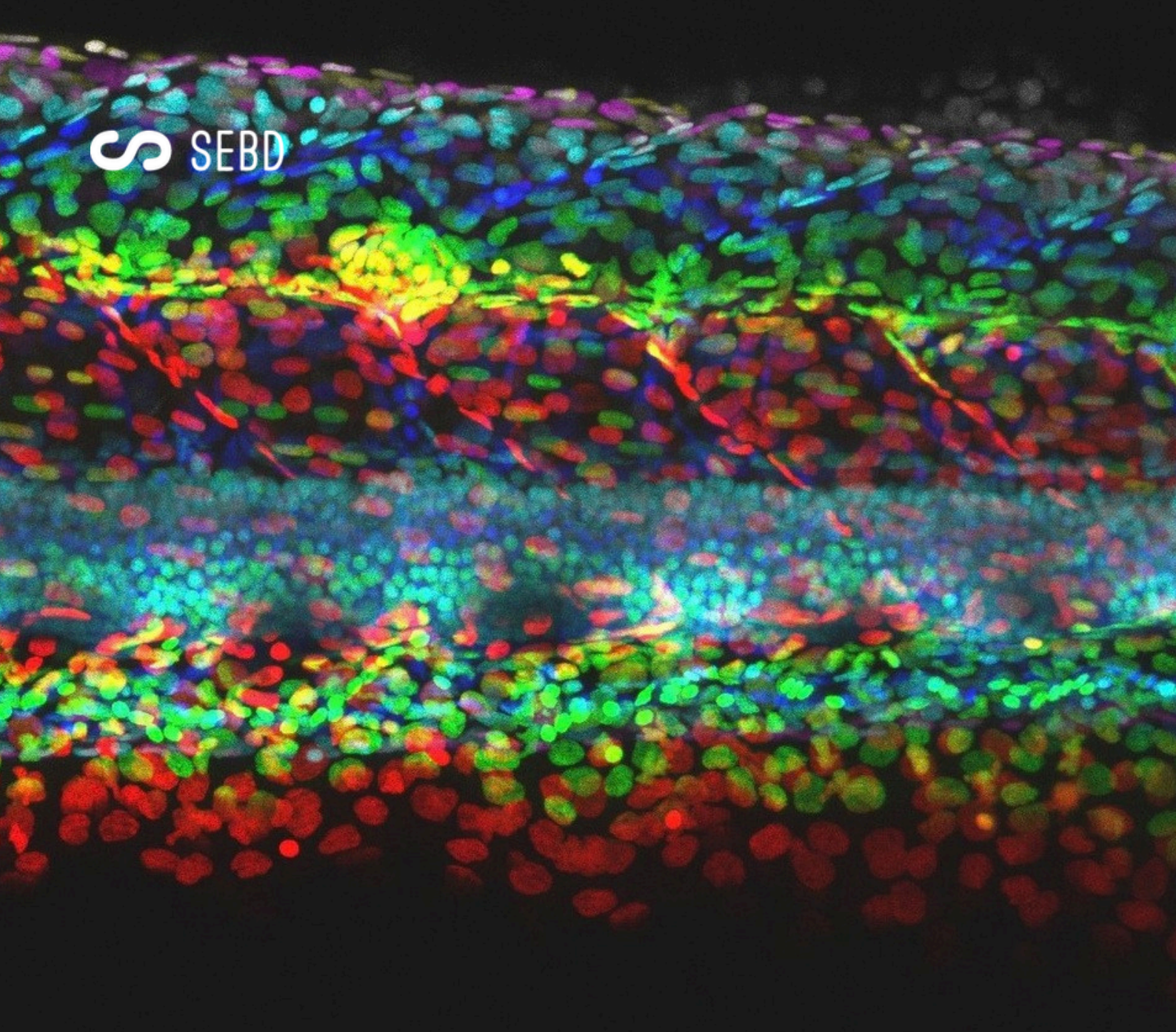
KEYNOTES · DAY 3

The complexity of making Blood Stem Cells: from the embryo to the dish

Anna Bigas.

Hospital del Mar Research Institute Barcelona and Josep Carreras Leukemia Research Institute. CIBERONC.

Hematopoietic stem cells (HSCs) arise during embryonic development through a tightly orchestrated process that transforms endothelial cells into blood-forming stem cells via the endothelial-to-hematopoietic transition (EHT). This process, occurring in specific anatomical niches such as the aorta-gonad-mesonephros (AGM) region and subsequently expanding in the fetal liver, depends on the coordinated action of signaling pathways, including Notch, Wnt and inflammatory signals, that steer the transcriptional programs that establish stem cell identity and self-renewal capacity. Despite decades of progress in understanding these developmental mechanisms, recapitulating the generation of fully functional, transplantable HSCs in vitro from pluripotent stem cells remains an unresolved challenge. This gap reflects the intricate, multi-layered nature of HSC ontogeny, where spatial, temporal, and molecular cues must converge precisely to generate durable stem cell function. Bridging our mechanistic understanding of embryonic hematopoiesis with stem cell engineering approaches holds promise for overcoming current barriers, with significant implications for regenerative medicine.



SEBD

04 / 09

Invited talks

INVITED TALKS · Session 1 · Early Embryonic Development: Gastrulation and MZT

Evolutionary landscapes of zygotic genome activation across animals

Manuel Irimia (1)

(1) UPF - CRG

During early animal embryogenesis, gene expression control shifts from maternally deposited products to newly transcribed RNA through zygotic genome activation (ZGA). Although essential and universal, ZGA remains poorly understood outside a few model species. Here, we generated a transcriptomic atlas of early embryogenesis across 61 animal species from 13 phyla and used a unified computational framework to infer ZGA timing systematically. We find extensive variation in ZGA onset across animals, but show that the ratio between genome size and egg cytoplasmic volume, a proxy for the nuclear-to-cytoplasmic ratio, robustly predicts when genome activation begins. We also reveal that zygotic genes also differ from maternal transcripts in genomic architecture, function and evolutionary conservation, suggesting that ZGA follows a conserved molecular logic despite flexible transcriptomic outputs.

INVITED TALKS · Session 1 · Early Embryonic Development: Gastrulation and MZT

How the microenvironment shapes early development.

Esteban Hoijman (1)

(1) Insituto de Biología Molecular de Barcelona

The dynamics of embryonic cells are typically studied under laboratory conditions using cell and embryo culture systems. However, how the native microenvironment of early embryos contributes to developmental programs remains poorly understood. Our research aims to uncover how the chemical, mechanical, and microbiological environments shape embryogenesis in its natural context. Here I will present our recent work revealing the earliest interactions between embryos and bacteria. In humans, preimplantation embryos can be exposed to bacterial infections associated causing prevalent uterine diseases associated to miscarriages and infertility. To investigate embryo–bacteria interactions, we initially developed infection models in early zebrafish embryos, which naturally encounter a complex microbial niche. Using quantitative live imaging across scales, we reveal that the embryonic surface epithelium is highly phagocytic, internalizing and destroying bacteria with an efficiency comparable to that of professional immune cells. We identified specialized zippering protrusions that mediate bacterial uptake and trigger an immune gene program in epithelial cells, a response that proves essential for normal development under infectious conditions. We found that this phagocytic activity is conserved in mouse embryos. Remarkably, extending these observations to humans, we performed functional assays on donated human embryos and demonstrated that they can internalize and destroy bacteria implicated in uterine diseases. Together, these findings reveal an unexpected role for embryonic tissues in host defense and illustrate how interactions with the native microenvironment can influence developmental trajectories. They also highlight how much remains to be learned about embryogenesis in its natural ecological context.

INVITED TALKS · Session 2 · Early Embryonic Development: Stem Cells

Embryo patterning signals oversee genome maintenance during cell lineage specification

Sergio Pérez Acebrón (1)

(1) University of Basque Country (EHU)

Keywords Patterning signals, WNT, cell lineage specification, stem cells, genome maintenance, chromosome segregation fidelity

Organismal development and homeostasis rely on the correct replication, maintenance and segregation of our genetic blueprints. How these intracellular processes are monitored across different human cellular lineages, and why the spatio-temporal distribution of mosaicism varies during development remain unknown. Using human and mouse pluripotent stem cells, we reveal that antero-posterior patterning signals display distinct antagonising roles in genome maintenance during early lineage specification. Mechanistically, we uncover that DKK1, Chordin, Cerberus, Noggin, LEFTY-A, BMP4 and WNT3A form a signalling “rheostat” that monitors ATM-dependent DNA replication stress and damage during S-phase, which in turn controls spindle dynamics and chromosome segregation fidelity in mitosis. Cell signalling control of genome and chromosomal stability declines after specification into the three human germ layers and subsequent lineages, following the withdrawal of ATM signalling as first responder to DNA replication stress, but reemerges in neural progenitors. In particular, we show that the brain patterning signal FGF drives replicative stress and chromosome missegregation during mouse and human neurogenesis. Our results highlight moonlighting roles of patterning signals and cell fate in genome maintenance that contribute to somatic mosaicism during i) human early lineage specification, with important implications to understand how mosaicism arises in peri-implantation embryos: the leading cause of miscarriage in humans; and ii) mammalian neurogenesis, which could provide a rationale for the high levels of mosaicism found in the developing brain.

References 1 de Jaime-Soguero A, Hattemer H, Bufe A, Haas A, van den Berg J, van Batenburg V, Das B, di Marco B, Androulaki S, Bohly N, Landry JJM, Schoell B, Rosa VS, Villacorta L, Baskan Y, Trapp M, Benes V, Chabes A, Shahbazi M, Jauch A, Engel U, Patrizi A, Sotillo R, van Oudenaarden A, Bageritz J, Alfonso J, Bastians H, and Acebron SP ‡. 2024. Developmental signals control chromosome segregation fidelity during pluripotency and neurogenesis by modulating replicative stress. Nature Communications. 15:7404. PMID: 39191776 2 Acebrón SP‡, Hattemer J, Rausch T, de Jaime-Soguero A. 2026. Morphogen Gradients as Drivers of Mosaicism During Early Human Development Bioessays. 48(3):e70128. PMID: 41879524

INVITED TALKS · Session 2 · Early Embryonic Development: Stem Cells

From single cells to symmetry breaking: decoding human gastrulation across scales

Elena Camacho Aguilar (1)

(1) Universidad de Sevilla

Keywords human development, human pluripotent stem cells, cell state transitions, signalling dynamics, mathematical modelling

A fundamental question in developmental and quantitative biology is how robust spatial patterns and symmetry breaking emerge from initially homogeneous cell populations. Answering this requires understanding multiple coupled levels of complexity (from single-cell regulatory logic and dynamic signalling to spatial constraints and cell-cell interactions) and how they are integrated across scales.

At around day 14 of development, the human epiblast disk breaks symmetry to form the primitive streak on one side, defining the posterior end of the embryo and initiating gastrulation. This process is initiated by a cascade of signals involving BMP4 from the surrounding extraembryonic tissues and endogenous WNT, NODAL and FGF signalling. Moreover, mouse studies and stem-cell-based embryo models suggest that interactions with juxtaposed extraembryonic tissues play a key role in spatially controlling symmetry breaking. However, the mechanisms governing this process remain largely elusive.

Human pluripotent stem cells offer a powerful model to study this process in a controlled, stepwise manner, allowing us to take a bottom-up approach to understand how the different levels of complexity are integrated to give rise to the AP axis of the embryo.

In this talk, I will present our lab's progress towards a multiscale quantitative framework for human gastrulation. Building on our previous work establishing how the dynamic interplay of BMP and WNT signalling controls cell fate specification at this stage, I will present our current progress across three scales: single-cell fate decisions, collective colony patterning, and embryonic-extraembryonic tissue interactions, combining live-cell imaging, single-cell transcriptomics, and multiscale computational modelling.

References 1 Camacho-Aguilar E, Yoon ST, Ortiz-Salazar MA, Du S, Guerra MC, Warmflash A. Combinatorial interpretation of BMP and WNT controls the decision between primitive streak and extraembryonic fates. Cell Systems. 2024 May 15;15(5):445-61.

INVITED TALKS · Session 3 · Morphogenesis and Organogenesis I

CNS structural and functional optimization in Drosophila embryo

Katerina Karkali
Centro de Biología Molecular Severo Ochoa – CSIC, Madrid, Spain

CNS morphology is evolutionarily selected to optimally support its function. In Drosophila, the embryonic Ventral Nerve Cord (VNC) undergoes dramatic condensation midway through embryogenesis, tightly coupled to motor coordination maturation and embryo hatching, providing an ideal framework to investigate structure–function relationships in the developing CNS. Through kinematic, biophysical and genetic analyses, we established that condensation is driven by coordinated neuronal and glial contractility, and that precise JNK signaling in pioneer neurons controls VNC architectural organization and motor output independently of electrophysiological properties.

These findings revealed that CNS structural organization actively influences circuit functionality, raising a fundamental question: how is tissue architecture developmentally established and maintained?

We hypothesized that glia and the extracellular matrix (ECM) constitute a dynamic mechanical unit that actively shapes CNS geometry during morphogenesis. To test this, we combined protein-trap reporter lines for major ECM components with a custom quantitative image analysis pipeline — including the ridge-detection algorithm TWOMBLI — to characterize matrix architecture dynamics across condensation stages, alongside targeted genetic manipulation in glia of ECM components and modifying enzymes. This revealed that stereotyped spatiotemporal ECM remodeling during active VNC condensation correlates with sub-perineurial glial expansion, identifying this population as a key modulator of matrix composition and architecture. Perturbation of Nidogen levels in glia alters matrix organization and the dynamics of VNC condensation. Ongoing experiments are assessing consequences for embryonic motor coordination.

In conclusion, the glia–ECM unit regulatory interplay is an active contributor to CNS morphogenesis and functional circuit optimization, with broad implications for ECM-related neurodevelopmental disorders.

INVITED TALKS · Session 4 · Morphogenesis and Organogenesis II

Self-organisation in neural tube organoids

Hannah Stuart
EMBL, Barcelona

The reliable generation of diverse cell types in precise proportions is essential for the formation of functional tissues during embryonic development. Three-dimensional organoid models derived from pluripotent stem cells (PSCs) provide powerful systems for identifying principles governing tissue self-organisation. Neural tube organoids (NTOs), initiated from single PSCs with a pulse of retinoic acid (RA), self-organise into structures containing floorplate cells that secrete SHH morphogen to pattern adjacent neural tissue. Yet, how the initial cellular diversity arises and how appropriate cell-type proportions are allocated has remained unclear. Here, using time-resolved single-cell transcriptomics, quantitative immunofluorescence, and dynamical systems modelling we show that RA triggers a transient co-expression state of the transcription factors PAX6 and FOXA2 from which cells asynchronously resolve into two opposing fates: PAX6^{+} neural precursors and FOXA2^{+} floorplate precursors. PAX6 and FOXA2 are both necessary and sufficient to reconstitute self-organisation, establishing these transcription factors as key determinants of emergent tissue pattern. Rather than operating as a simple feed-forward system in which cells are guided solely by RA, feedback between the alternative cell fates, mediated by BMP signalling from floorplate precursors, determines cell type proportions and ensures reproducible cell-type diversity in each NTO. This dual expression state was also identified in mouse embryos, demonstrating how in vitro models inform in vivo biology. These findings establish a general design strategy – symmetry breaking through opposing fate determinants coupled to proportioning via signal feedback control – that may operate broadly across developmental contexts to generate tissues with predictable cellular compositions.

INVITED TALKS · Session 4 · Morphogenesis and Organogenesis II

Regulation of tissue integration in emerging organs

Noel Blanco-Touriñán
Instituto de Biología Molecular y Celular de Plantas, CSIC–UPV, Valencia, Spain

The formation of new organs requires more than the specification of new cell identities. Emerging organs must become functionally integrated with the tissues of the organism, establishing continuity with transport systems and cellular barriers. This poses a common developmental challenge across multicellular organisms: new tissues must connect to pre-existing networks without compromising their organization or function. Plants provide a particularly striking example, as they continuously generate new organs throughout their life.

We investigate how newly formed organs establish continuity with pre-existing tissues, focusing on two essential networks: the vasculature, which mediates long-distance transport, and the endodermis, a polarized barrier tissue functionally reminiscent of animal epithelia. By comparing the cellular and molecular mechanisms underlying these connections across different plant species, we find that conserved regulatory mechanisms can give rise to distinct developmental strategies while converging on the same physiological outcome: the functional integration of new organs into the existing body plan.

INVITED TALKS · Session 5 · Neurodevelopment

How early embryos shaped vertebrate brain evolution

Fernando Garcia-Moreno (1)

(1) Achucarro Basque Center for Neuroscience

Keywords Neurodevelopment, evodevo, brain evolution,

conservation of their embryonic organization, the brain bauplan. Yet, both the cellular architecture of this conserved blueprint and the mechanisms underlying its persistence have remained elusive. In this talk, I will present comparative single-cell, spatial transcriptomic and epigenomic analyses across five vertebrate species that reconstruct the early embryonic brain at cellular resolution. Cross-species comparisons identify conserved progenitor domains that define homologous neuromeres across vertebrates and refine long-standing questions regarding forebrain organization. Beyond this shared architecture, gene regulatory network analyses reveal two independent sources of evolutionary constraint. Regional identity programs, enriched in essential developmental genes, preserve the brain bauplan, whereas a deeply conserved pan-progenitor network, composed of highly pleiotropic genes, constrains proliferating cell states. In contrast, later functional and neuronal programs are less constrained and more evolutionarily flexible. Together, these findings provide a mechanistic framework for understanding how deeply conserved embryonic programs have shaped vertebrate brain evolution while allowing the emergence of extensive adult diversity.

INVITED TALKS · Session 5 · Neurodevelopment

Cis-regulatory strategies for cell fate choice: spatial, temporal and synthetic patterning in the vertebrate neural tube

M Joaquina Delas Vives (1)

(1) LMCB, UCL

Keywords cis-regulation, neural tube, morphogens

During development, the patterns of gene expression that assign cell fate are directed by signaling cues. Cis-regulatory elements (CREs) integrate tissue and cellular inputs to direct gene expression, but how this is accomplished remains poorly understood. To date most focus has been placed on activating CREs (enhancers) and transcription factors (TFs) that drive gene expression, yet repressive TFs also mediate decisions between alternative cell fates in development. Using the developing neural tube, we identified two distinct ways in which CREs can drive cell fate choice. During spatial patterning in response to morphogen gradient Shh, most neural progenitors open the same CREs and it is the differential binding of cell-type-specific repressors that control gene activity. In contrast, temporal patterning, controlling the timing and order of cell type generation, requires the opening and closing of CREs. We find this temporal chromatin program is used across neural tube spatial progenitors and is conserved across other central nervous system regions.

To investigate how CREs integrate inputs to direct precise gene expression in time and space, we developed a high-throughput combinatorial screening strategy, that we term NeMECiS, to investigate signal-dependent cis-regulatory activity during differentiating mammalian stem cells. Our work concatenating fragments from existing Shh-responsive CREs generated thousands of signal-responsive synthetic CREs, many exceeding the activity of natural sequences. We find CRE activity can be decomposed into specific quantitative contributions, combined in a multiplicative way, and tuned by their relative positioning and spacing. This framework additionally allowed us to re-design CREs for pattern engineering.

References 1 Predictable Engineering of Signal-Dependent Cis-Regulatory Elements Jake Cornwall-Scoones, Dirk Benzinger, Tianji Yu, Alberto Pezzotta, Andreas Sagner, ..., Manuela Melchionda, Oliver C. K. Inge, Bianca Dumitrascu, James Briscoe, M. Joaquina Delás Pre-print on biorxiv · 07 Mar 2025 · doi:10.1101/2025.03.07.642002 2 The cis-regulatory logic integrating spatial and temporal patterning in the vertebrate neural tube Isabel Zhang, Giulia L.M. Boezio, Jake Cornwall-Scoones, Thomas Frith, Elizabeth Finnie, ..., Michael Howell, Robin Lovell-Badge, Andreas Sagner, James Briscoe, M. Joaquina Delás Developmental Cell · 01 Nov 2025 · doi:10.1016/j.devcel.2025.06.029 3 Developmental cell fate choice in neural tube progenitors employs two distinct cis-regulatory strategies M. Joaquina Delás, Christos M. Kalaitzis, Tamara Fawzi, Madeleine Demuth, Isabel Zhang, Hannah T. Stuart, Elena Costantini, Kenzo Ivanovitch, Elly M. Tanaka, James Briscoe Developmental Cell · 01 Jan 2023 · doi:10.1016/j.devcel.2022.11.016

INVITED TALKS · Session 6 · Evo-Devo

Regulatory mechanisms underlying limb adaptation in moles

Francisca Martínez Real
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Embryonic development relies on precise gene expression patterns, orchestrated by non-coding cis-regulatory elements (CREs) that determine when and where genes are active. Developmental genes often reside within large regulatory landscapes containing multiple CREs that interact with their target promoters through 3D chromatin folding to generate highly pleiotropic transcriptional patterns. Mutations that alter the composition or architecture of these regulatory landscapes can cause aberrant gene expression and developmental defects, yet they can also drive adaptive trait evolution. How such regulatory changes reshape gene expression to generate phenotypic diversity remains a central question in evolutionary biology.

Moles provide a striking natural system to address it. Adapted to life underground, they have evolved a remarkable suite of traits, including hypoxia tolerance, eye degeneration, female intersexuality, and forelimbs radically remodeled for digging, with an enlarged humerus, short and broad forepaws, and even an extra “thumb.” To uncover the regulatory basis of these limb modifications, we generated single-cell transcriptomic and epigenomic datasets from embryonic mole and mouse limbs, complemented by bulk 3D chromatin contacts datasets, and used comparative approaches to identify regulatory changes associated with mole limb development. We find that changes in 3D chromatin interactions and transcription factor binding motifs are associated with mole-specific expression patterns of key limb developmental genes. These results suggest how evolutionary changes in regulatory landscapes can rewire developmental gene expression and give rise to novel traits.

INVITED TALKS · Session 6 · Evo-Devo

The rise of auxin as a signal during complexification

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(1) Centro de Biotecnología y Genómica de Plantas (CBGP)

Keywords Auxin, Plants, Molecular Evolution

The emergence of complex multicellular organisms required the evolution of mechanisms capable of coordinating cell behaviour across space and time. In plants, the signalling molecule auxin occupies a central position in this process, functioning as a regulator of growth, pattern formation and developmental plasticity. Yet many of the molecular components underlying auxin signalling predate the origin of land plants, raising a fundamental evolutionary question: how did an ancient set of molecular tools become integrated into the developmental systems that generate modern plant forms?

I will discuss how comparative genomics, evolutionary reconstruction and functional studies across diverse plant lineages are helping to unravel the origin of the auxin signalling pathway. Particular attention will be paid to the emergence of auxin perception and transcriptional regulation, and to growing evidence that key components of the pathway arose through the evolutionary co-option—or molecular piracy—of pre-existing cellular machinery. By tracing the assembly of auxin signalling from its ancestral building blocks, we can begin to reconstruct how a relatively simple molecular pathway was transformed into a versatile developmental system and repeatedly deployed to regulate new developmental processes throughout plant evolution.

By examining auxin signalling across deep evolutionary timescales, we gain insight into the origins of developmental robustness, plasticity and innovation in plants. I will argue that the auxin signalling pathway provides a powerful framework for understanding how both molecular and developmental mechanisms evolve and how conserved molecular components can generate the extraordinary diversity of forms observed across extant plant lineages.

INVITED TALKS · Session 7 · Developmental Disorders

Nipbl disruption in Cornelia de Lange Syndrome alters chromatin organisation

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(1) Instituto de Biomedicina de Valencia (IBV-CSIC)

Keywords Chromosome segregation, rare disease, developmental disorder, Cornelia de Lange Syndrome

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.

References 1 García et al., 2021 Nat comm

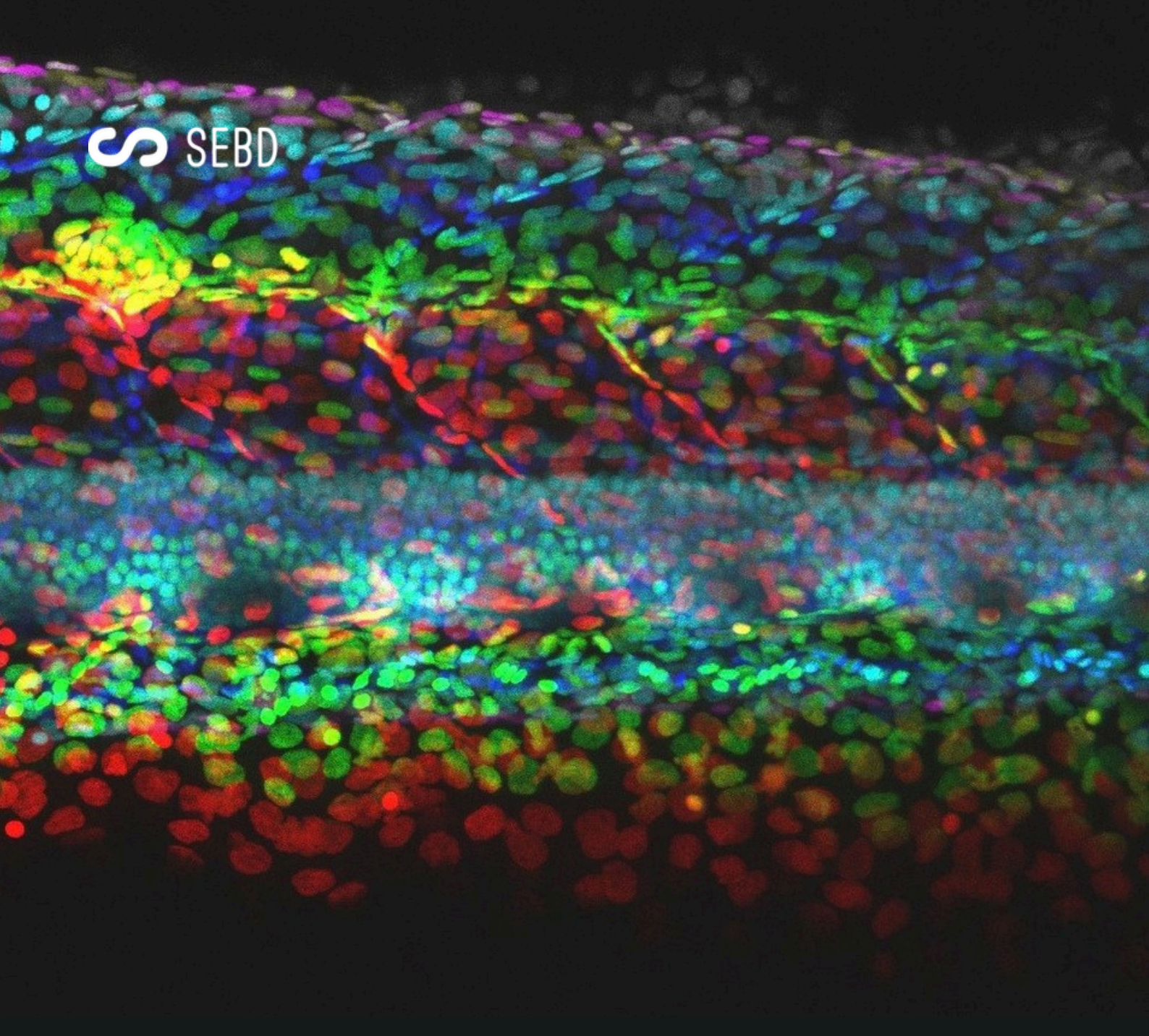
INVITED TALKS · Session 7 · Developmental Disorders

From Building to Breaking: Human-Specific Cellular Mechanisms in Neurodevelopment

Paula España Bonilla (1), Glòria Casas Gimeno (1), Axelle Wilmerding (1), Rebeca Ruiz Delgado (1), Tatiana Pérez Castellanos (1), Naroa Jara Ortega (1), Natalia Igual Nevot (1), Susana Usieto (1), Murielle Saade (1)

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The expansion of the human central nervous system (CNS) depends on cellular programs that coordinate neural progenitor cell (NPC) proliferation, differentiation, polarity, and tissue architecture. Our work defines how the molecular composition and dynamic remodeling of intracellular organelles - the “Neural Architects”- drive these programs, and how their perturbation converts developmental innovations into vulnerabilities. We focus on the centrosome–cilium–microtubule axis and associated cytoskeletal and polarity networks, combining live and super-resolution imaging with human pluripotent stem-cell models, 3D neural organoids, and vertebrate embryos. We investigate how organelle behavior, topology, and protein composition are remodeled during NPC division, neuronal differentiation, and delamination, and how this plasticity supports adaptive CNS growth. A central theme is the human-specific diversification of these Neural Architects. At the 1q21 locus, we dissect dosage-sensitive, human-biased genes - including PDE4DIP and the FAM72 paralogs - and their roles in centrosomal organization, microtubule dynamics, NPC division modes, and progenitor expansion. In parallel, we study the ciliary rootlet protein CROCC (Rootletin), defining how rootlet remodeling coordinates neuroepithelial architecture and neuronal delamination. Finally, we probe how these human-specific programs respond to environmental and genetic perturbations. We model two neurodevelopmental-disorder contexts- Zika virus infection and 1q21.1 CNV- to test robustness and vulnerability of centrosome-ciliummicrotubule programs. In parallel, we employ comparative primate systems to introduce human-specific 1q21 genes into non-human contexts, asking whether their expression alters NPC division modes and progenitor expansion. Together, these approaches reveal how evolutionary innovations in organelle regulation can be both adaptive and vulnerable when challenged, providing mechanistic insight into the cellular basis of human CNS expansion.



05 / 09

Selected talks

SELECTED TALK · Session 1 · Early Embryonic Development

Visualising the subnuclear distribution of open chromatin in situ during early Drosophila embryogenesis

Blanca Pijuan-Sala (1), Katharina Bender (1), Emilio Benedum, Christoph Schaub (1), Eileen E. Furlong (1)
(1) EMBL

The acquisition of multicellular complexity is a hallmark of embryonic development. As development progresses, the chromatin landscape is established to generate the diverse gene expression programmes that specify distinct cellular identities in a precise and robust manner. Previous single-cell sequencing studies have shown that chromatin accessibility states are closely linked to cellular identity. However, how chromatin accessibility is spatially distributed within nuclei in the context of morphogenetic events during embryonic development remains largely unexplored. To begin addressing this question, we developed modular ATAC-see, an imaging-based method for visualising chromatin accessibility globally in fixed whole embryos while preserving its subnuclear and embryonic spatial context. Applying modular ATAC-see to early Drosophila embryos, we identified a polarised distribution of accessible chromatin within blastoderm nuclei, which is affected by the disruption of proteins required to establish the chromatin landscape at this developmental stage. More broadly, modular ATAC-see complements sequencing-based approaches by revealing the global spatial distribution of accessible chromatin within intact embryos, providing a new framework for investigating how chromatin accessibility patterning is coordinated across subnuclear, cellular, and embryonic scales.

SELECTED TALK · Session 1 · Early Embryonic Development

Uncovering the role of post-translational modifications in regulating the maternal-to-zygotic transition

Marion Thompson (1), Daniel Nahón-Cano (1), Alberto Cañibano-Hernandez (1), Carmen Espejo-Serrano (2), Ariel Bazzini (3), Román González-Prieto (2), Miguel Moreno-Mateos (1)

(1) Andalusian Center for Developmental Biology (CABD), (2) Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER), (3) Stowers Institute for Medical Research

Keywords MZT ZGA Zebrafish RfxCas13d Phosphorylation Ubiquitination Post-translational modifications

The maternal-to-zygotic transition (MZT) is a universal process that is essential during early development. It involves two distinct processes: degradation of maternally provided protein and RNA, and zygotic genome activation (ZGA). Here, we investigate how post-translational modifications (PTMs) may be involved in regulating these two processes. Phosphorylation is a PTM that is able to affect protein stability, activity and localisation, and previous work in our lab has demonstrated how kinases can have essential roles during ZGA in teleosts (Hernandez-Huertas L. et al., 2025). With this in mind and in collaboration with the Bazzini lab, we generated the first phospho-proteome covering different time points during MZT in zebrafish as vertebrate model. A global analysis of this data suggests that there is massive protein phosphorylation reprograming occurring, that could potentially play a major role during early embryogenesis. Furthermore, we are using this data to identify kinases and phosphatases that may be important regulators of these changes in protein phosphorylation. In addition, we also focused on protein ubiquitination, another PTM poorly understood during MZT. To investigate this, we applied our optimized CRISPR-RfxCas13d system to perform a screen targeting maternally-provided mRNAs involved in protein ubiquitination in zebrafish and selected an E3 ubiquitin ligase, Rfwd3, as our main candidate since its knockdown caused early developmental defects. Through transcriptomics, genomics and proteomics approaches we are currently exploring the role of Rfwd3 during MZT. Altogether, this project aims to systematically identify and characterized novel post-translational modifiers and how this mode of regulation controls early embryogenesis.

References 1 Hernández-Huertas, L., Moreno-Sánchez, I., Crespo-Cuadrado, J., Vargas-Baco, A., da Silva Pescador, G., Zhang, Y., Wen, Z., Florens, L., Santos-Pereira, J. M., Bazzini, A. A. and Moreno-Mateos, M. A. (2025). 'CRISPR-RfxCas13d screening uncovers Bckdk as a post-translational regulator of maternal-to-zygotic transition in teleosts'. The EMBO Journal 2025 44:23. Springer, 44 (23), pp. 7021–7059. doi: 10.1038/S44318-025-00617-8.

SELECTED TALK · Session 2 · Stem Cells

YAP1, its isoforms and interactors as critical regulators of ES cell pluripotency

Thomas Spruce (1,2), Niccolo Arecco (2), Marta Escobar (1), Marta Miret (2), Iris Guerrero (1), Lucia Goyeneche (2), Barbara Pernaute (1,2), Manuel Irimia (2,3)

(1) Centro Andaluz de Biología del Desarrollo (CABD), CSIC/UPO/JA, Seville, Spain. (2) Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology, Barcelona, Spain. (3) Universitat Pompeu Fabra, Barcelona, Spain.

Keywords YAP1, Embryonic stem cells, Pluripotency, Alternative Splicing, Transcriptional regulation.

The pluripotent period extends from around 3.5 to 7.5 days post coitum (dpc) in the mouse embryo. Although pluripotency is maintained throughout this time cellular properties change dynamically and distinct pluripotency states can be captured in cell culture. The Hippo pathway effector YAP1 is a transcriptional co-factor that plays important roles in numerous fundamental biological processes. Several splice variants have been described for YAP1, among the most common include one in which exon 6 is included or excluded, modulating a leucine zipper that sits next to YAP1's transcriptional activation domain. We have observed that whilst both these YAP1 splicing variants coexist in all tissue types the balance between them is tightly regulated during mammalian development, with the inclusion isoform dominating during early/naïve pluripotency and the skipping isoform dominating as differentiation proceeds. Using a combination of knockout and overexpression systems we have found that whilst both variants increase ES cell proliferation and inhibit ES cell differentiation into primitive endoderm, the skipping isoform additionally promotes pluripotency exit due to enhanced transcriptional activator activity. Our results from isoform specific Immunoprecipitation Mass Spectrometry and ongoing functional experiments suggest this enhanced activator activity results from the skipping isoform having increased affinity for a subset of transcriptional regulators. Altogether, our results show a tight regulation of Yap1 exon 6 splicing is needed to maintain stem cell pluripotency and homeostasis and opens the possibility of this happening in other contexts where YAP1 function is also essential.

SELECTED TALK · Session 2 · Stem Cells

HLH-30/TFEB orchestrates chromatin reorganization and maintains stem cell quiescence during developmental arrest in C. elegans

Marta Muñoz-Barrera (1), Alejandro Mata-Cabana (1), Almudena Moreno-Rivero (1), Francine A. Piubeli (1), Beatriz Ren-Barroso (1), Nada Al-Refaie (2), Gabriel Gutierrez (1), Daphne S. Cabianca (2), María Olmedo López (1)

(1) Universidad de Sevilla, (2) Helmholtz Zentrum Munich

Keywords Quiescence, chromatin, transcription factors, nutrient sensing

Cellular quiescence is a fundamental property of stem cells that preserves tissue homeostasis and regenerative potential under adverse conditions. However, how environmental cues are integrated with chromatin organization to maintain quiescence remains poorly understood. Using the Caenorhabditis elegans L1 starvation-induced arrest as an in vivo model, we investigated the role of the evolutionarily conserved transcription factor HLH-30/TFEB in stem cell quiescence.

We show that HLH-30 cooperates with DAF-16/FOXO to promote survival during nutrient deprivation and that both factors reciprocally regulate each other's activity. Transcriptomic analyses identify HLH-30 as a major regulator of genes involved in chromosome organization and cell-cycle control. Consistent with this, nuclear architecture analyses reveal that HLH-30 is required for the large-scale chromatin reorganization triggered by starvation.

Importantly, loss of HLH-30 disrupts quiescence in seam cells, the epidermal stem cell-like lineage of C. elegans, leading to inappropriate activation of developmental programs under nutrient-restricted conditions. Mechanistically, these defects are associated with overactivation of the pioneer transcription factor BLMP-1, uncovering a previously unrecognized pathway linking nutrient sensing, chromatin architecture, and developmental timing.

Together, our findings identify HLH-30/TFEB as a key regulator of genome organization and stem cell quiescence in vivo. These results reveal conserved mechanisms by which environmental signals shape stem cell behavior, with potential implications for tissue regeneration, aging, and age-related disease.

SELECTED TALK · Session 3 · Morphogenesis and Organogenesis I

A natural programmable metamaterial controls 3D curvature of compound eyes

Juan Garrido-García (1), Rhian F. Walther (2), Jesús Torres-Tirado (3), Jesús A. Andrés-San Román (4), Jose A. Sanz- Herrera (5), Franck Pichaud (2), Fernando Casares (3), Luis M. Escudero (4)

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Keywords Metamaterial, Morphogenesis, Drosophila, eye.

A fundamental research area in materials science focuses on metamaterials: systems whose properties do not depend so much on the substance they are made of, but rather on how they are organized. A metamaterial features a precise geometric design, and it is this design which dictates the system behaves in a specific way. Within this field, a particularly interesting advancement is that of programmable metamaterials: structures designed to alter their shape in a predictable manner when activated by external stimuli. Until now, creating something so complex was considered an exclusive achievement of human-designed technology.

I will present our current work focused on the morphogenesis of the Drosophila compound eye. We investigate the unknown mechanisms encoding the organ’s precise 3D curvature, which is essential for panoramic vision. Our key finding is that the developing eye functions as a biological programmable metamaterial. This mechanism is controlled by a specific pattern of triangle sizes in a supracellular mesh located in the basal retina. Computational and genetic evidence shows that this micropattern is essential for establishing local curvature, revealing a novel principle of morphogenesis control.

Our research describes the first natural metamaterial, demonstrating that biology uses engineering principles previously thought to be exclusive to humans.

References 1 Garrido-García et al. 2026. Nature Communications, in press.

SELECTED TALK · Session 3 · Morphogenesis and Organogenesis I

Shade enhancers, a novel class of redundant shadow enhancers uncovered by functional JAK-STAT signalling pathway dissection in Drosophila

James C-G Hombría (1), Carlos Sánchez-Higueras (1), Laura Terrón (1), Daniel Barcenilla-Merino (1), Mar García- Ferrés (1), Álvaro Marrufo (1), Sol Sotillos (1)

(1) CABD, CSIC-JA-UPO

Keywords Functional analysis, JAK-STAT, Upd ligand, shadow enhancers, Drosophila, gene regulation

JAK-STAT signalling pathway activation relies mostly on the transcriptional regulation of its ligands. In contrast to the complexity of the vertebrate pathway, with a multiplicity of ligands, receptors, kinases and transcription factors, the simplicity of the Drosophila pathway with only three ligands (Upd1, Upd2 and Upd3) facilitates the genetic study of its multiple functions. To discover how, when and where the Upd1 ligand performs each of organogenetic function, we searched for the enhancers controlling its dynamic spatio-temporal regulation, and used them to generate minigenes replicating specific stages of Upd expression. The combination of these minigenes with mutations deleting all three Upd ligands, allowed us to identify the timing when the various embryonic functions are mediated, including tracheae, corpora allata and prothoracic gland specification, head involution, posterior spiracle formation and hind gut elongation. We uncover most of the enhancers controlling STAT activation during embryonic organogenesis and those driving activation in the adult testis and the ovary. As expected, we identified several redundant shadow enhancers but, interestingly, we found a different type of redundant enhancers that mediate identical developmental functions although driving completely different expression patterns. We name this new class of functionally redundant enhancers driving different expression as shade enhancers. We propose shade enhancers provide evolutionary plasticity as they allow the spatial pattern of gene expression diversification without altering the underlying body plan.

SELECTED TALK · Session 3 · Morphogenesis and Organogenesis I

Early development of the early otic placode and the morphogenetic balance between the asymetric epithelizing OEPD and migrating neural Crest.

Lucas Cunha (1), Berta Alsina (2)
(1) UPF, (2) UPF-MELIS

Keywords Morphogenesis, Inner ear, 3D-segmentation, Neural Crest, Live-imaging
The inner ear is the sensory organ for hearing and balance. Across vertebrates, specified ectodermal domains called cranial placodes undergo morphogenesis to generate these systems (1). In zebrafish, the otic placode (OP) arises from a homogeneous progenitor population expressing Pax2/Pax8, Gata3, and Foxi1, known as the posterior placodal region (PPR). Otic placode morphogenesis (OPM) is a swift, 4-hour process (12–16 hpf) that segregates the PPR into three distinct Pax2a+ placodes (2, 3). Because traditional research has largely bypassed early OPM mechanics in favor of sequential gene expression, we combined high spatio-temporal imaging with advanced quantitative image analysis to elucidate the physical forces driving early development.

We identified two distinct tissue dynamics governing OPM. First, using an in-house single-cell 3D segmentation tool, we quantitatively demonstrate for the first time that OPM involves stepwise epithelialization, with ventral-medial cells being geometrically distinct from dorsal-lateral counterparts. We are currently testing how inducing morphogens, like FGF, manipulate this tissue architecture using mutant placodes. Second, we show for the first time that migrating Neural Crest Cells (NCCs) of the R4-5 and R5-6 streams synchronize precisely during OPM. This migration is linked to OP anteroposterior (AP) compaction, which is directly altered when NCC migration is inhibited. We are currently testing whether this disruption confers a cell-sorting phenotype in the PPR, evaluating the "chase and run" hypothesis (4) in vivo.

Ultimately, this work introduces novel methodologies and advances current paradigms, helping move cranial placode development toward a holistic, mechanochemical understanding.

References 1 Whitfield TT, Riley BB, Chiang MY, Phillips B. Development of the zebrafish inner ear. Dev Dyn. 2002;223(4):427-458. doi:10.1002/dvdy.10073 2 Groves AK, Fekete DM. Shaping sound in space: the regulation of inner ear patterning. Development. 2012;139(2):245-257. doi:10.1242/dev.067074 3 Hoijman E, Rubbini D, Colombelli J, Alsina B. Mitotic cell rounding and epithelial thinning regulate lumen growth and shape. Nat Commun. 2015;6:7355. Published 2015 Jun 16. doi:10.1038/ncomms8355 4 Theveneau E, Steventon B, Scarpa E, et al. Chase-and-run between adjacent cell populations promotes directional collective migration. Nat Cell Biol. 2013;15(7):763-772. doi:10.1038/ncb2772

SELECTED TALK · Session 4 · Morphogenesis and Organogenesis II

Spatio-temporal synchronisation of caspase activation and its impact on cell death pattern

Antonio Jesus Tagua Jañez (1), Tom Cumming (2), Alexis Villars (3), Anđela Davidović (1), Romain Levayer (1)

(1) Institut Pasteur, (2) University of Glasgow, (3) ETH Zurich

Keywords Apoptosis / Epithelium / Caspases / Self-organisation / optogenetics

Programmed cell death (apoptosis) is essential for epithelial tissue homeostasis and organ function. Although the molecular regulators of apoptosis are well described, the spatiotemporal patterns of cell death within tissues remain poorly understood, likely due to complex intercellular interactions and feedback mechanisms that locally regulate apoptotic rates1.

Recent studies have revealed a conserved negative feedback mechanism in which ERK activation near dying cells locally blocks cell death and disperses apoptotic events in time and space2. However, in the absence of this negative feedback, the dispersion of cell death is lost, leading to a significantly higher degree of cell death clustering than would be expected by random chance. Using live imaging in the Drosophila pupal notum combined with optogenetics2, live caspase sensors3, and genetic perturbations, we investigated the dynamics of caspase activation and determined whether specific local mechanisms promote this clustering of cell death. Our results reveal that caspase activity is locally clustered, consistent with a potential propagation mechanism, and suggest that synchronised cell division contributes to coordinated caspase activation events.

Together, this work identifies multiple regulators of caspase dynamics with important implications for tissue homeostasis.

References 1 Cumming, T., & Levayer, R. (2024). Toward a predictive understanding of epithelial cell death. Seminars in Cell & Developmental Biology, 156, 44–57. https://doi.org/10.1016/j.semcdb.2023.06.008. 2 Valon, L., Davidović, A., Levillayer, F., Villars, A., Chouly, M., Cerqueira-Campos, F., & Levayer, R. (2021). Robustness of epithelial sealing is an emerging property of local ERK feedback driven by cell elimination. Developmental Cell, 56(12), 1700–1711.e8. https://doi.org/10.1016/j.devcel.2021.05.006 3 Schott, S., Ambrosini, A., Barbaste, A., Benassayag, C., Gracia, M., Proag, A., Rayer, M., Monier, B., & Suzanne, M. (2017). A fluorescent toolkit for spatiotemporal tracking of apoptotic cells in living Drosophila tissues. Development , 144(20), 3840–3846. https://doi.org/10.1242/dev.149807.

SELECTED TALK · Session 4 · Morphogenesis and Organogenesis II

Cell type-specific regulation of gene expression and chromatin accessibility by mechanical tension during gastrulation

Elena Sánchez (1), Jorge Corbacho (1), Rocío Polvillo (1), Javier Macho (2), Cielo Lucía Centola (1), Juan Ramón Martínez (1)

(1) CSIC-CABD, (2) CSIC-CABIMER

Keywords Mechanical regulation; single-cell multiome; chromatin accessibility; gene expression; embryonic development.

Genetic programs and mechanical forces during embryonic development are key drivers of tissue morphogenesis, with a strong bidirectional interplay that generates mechanical feedback loops. Despite their relevance in development, repair, and cancer1, 2, the underlying molecular mechanisms remain poorly understood. Our laboratory investigates in vivo “outside-in” mechanotransduction, focusing on how mechanical cues regulate chromatin and transcriptional programs that control cell mechanics, survival, proliferation, and fate. To achive this, we applied external tension to gastrulating embryos of Oryzias latipes (medaka) at 70% epiboly using a motorized mechanical tester (Univert; CellScale). Control and mechanically compressed embryos were then collected and Chromium 10x single-cell multiome experiments were performed. Cell annotation was performed using an orthology-based expression reference derived from zebrafish data available at the ZFIN repository, which allowed us to identify the major embryonic germ layers (ectoderm, endoderm, and mesoderm). Differential gene expression and chromatin accessibility analyses between compressed and control samples were performed for each cluster, revealing a stronger response to mechanical compression in non-neural and neural ectoderm cells. This response was also associated with differences in cell cycle state, leading compressed cells to accumulate in G1 phase, suggesting that mechanical compression favours cell identity of ectodermal cells. Notably, through GRN inference analysis, foxh1 emerged as a candidate transcription factor potentially implicated in mechanoregulation in gastrulating precursors. These preliminary results suggest that mechanical tension is differentially interpreted by germ layer precursors at the regulatory level, with important effects specifically on ectodermal identity.

References 1 Northcott JM, et al. 2018. Front Cell Dev Biol 6, 17. DOI: 10.3389/fcell.2018.00017 2 Zanconato F, et al. 2016. Cancer Cell 29, 783-803. DOI: 10.1016/j.ccell.2016.05.005

SELECTED TALK · Session 5 · Neurodevelopment

Organizers or disorganizers? A folate-sensitive cortical hem organizer dictates the divergent outcomes of one-carbon-limited brain development

Antonio Herrera (1)
(1) EPFL/UGR

Why does a simple vitamin make the difference between healthy brain development and catastrophic birth defects? Folate is best known as the nutrient that prevents neural tube defects (NTDs). More recently, folate deficiency has also been associated with neurodevelopmental disorders such as autism spectrum disorder, raising the question of why embryos respond so differently to folate limitation. To address this, we combined single-cell RNA and ATAC sequencing, large-scale spatial transcriptomics, and ex utero embryo culture to dissect the developing brain at cellular resolution and trace how folate deficiency perturbs its organization and developmental dynamics.

Rather than causing global metabolic collapse, folate deficiency disproportionately affects a discrete dorsal telencephalic organizer: the Wnt8b+ telencephalic roof plate and its cortical hem derivatives. This vulnerability bifurcates through two dissociable branches of one-carbon metabolism acting at distinct developmental stages. An early nucleotide-limited branch activates Ybx1-associated replication stress, depletes organizer progenitors, and leads to neural tube closure failure. Later, a methylation-dependent branch remodels membrane phospholipids and impairs maturation of cortical hem-derived radial glia, driving premature differentiation of adjacent dorsal telencephalic glutamatergic populations even in embryos that successfully complete neural tube closure.

Together, these results support a stage-dependent model in which early organizer loss produces lethal NTDs, whereas later defective organizer maturation yields morphologically silent cortical phenotypes. These findings show that successful neural tube closure does not guarantee cortical integrity, linking folate metabolism to neurodevelopmental risk beyond overt malformation and identifying a metabolic organizer as a privileged interface between nutrition and brain patterning.

SELECTED TALK · Session 5 · Neurodevelopment

Early diversification of neural progenitor cells in the dorsal pallium of amniotes

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Keywords neurodevelopment, amniotes, cerebral cortex, neural progenitor cells
The phenomenal size and cellular diversity of the human cerebral cortex is the outcome of an elaborate developmental program sculpted by evolutionary forces during millions of years. This evolution involved changes in neural progenitor cells, which compared to reptiles and birds acquired the capacity to generate larger numbers of neurons and in greater diversity. Genetic causes of increased progenitor cell proliferation during evolution have been identified, but mechanisms involved in the diversification of neural progenitor cells remain unknown. To address this question, we performed the first integrated, multi-level, comparative study of neural progenitor cells and lineages in the embryonic cortex across amniotes. The analysis was focused on four strategic species that cover the spectrum of cortical phenotypes: house snake, chick, mouse and ferret. Our results revealed an unsuspected diversity of basal progenitor cell types in chick, superior to mouse and with similarities to the more complex ferret cortex. Chick dorsal pallial progenitors included subapical and basal Radial Glia, and co-expressed Pax6 and Tbr2, similar to ferret but unlike mouse, where these are largely absent. Our findings challenge current concepts on the evolutionary trajectory of the cerebral cortex, revealing a very early diversification of cortical progenitor cell types in amniotes that predated the last common ancestor of mammals and birds.

SELECTED TALK · Session 6 · Evo-Devo

Gene duplication and co-option originated a male specific visual system in mayflies

Isabel Almudí Cabrero (1)
(1) IRBio/UB

Morphological novelties represent key evolutionary transitions, yet their emergence within highly conserved body plans remains poorly understood. Here, we investigate the origin of the turbanate eyes (TurbEyes) of mayflies, an extreme example of a novel, male-specific visual system. Using single-cell transcriptomics, chromatin accessibility, and gene regulatory network reconstruction, we show that TurbEye development results from the co-option and partial rewiring of the conserved Retinal Determination Gene Network (RDGN). We identify a novel, male-specific paralogue of the sex-determination transcription factor doublesex (dsxM) as a key upstream regulator that recruits this network by directly activating eyeless during early embryogenesis. This co-option is accompanied by expansion of male-specific regulatory programs during nymphal development, supporting the progressive formation of a duplicated visual system including its associated neural circuitry. Our findings reveal that the integration of gene duplication and sex-specific regulation enables the redeployment of conserved developmental programs. Together, these findings show how conserved developmental systems can be redeployed to generate striking morphological innovations.

SELECTED TALK · Session 6 · Evo-Devo

Characterizing the small-spotted catshark embryonic fins at single-cell resolution

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Keywords Gene regulation, Development, Evolution, Single-cell technologies, Fin-to-limb transition

During fin- to- limb transition, gene networks are believed to be conserved, yet rewired. Indeed, transcriptional control of the same genes by different combinations of cis-regulatory elements (CREs) bound by tissue-specific transcription factors (TFs) modulates their expression patterns. Despite of decades of investigation of this popular textbook evolutionary process, our knowledge is still limited.

Here, we employ the recently sequenced non-model species small-spotted catshark (S. canicula), a Chondricthyan representing the most ancestral lineage of jawed vertebrates. Using simultaneous single-cell RNA- and ATAC-seq in paired catshark fins in two developmental stages, we aim at characterizing the cell populations in those tissues. Our analyses reveal cell populations resembling tetrapod appendages, with key appendage patterning genes exhibiting conserved expression patterns. Meanwhile, we also detect genes with altered or heterochronic expression compared to the mouse limb.

Overall, we explore the expression domains of patterning genes and the cis-regulatory elements controlling those in Chondrichthyan fins. Next, we wish to identify key regulatory differences underlying the gene expression pattern alterations between fins and limbs and eventually, elucidate some of the events of the fin-to-limb transition.

SELECTED TALK · Session 7 · Developmental Disorders

A Neurodevelopmental Syndrome Arising from Enhancer of Polycomb Homologue 1 Haploinsufficiency

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Keywords neurodevelopmental disorders, NuA4/TIP60 complex, neuroepigenetics, EPC1 haploinsufficiency, motor function, cognitive impairment

Epigenetic regulation is essential for brain development, yet how distinct chromatin-modifying activities are integrated to shape neural circuits remains poorly understood. We identify haploinsufficiency in enhancer of polycomb homologue 1 (EPC1), a core subunit of the NuA4/TIP60 complex, as a conserved, dosage-sensitive regulator producing locomotion-related phenotypes across species. De novo truncating variants in EPC1 were found in individuals with early-onset neurodevelopmental syndromes featuring prominent motor impairment. Locomotor and motor-coordination deficits are recapitulated in mice and C. elegans models of reduced EPC1/Epc1 dosage, suggesting a deeply conserved requirement for EPC1 in neurodevelopment.

Mechanistically, reduced dosage of EPC1 leads in mice to cortical hypoacetylation, dysregulated developmental gene programs, altered excitatory–inhibitory neuronal balance and reduced recruitment of neural networks upon cognitive demands. These developmental defects manifest as impairments in motor coordination, cognition, and behavioral flexibility. Together, our results establish EPC1 haploinsufficiency as a dosage-sensitive disruption of neurodevelopmental programs and uncover conserved epigenetic control of motor circuit formation as a core disease-relevant mechanism.

SELECTED TALK · Session 7 · Developmental Disorders

A progeria mutation in baf-1 confers neuroprotection against polyQ-based aggregates

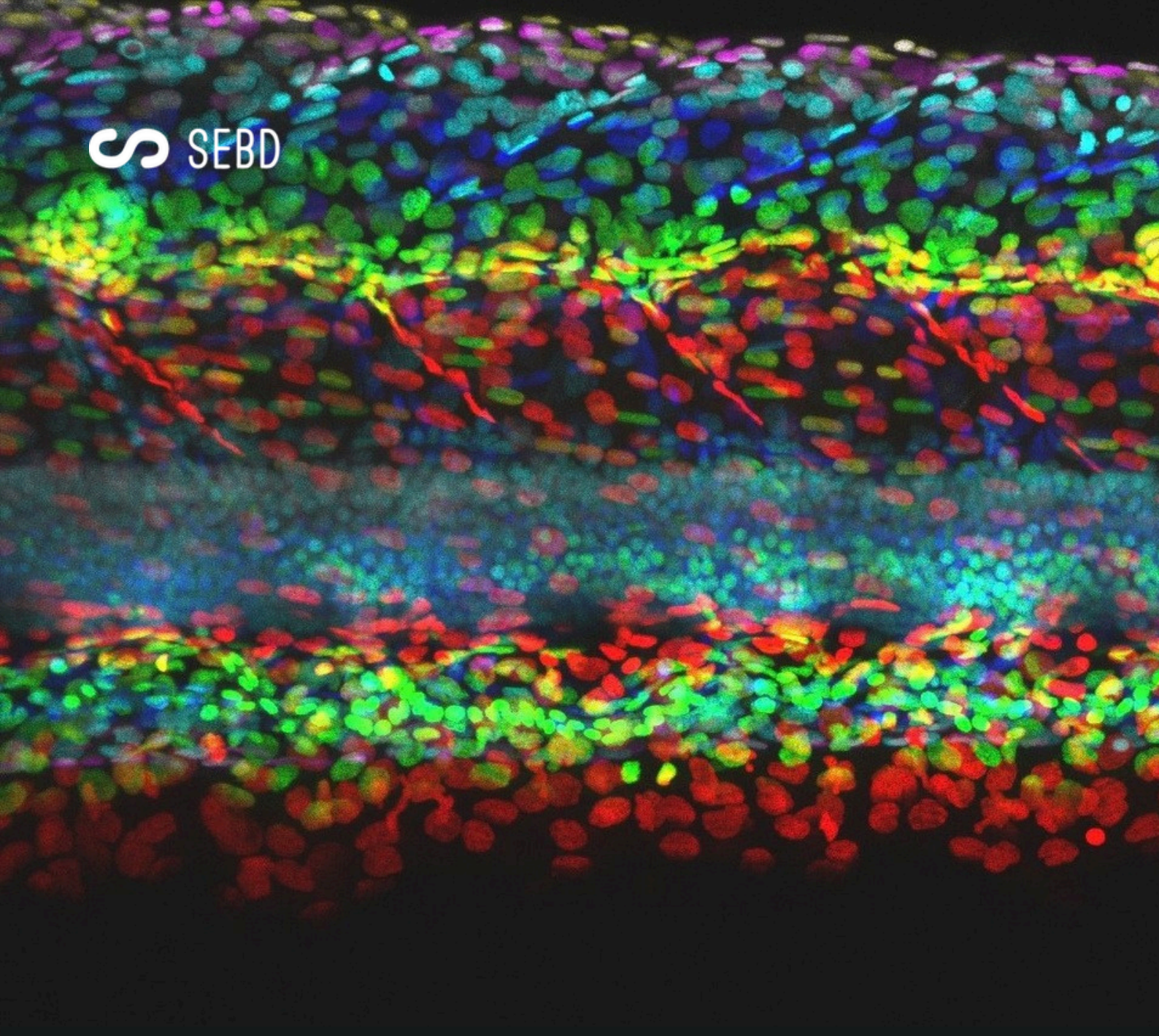
Adrián Fragoso-Luna (1), Andrea Del Valle Carranza (2), María Luque-Jiménez (1), Anita G Fernandez (3), Patricia De La Cruz-Ruiz (4), Jesús Fernández-Abascal (1), Cristina Ayuso-García (1), Antonio Miranda-Vizuete (4), Ángeles Ortega-De La Torre (1), Rafael Vázquez-Manrique (2), Peter Askjaer (1)

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Keywords Accelerated aging; Progeria; Nuclear envelope; Chromatin organisation; Neurodegeneration

Progerias are rare syndromes that cause accelerating ageing since early childhood, with an average lifespan close to 15 years. The aetiology lies in mutations within components of the nuclear envelope, like lamin (Hutchinson-Gilford Progeria Syndrome) and BAF (Néstor-Guillermo Progeria Syndrome). Patients suffer typical problems linked to ageing, like osteoporosis, atherosclerosis, lipodystrophy, sarcopenia, hair loss, etc. Paradoxically, it is remarkable that they do not develop neurodegeneration, a classical hallmark of ageing.

Using a C. elegans model of Néstor-Guillermo Progeria Syndrome (baf-1(G12T)), we have observed a significant resistance to polyQ-associated neurodiseases like, Huntington and Joseph-Machado ataxia. The frequency of protein aggregates is reduced specifically in neurons of baf-1(G12T) mutants, accompanied by an improvement in motility and touch response. Other neurodegenerations, as Alzheimer, Parkinson and ALS are also triggered by aggregation of aberrant proteins, that interfere with the correct physiology of the cell. Specifically, many studies suggest that the main intracellular process affected is endolysosomal trafficking, key in neurons. Interestingly, our work indicates that Néstor-Guillermo Progeria Syndrome also affect endolysosomal trafficking both in C. elegans and human patient cell lines. Analysis of chromatin accessibility in neurons reveals that the progeria mutation induces opening of genes involved in proteostasis. We postulate that accelerated-ageing disorders antagonize neurodegeneration through endolysosomal trafficking.



06 / 09

Awards

Gómez-Skarmeta and thesis prizes

AWARDS · JL GOMEZ SKARMETA AWARD

Aneuploidy and its impact on human development

Martha Shahbazi
(MRC-LMB. CAMBRIDGE, UK)

Chromosome segregation errors are unusually frequent in early human embryos compared with other mammals, resulting in a remarkably high incidence of aneuploidy. This contrasts with the low frequency of aneuploid cells detected at birth, suggesting active quality-control mechanisms eliminate or tolerate abnormal cells during development. How the fate of individual aneuploid cells is determined, and how these fate decisions influence the developmental potential of embryos remain largely unknown. Intriguingly, aneuploid cells are commonly found in the placenta, without overtly compromising its function, pointing to tissue-specific mechanisms of aneuploid tolerance.

Our laboratory investigates how different embryonic and extra-embryonic tissues respond to chromosome imbalance. In this talk, I will discuss recent advances in developing aneuploid placental organoid models and genomic screens to understand the molecular mechanisms that govern the fate of aneuploid cells during early human development. Ultimately, this work aims to provide a mechanistic framework for understanding how aneuploidy influences cell function, tissue formation, and the establishment of a healthy pregnancy.

AWARDS · BEST THESIS 2024

Mammalian digit tip regeneration: dissecting the roles of dorsoventral patterning, Lmx1b, and nail-derived signaling

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The digit tip is the only region of the mammalian limb, including mammals, capable of regenerating after amputation, a phenomenon closely linked to the presence of the nail organ. To investigate this process, we analyzed LARM1/2 mutants, which lack both the nail organ and dorsoventral patterning due to the loss of Lmx1b, a transcription factor responsible for establishing limb dorsality. In these mice, nail-less digits fail to regenerate, and this failure is not due to the loss of dorsoventral polarity, demonstrating that the nail organ is required for digit tip regeneration independently of dorsoventral patterning. We further found that Lmx1b is expressed in the dermis underlying the nail and in the regenerative blastema, suggesting a possible direct role for this gene in postnatal regeneration distinct from its classical embryonic function in dorsoventral patterning. To disentangle the contribution of Lmx1b from that of the nail itself in LARM1/2 mutants, we induced nail signals, namely Wnt/β-catenin and Fgf. While activation of β-catenin failed to restore regeneration, exogenous FGF treatment promoted blastema expansion and bone regeneration. These results indicate that FGF is able to bypass Lmx1b deficiency, suggesting that FGF may act downstream or independent of Lmx1b. Current experiments are aimed at determining whether restoring Lmx1b expression in LARM1/2 mutants is sufficient to rescue digit regeneration independently of the nail.

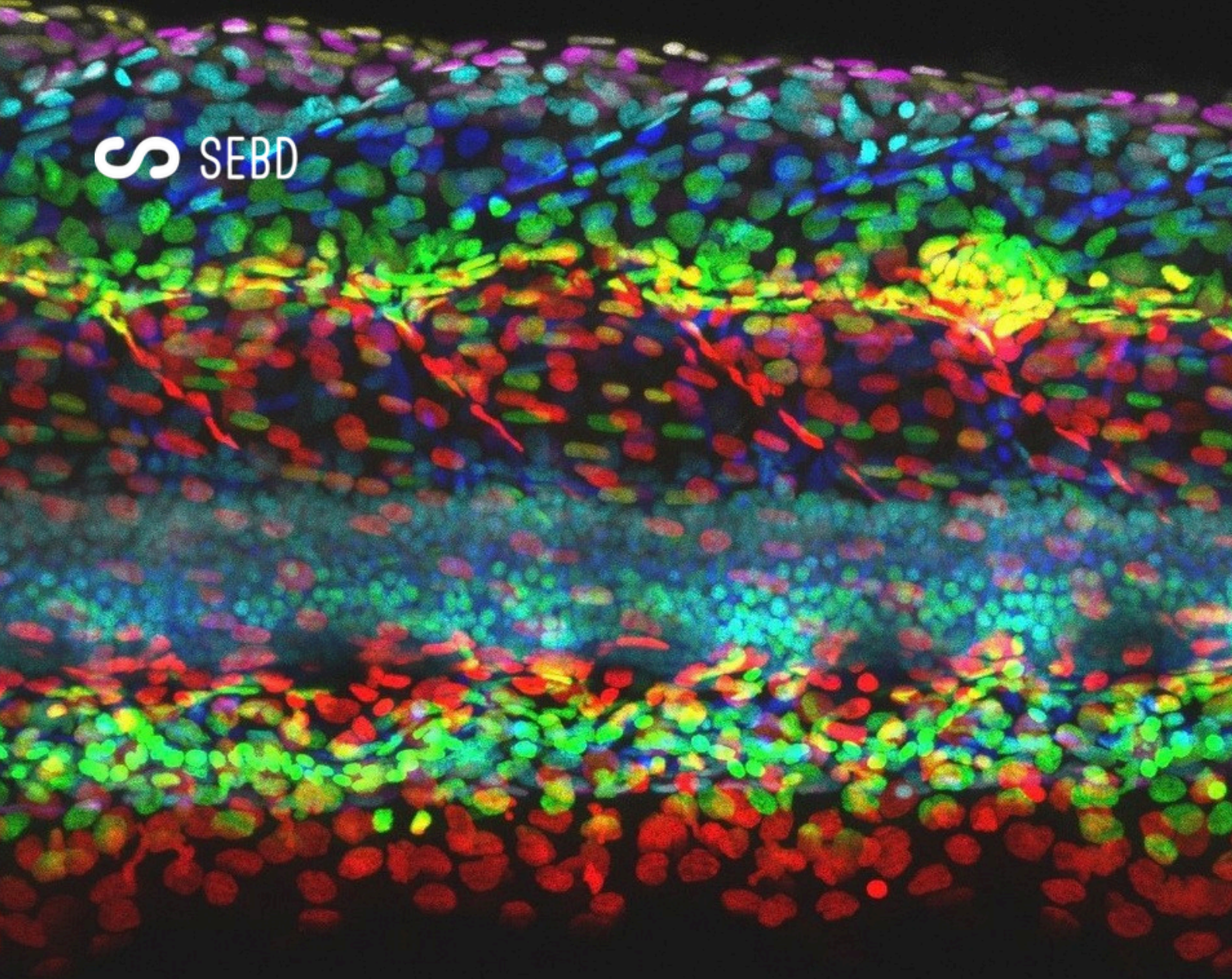
AWARDS · BEST THESIS 2025

CRISPR-RfxCas13d screening uncovers Bckdk as a post-translational regulator of maternal-to-zygotic transition in teleosts

Luis Hernández-Huertas

(CABD, Sevilla, Spain)

The maternal-to-zygotic transition (MZT) is a reprogramming process encompassing zygotic genome activation (ZGA) and the clearance of maternally provided mRNAs. While some factors regulating the MZT have been identified, there are thousands of maternal RNAs whose functions remain uncharacterized. To address this gap, we implemented the CRISPR-RfxCas13d system as an effective and precise tool for depleting specific RNA transcripts in zebrafish and other vertebrate embryos, such as medaka, killifish, and mouse. Using this technology, we performed a maternal RNA screen targeting mRNAs that encode kinases, phosphatases, or their regulatory proteins in zebrafish embryos. This screen identified Bckdk as a novel post-translational regulator of the MZT. Maternal bckdk knockdown caused epiboly defects, ZGA dysregulation, global H3K27ac reduction, and a partial impairment of miR-430 processing. Phosphoproteomic analysis revealed that the chromatin remodeling factor Phf10/Baf45a is less phosphorylated upon Bckdk depletion. Furthermore, phf10 knockdown also disrupted ZGA, whereas the expression of a phosphomimetic Phf10 mutant rescued the developmental defects induced by bckdk depletion and restored H3K27ac levels. Altogether, our results demonstrate the power of CRISPR-RfxCas13d screening to uncover novel regulators of early vertebrate development and shed light on the post-translational control of the MZT mediated by protein phosphorylation.



SEBD

07 / 09

Poster flash talks

FLASH TALKS · ODD: DAY 1 · Poster number 1

Developmental Roles of Non-canonical DNA Methylation at Satellite DNA Repeats

Thirsa Brethouwer (1), Álvaro Pérez Martos (1), Laura Romero Montañez (1), Anne Jung (2), Daniel Summerer (2), María Almuedo Castillo (1), Ozren Bogdanovic (1)

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Keywords Non-canonical DNA Methylation, H3K9me3, Embryonic Development, TALE imaging

DNA methylation is a major epigenetic modification of vertebrate genomes, mainly found in the CpG dinucleotide context. DNA methylation in the non-CpG context (mCH, H=A, C or T) has been identified in the vertebrate subphylum, however, at much lower quantities and in a tissue-restricted manner. One of the major impediments of studying the function of mCH in mammals is that it cannot be uncoupled from mCG, as both are written by DNMT3A/B. Our lab has recently described a peculiar mCH reprogramming event that occurs at mosaic satellite repeats (MOSAT) in teleosts, for which, in zebrafish, Dnmt3ba was found to be specifically responsible. This offers a unique opportunity to study the independent function of mCH. Here we investigate to what extent mCH reprogramming at MOSAT repeats affects early zebrafish embryonic development. Using base-resolution DNA methylome quantification, TALE-based imaging and transcriptome and DNA accessibility profiling, in zebrafish loss of function models, we are working towards understanding the function of mCH. We have observed an acceleration in early embryonic development, altered transcriptomes and an increase in MOSAT repeat accessibility in zebrafish models deficient in mCH at MOSAT repeats. In addition, imaging using MOSAT specific TALE's shows reduced localisation of MOSAT repeats to the nuclear periphery in these mutants. Finally, we describe an inverse relationship between mCH and H3K9me3 at MOSAT repeats, suggesting a structural role for mCH in early embryonic development. Together these results contribute to establishing novel functions for one of the oldest, and best described gene-regulatory marks.

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FLASH TALKS · ODD: DAY 1 · Poster number 3

Uncovering how cellular connections and mechanical inputs impact gene regulation during embryonic development

JUAN APERADOR REDONDO (1), JESÚS GARCÍA-JUNCO (1), ALBERTO PÉREZ-POSADA (1), ESTEFANÍA SANABRÍA REINOSO (1), GIULIO STORARI (1), NICHOLAS DE JAGER (1), ANDREA MARTÍNEZ VARA (1), THIRSA BRETHOUWER (1), OZREN BOGDANOVIC (1), MARÍA ALMUEDO CASTILLO (1)

(1) CENTRO ANDALUZ DE BIOLOGÍA DEL DESARROLLO

Keywords Mechanotransduction, early embryonic development, single-cell, DSP fixation

During embryonic development, apparently identical cells differentiate into distinct types with specific functions and well-defined morphologies. For instance, stem cells exhibit rounded geometries with relaxed nuclei, whereas differentiated cells acquire complex shapes. Extracellular matrix (ECM) changes are transmitted to the cell membrane and nucleus, activating specific transcriptomic programs. However, how cells coordinate mechanical sensing with gene regulation remains poorly understood.

Although single-cell omics are essential for studying cellular heterogeneity, the required cell dissociation step disrupts rapid and reversible mechano-regulated processes. To overcome this limitation, we utilized dithiobis(succinimidyl propionate) (DSP) as a genomic fixator prior to dissociation.

To identify genes modulated by cellular connections and geometries (the "cellular connexome"), we compared fresh and DSP-fixed dissociated cells from early zebrafish embryos. Our bulk and single-cell RNA-seq data reveal that DSP-fixed cells retain significantly higher expression of genes related to cell-cell and cell-ECM contacts. Transcription factors (TFs), intermediate filaments, and ion channels are also better preserved, suggesting their mechanosensitivity.

Comparative scRNA-seq cluster analysis shows that the largest transcriptomic differences correspond to cell populations undergoing long, rapid migration during epiboly. We are currently validating these cellular connexome components via in situ hybridization, in vivo TF-GFP tracking, and CRISPR-Cas9 knockouts.

Ultimately, this optimized DSP-fixation protocol enables unprecedented resolution for assessing how cellular connections and mechanical states regulate gene expression at the single-cell level during embryonic development.

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FLASH TALKS · ODD: DAY 1 · Poster number 5

Calcium dynamics control the ovarian stem cell niche homeostasis during ageing

Helena Sánchez Gómez (1), Judith Huertas, Dilamm Even Ros, Acaimo González-Reyes (1), Salvador C. Herrera (1)

(1) CABD

Keywords Drosophila melanogaster, germline stem cells, calcium dynamics, calcium transients.

Ageing is concomitant with altered tissue maintenance and repair. Because stem cell activity is key to maintaining tissue homeostasis and since tissue attrition correlates with decreased stem cell homing, self-renewal, and repopulation capacity, stem cell ageing lies beneath tissue ageing. During ageing, both stem cells and their niches undergo changes, resulting in impaired niche activity and the waning of resident stem cell populations. To identify new genes required in niche support cells and involved in age-associated germline stem cell (GSC) depletion, my group embarked upon a transcriptomic analysis of ageing niche support cells in the Drosophila ovary and found a discrete number of candidate genes whose transcription was regulated with time1. These included genes related to calcium dynamics. We thus decided to study the effect of calcium metabolism during female germline niche ageing.

During in vivo recordings using a calcium fluorescent reporter, we observed that niche cells present calcium transients. We quantified several parameters and found significant differences between young and old cells. To assess the effects of calcium manipulation, we used RNA interference to knock down genes involved in calcium dynamics in niche support cells. We discovered that calcium channels such as RyR and Itpr; calcium-binding proteins including Cam and CanB; and its downstream signalling components, such as CaMKII, are essential in niche cells for the proper maintenance of GSCs, preserving their population size and preventing excessive expansion in young and old flies. Our latest findings support a model in which calcium dynamics regulate niche homeostasis during ageing.

References 1 Even-Ross, D., et al. (2025) Nat. Comms. 16:2596. doi:10.1038/s41467-025-57901-8

FLASH TALKS · ODD: DAY 1 · Poster number 7

Matrix Stiffness as a Mechanobiological Regulator of Cardiomyocyte Response During Zebrafish Heart Regeneration

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(1) Centro Andaluz de Biologia del Desarollo

The zebrafish (Danio rerio) heart serves as a valuable model for investigating the cellular and molecular mechanisms underlying cardiac development and regeneration. In contrast to mammals, zebrafish possess a robust capacity to fully regenerate cardiac tissue following injury, making them an optimal system for studying the regulatory processes involved. A key aspect of regeneration is the dynamic remodelling of the extracellular matrix (ECM), which progresses through distinct mechanical states: an initial softening phase associated with inflammatory infiltration, a stiffer fibrotic intermediate resulting from collagen and fibrin deposition, and a gradual restoration of baseline compliance as tissue repair advances. These alterations in ECM stiffness function as instructive mechanobiological signals that directly modulate cardiomyocyte behaviour, proliferation, and fate.

Building on this background knowledge, my research investigates how cardiomyocytes sense and respond to mechanical stimuli during regeneration by employing hydrogels with tunable stiffness that mimic the mechanical progression of the injured myocardium. Phenotypic and multi-omics analyses are integrated to map the mechanosensitive gene regulatory networks activated under varying mechanical conditions.

In parallel, this research explores how tissue-level mechanical forces, including hemodynamic flow and myocardial tension, shape early embryonic heart looping, a critical morphogenetic event that establishes cardiac asymmetry and chamber organization. Taken together, this work seeks to define the mechanobiological logic that connects physical forces to cardiac form and function, with broader implications for regenerative medicine

FLASH TALKS · ODD: DAY 1 · Poster number 9

Unlikely allies: non-canonical role for MMP14 and N-cadherin during Xenopus laevis neural crest migration

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(1) Center for Integrative Biology Toulouse

Keywords epithelial-mesenchymal transition, cell migration, cell motility, matrix-metalloproteases, cadherins, Xenopus laevis, neural crest, morphogenesis

Neural crest cells are a population of embryonic pluripotent cells that undergo epithelial-mesenchymal transition (EMT) and migrate to give rise to a wide array of cell types. EMT is a critical process in which a static epithelial cell becomes motile by acquiring a mesenchymal phenotype. To start migrating, cells change their adhesion repertoire to transition from stable to transient cell-cell interaction. This is performed notably through a switch between E-cadherin and N-cadherin. Additionally, to remodel the extracellular environment, cells begin to express and secrete Matrix Metalloproteases (MMPs). MMP14 is a membrane-bound MMP that is often expressed during EMT in both physiological and pathological settings. MMPs are primarily known for their involvement in extracellular matrix remodeling, but are now understood as versatile proteins. They can process a large variety of substrates outside their scope, perform non-canonical functions, both dependently and independently of their catalytic activity, and can even traffic to the nucleus. Here, using Xenopus laevis neural crest cells as a model, we combined knockdown experiments with biochemical and microscopy analysis both in vivo and ex vivo, and uncovered two unexpected roles for MMP14 and N-cadherin during cell migration. Functionally, MMP14 and N-cadherin are part of the same pathway. However, MMP14 does not act via matrix remodeling, and N-cadherin does not act through its expected homotypic binding but affects adhesion and motility cell-autonomously. These previously undocumented functions provide new insight into the network at play during cell migration.

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FLASH TALKS · ODD: DAY 1 · Poster number 11

Metabolic control of multicellular transition in a close unicellular relative of Metazoa

Eudald Pascual Carreras (1), Elena Casacuberta (2), Iñaki Ruiz-Trillo (2), Vikas Trivedi (1)

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Keywords Multicellularity, Capsaspora, aggregation, metabolism

The emergence of multicellular animals from unicellular ancestors remains a mystery. Current research has focused on key processes for multicellular life, such as cell adhesion, cell signaling and transcriptional regulation. However, the potential role of metabolism in this transition has not been studied. The filasterean Capsaspora owczarzaki, a close unicellular relative of animals, provides a powerful model to address this gap, as it exhibits three well-defined life stages: a quiescent, resistant cystic stage; a proliferative filopodiated stage; and a multicellular stage acquired through chemically induced aggregation (e.g., fetal bovine serum). Previous transcriptomic analyses revealed marked differences between these stages. Here, we extend this work by integrating lipidomic and metabolomic analyses to uncover metabolic remodeling across the unicellular-to-multicellular transition. Lipidomics reveals pronounced but stage-specific membrane reorganization. Cystic cells display rigid, storage-oriented membranes and reduced mitochondrial activity. Filopodiated cells initiate membrane remodeling, coupled to mitochondrial activation. In aggregates, cells undergo large-scale membrane expansion while maintaining lipid homeostasis. Metabolomic profiling further shows that the transition to multicellularity is accompanied by the upregulation of reductive metabolic pathways, including the pentose phosphate pathway (PPP) and glutathione metabolism, suggesting enhanced buffering of reactive oxygen species to promote survival in the multicellular state. Finally, pharmacological inhibition experiments demonstrate that PPP is required for aggregation formation and maintenance. Together, these results highlight metabolism as an active driver of the transition to animal multicellularity, opening new avenues to explore how metabolic innovation contributed to the evolutionary origins of multicellular life.

FLASH TALKS · ODD: DAY 1 · Poster number 13

Metabolic reprogramming during early planarian regeneration: a single-cell transcriptomic perspective

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What metabolic programmes does a planarian activate in the first hours after amputation? Despite extensive knowledge of the signalling pathways triggered by injury, the metabolic state of the tissue at the moment of wounding — and how it shifts in response — remains poorly understood. In stem cell-based regeneration, metabolic reprogramming may determine which cell types respond to injury and how, acting as an early instructive input rather than a downstream consequence of tissue growth [1,2]. Planarians (Schmidtea mediterranea) offer a tractable system to address this: regeneration depends on adult stem cells (neoblasts) [3], animals tolerate a wide range of nutritional states [4], and the early transcriptional response to amputation can be captured at single-cell resolution [5].

Using fGSEA-based pathway enrichment on single-cell transcriptomic data spanning five post-amputation time points (1.5–24 hpa), we characterise the dynamics of broad metabolic programmes after injury. Our analysis reveals that glycolytic programmes are suppressed across several cell types during the early regenerative response, while beta-oxidation shows context-dependent activation at specific time points. These pathway-specific and temporally dynamic changes are not consistent with the glycolysis-biased metabolism typically associated with stem cell activation and proliferation [6,7], pointing to metabolic dynamics during regeneration initiation that warrant further investigation. Ongoing analyses including metabolic scoring, trajectory analysis and integration with functional and lipidomics data will determine how these transcriptional metabolic dynamics connect to cell fate decisions and regenerative signalling.

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FLASH TALKS · EVEN: DAY 2 · Poster number 2

An endogenous retrovirus plays an essential role during zebrafish morphogenesis

Ana Ariza Cosano (1), Pilar G. Marchante (1), Ana Gázquez-Gutiérrez (1), Ana Colomer-Boronat (1), Francisco J Sánchez-Luque (2), Miguel Ángel Moreno Mateos (3), Sara R. Heras (1)

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Keywords Endogenous retrovirus, morphogenesis

Transposable elements, and particularly endogenous retroviruses (ERVs), are increasingly recognized as key contributors to vertebrate genomes. They have gained attention for their roles during embryogenesis, especially the ancient, inactive copies that have been co-opted for pluripotency. However, two major questions remain underexplored: whether young, recently active ERVs can be functionally integrated into host developmental programs, and whether ERVs can play essential roles during development, such as tissue morphogenesis. Here, we address both questions by studying ERV1-3, a recently active subfamily that is specifically expressed in the paraxial mesoderm and somites during morphogenesis in zebrafish. We find that this expression is mainly driven by three recently inserted, full-length copies with only a fragmented pol gene and whose LTRs harbour a unique combination of binding sites for specific mesodermal transcription factors. These include a Nodal-stimulated
genes, and as such we demonstrate that ERV1-3 is essential for mesoderm development downstream of Nodal signalling. Knockdown of ERV1-3 RNA using CRISPR-Cas13d resulted in early defects in axial and paraxial mesoderm development and later produced somite and midline phenotypes that closely resemble those seen in Nodal mutants. Importantly, these phenotypes can be partially rescued by injecting a transcript predicted from the highly expressed copies, demonstrating that ERV1-3 RNA is functionally required for proper mesoderm development. Taken together, our findings reveal that a recently active endogenous retrovirus has become integrated into a conserved vertebrate developmental programme and uncover an essential role for ERV-derived transcripts in tissue morphogenesis.

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FLASH TALKS · EVEN: DAY 2 · Poster number 4

Investigating the impact of ageing on 3D genome architecture in adIRD models in the turquoise

Juan Ramon Martinez (1), Javier Macho (2), María del Valle Hernández (1), Lucia Muñoz (1), Sofia Manzo (1), Lorena Ardila (1), Silvia Naranjo (1), Juan Tena (1)
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Keywords Retinal ageing, chromatin remodeling, turquoise killifish, photoreceptor degeneration, 3D genome architecture, epigenomics

The retina is a highly specialized neural tissue whose function depends on the lifelong maintenance of photoreceptor identity and chromatin organization. Inherited Retinal Diseases (IRDs) are among the leading causes of blindness worldwide, affecting more than 5 million people, yet the epigenomic mechanisms underlying their progression remain poorly understood. To investigate how chromatin regulation contributes to retinal homeostasis and degeneration, we use the turquoise killifish, a short-lived vertebrate model that recapitulates key hallmarks of human ageing. We generated a transgenic killifish line carrying a human rhodopsin mutation that induces progressive photoreceptor degeneration. Using bulk RNA-seq, ATAC-seq and Hi-C, we characterized transcriptional and chromatin landscape changes associated with retinal ageing and degeneration. Our analyses revealed widespread alterations linked to loss of retinal identity, activation of immune and stress-response pathways, and remodeling of chromatin accessibility. Alternative splicing analyses further uncovered conserved molecular programs shared between physiological ageing and neurodegeneration, including signatures also observed in zebrafish. Together, our results provide new insight into the gene regulatory and chromatin-based mechanisms that maintain photoreceptor identity and retinal function. These findings establish the turquoise killifish as a powerful vertebrate model to study how disruption of conserved regulatory programs contributes to neural tissue ageing and disease.

FLASH TALKS · EVEN: DAY 2 · Poster number 6

Mitochondrial metabolism defines developmental plasticity during nervous system development

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Keywords Critical periods; developmental plasticity; neurodevelopment; mitochondrial metabolism; reactive oxygen species; HIF-1α; Drosophila.

Critical periods are among the most remarkable phenomena in biology: brief developmental windows during which experience can permanently shape the nervous system. How these windows are established and ultimately closed remains one of the central unanswered questions in developmental biology. We hypothesised that mitochondrial metabolism acts as a developmental switch that determines when neural circuits are permissive to long-lasting plasticity. Using the Drosophila melanogaster locomotor network as a genetically tractable model, we combine precise temporal genetic manipulations with quantitative analyses of synaptic development, neuronal physiology and behaviour to investigate how metabolic state regulates critical period plasticity. Mechanistically, we identify mitochondrial reactive oxygen species generated by reverse electron transport at Complex I as instructive developmental signals that activate HIF-1α-dependent transcriptional programmes to couple transient environmental and activity-dependent inputs to long-lasting changes in neuronal properties, synaptic connectivity and behaviour. Ongoing work further indicates that critical period perturbations induce persistent metabolic reprogramming, supporting the idea that transient metabolic states determine whether developmental experience becomes permanently encoded. Together, our findings establish mitochondrial metabolism as a central regulator of developmental plasticity and support a new conceptual framework in which critical periods represent transient metabolically defined developmental states. This work provides a mechanistic basis for how early-life experience is permanently integrated into developing neural circuits and offers new perspectives for understanding neurodevelopmental disorders associated with disrupted critical period plasticity.

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FLASH TALKS · EVEN: DAY 2 · Poster number 8

Cnidarian HOX-DNA binding preferences reveal the shared logic of eumetazoan axial patterning

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Keywords HOX transcription factors, DNA binding specificity, Transcription factor evolution, Cnidaria-Bilateria divergence, Common ancestor Cnidaria-Bilateria

HOX genes encode an evolutionarily conserved family of homeodomain-containing transcription factors that pattern the anterior-posterior (AP) axis of bilaterians by regulating distinct gene networks. Outside of Bilateria, Cnidaria is the only other phylum with bona fide HOX genes, where they are used to pattern two orthogonal body axes. Sequence-based comparisons suggest that cnidarian genomes lack the full range of HOX DNA binding specificities present in bilaterians, raising the question of how HOX factors pattern cnidarian body axes. Here we integrate quantitative measurements of DNA binding specificity, high-resolution genome-wide binding experiments, and functional enhancer assays to demonstrate that cnidarian HOX proteins from the sea anemone Nematostella vectensis have the full range of binding specificities present in bilaterians. We further show that some Nematostella HOX proteins have a less restrictive binding specificity than their bilaterian counterparts. Thus, the full regulatory potential of HOX-mediated specification of the bilaterian AP axis is present in cnidarians, suggesting that the core features of HOX-DNA recognition were present in the eumetazoan common ancestor over 600 million years ago.

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FLASH TALKS · EVEN: DAY 2 · Poster number 10

Sex-specific programmed genome elimination in the sea lamprey

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Keywords sea lamprey, PGR, DNA elimination

The sea lamprey (Petromyzon marinus) is an ancient vertebrate lineage that provides a model to study early genome evolution. In most organisms, genome content and structure are maintained throughout development. However, some species, such as lamprey, undergo programmed genome rearrangement (PGR), in which specific DNA sequences are selectively eliminated during early embryogenesis. While PGR has been well characterized in the male lamprey germline, its occurrence in the female germline has remained unresolved, largely due to the technical challenges associated with isolating female germline DNA. To address this gap, we optimized a protocol to isolate oocyte DNA from sea lampreys and generated matched male germline samples from the same population. These samples were sequenced using combined long and short read approaches and compared to publicly available male germline and somatic datasets from a distinct population, aligning all datasets to both reference assemblies to enable cross-population comparisons. Analyses revealed that oocyte genomic profiles closely resemble somatic tissues and are distinct from the male germline. We identified genomic regions present in sperm but absent from both oocytes and soma, suggesting that these sequences are eliminated through PGR. These patterns are consistent across populations, indicating a conserved genome elimination mechanism that may help prevent the misexpression of male germline–restricted genes in inappropriate cellular contexts.

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FLASH TALKS · EVEN: DAY 2 · Poster number 12

Limb dorsalization: Lmx1b mode of actions during limb development

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The establishment of dorsoventral asymmetry during vertebrate limb development, leading to the formation of distinct dorsal and ventral compartments, is essential for proper locomotion and manipulation. The LIM homeobox transcription factor Lmx1b is expressed specifically in the dorsal limb bud mesenchyme and is both necessary and sufficient for specifying dorsal limb identity. Loss of Lmx1b function results in ventralization of dorsal structures, as demonstrated in mouse models and in Nail-Patella Syndrome in humans.

Despite its central role and previous genome-wide transcriptomic and ChIP-seq studies, the downstream gene regulatory network and direct targets of Lmx1b remain incompletely characterized.

To dissect the Lmx1b-mediated gene regulatory network in a systematic temporal manner, it is necessary to isolate Lmx1b-expressing cells. To achieve this, I generated the rLARM2-GFP mouse line, which drives GFP expression under the control of LARM2, a limb-specific Lmx1b enhancer. Characterization of GFP expression demonstrated faithful recapitulation of the dorsally restricted endogenous LARM2 activity, including its anterior bias. Additionally, I have established the Lmx1b-2A-Cre mouse line in the laboratory which, when combined with the ROSAmTmG reporter allele labels cells that have ever expressed Lmx1b. Using these genetic tools, we are starting to isolate dorsal limb mesenchymal cells and to characterize the transcriptional programs downstream of Lmx1b.

In parallel, I have obtained the only ChIP-grade validated Lmx1b antibody and started ChIP-seq experiments across multiple developmental stages.

Integration of these datasets will enable the identification of direct Lmx1b target genes and provide new insights into the gene regulatory mechanisms underlying limb dorsalization.

FLASH TALKS · EVEN: DAY 2 · Poster number 14

Exploring new NKTR functions associated with a rare neurodevelopmental disorder

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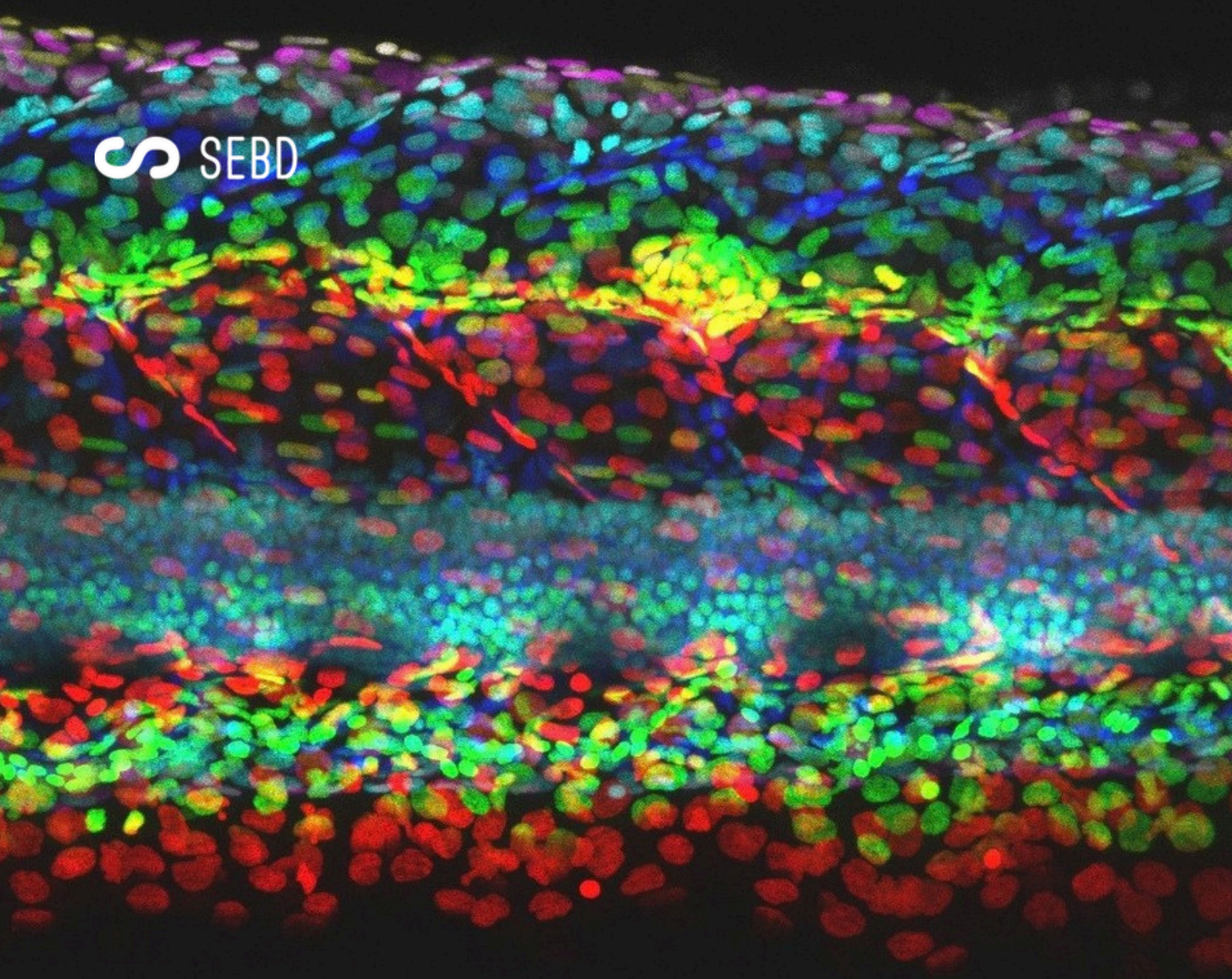
Keywords Neural Crest Cells, rare disease, craniofacial development, disease modeling

By analyzing exome sequencing data, we identified patients with NKTR (Natural Killer Triggering Receptor) mutations who were affected by a rare neurodevelopmental syndrome involving global developmental delay and craniofacial and brain abnormalities. NKTR encodes a poorly studied protein, which was initially described as a membrane co-receptor in natural killer cells. However, its role in neurodevelopment remains unknown.

Our study demonstrated the expression of mouse and zebrafish NKTR orthologues in the brain and neural crest cell (NCC)-derived structures during embryonic development suggesting a potential role for NKTR in craniofacial development. To model the human syndrome associated with NKTR mutations, we designed a CRISPR/Cas13d-based Loss of Function (LoF) strategy in zebrafish embryos to induce degradation of nktr mRNA. By using NCC markers, such as dlx2 and sox9a, we observed specific developmental alterations at different embryonic stages in nktr crispants compared to controls. Moreover, through morphometric measurements we corroborate a reduction of brain and head dimension in nktr crispants, correlating with microcephaly observed in patients.

Recently we imported a nktr knockout line characterized by a nonsense mutation that causes a premature stop codon thus possibly representing a LoF mutation like the one found in patients. Our preliminary data confirm brain and craniofacial abnormalities in homozygous mutant embryos when compared to controls.

To shed light on the molecular pathways altered in nktr mutant embryos, we performed an RNA-seq analysis that suggested a novel function for NKTR during neurodevelopment, paving the way for the design of future functional rescue strategies and therapeutic approaches.



08 / 09

Poster abstracts

POSTER · Session 1 · Early Embryonic Development · Poster number 119

Generation of synthetic enhancers with in vivo temporal and cell type specificity

Carla Victoria Jimenez-Medina (1), Ivan Barbera-Aura (1), Alicia Hurtado (1), Dario G. Lupiañez (1)

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Enhancers are cis-regulatory elements that control when, where, and to what extent genes are expressed. As enhancers drive highly restricted transcriptional patterns, they can be employed to direct transgene expression to specific cell types, tissues, or developmental stages. This aspect makes them valuable tools for lineage tracing, functional studies, gene editing or the generation of transgenic models. Yet, the use of enhancers as genetic tools has been largely limited to naturally occurring elements, as the cis-regulatory codes governing their activity remains poorly understood.

Recent advances in deep learning have provided novel means for decoding cis-regulatory codes and understand the logic underlying enhancer activity. Here we developed deep learning models from single-cell ATAC-seq data and applied them to design synthetic enhancers for distinct gonadal cell types. Using LacZ reporter assays in transgenic mice, we demonstrate that these synthetic sequences can drive robust transcriptional activity in vivo with temporal and cell type specificity.

Altogether, our findings demonstrate how AI-driven approaches can decipher the cis-regulatory code of gonadal cell types, enabling the engineering of synthetic enhancers with restricted spatiotemporal activity.

POSTER · Session 1 · Early Embryonic Development · Poster number 120

Elucidating the Cis-Regulatory Code of Sex Determination With Deep Learning Models

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Recent advances have highlighted the importance of the non-coding genome in regulating sex determination (1,2). However, the underlying rules, patterns, and sequence features underlying this activity are largely unknown. In this talk, I will present our recent efforts to decode the cis-regulatory code of sex determination by applying deep learning models to single-cell omics data (3). I will show how these approaches can uncover broad sequence motifs that pinpoint key transcriptional regulators and, most importantly, the combinatorial syntax through which they act. Beyond deciphering regulatory logic, I will demonstrate how deep learning models allow the design of synthetic cis-regulatory elements that can drive transcription in specific cell types, with spatial and temporal precision (4). All in all, these advances enable bottom-up strategies for dissecting and engineering the regulatory programs that control sex determination.

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POSTER · Session 1 · Early Embryonic Development · Poster number 105

Epigenomic and transcriptomic characterization of the ectopic expression of CTA genes in Danio rerio embryos

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The aberrant reactivation of genes normally restricted to the germline is a recurring hallmark of numerous tumoral processes, yet whether this reactivation is merely a subproduct of global epigenetic dysregulation or actively contributes to the acquisition of germline identity in somatic cells remains poorly understood. In this work, we analyze the molecular consequences of the ectopic expression of Cancer Testis Antigen (CTA) genes dazl, ddx4, and elavl2 in Danio rerio embryos, with the aim of evaluating their capacity to induce an early cellular reprogramming toward a germline-like identity, analogous to that observed in tumorigenesis. To this end, mRNA of these CTA genes was injected into zebrafish embryos. RNA-seq and whole genome bisulfite sequencing (WGBS) data were subsequently obtained at 24 hours post-fertilization (hpf) to identify differentially expressed genes (DEGs) and differentially methylated regions (DMRs). The results revealed a marked transcriptomic response driven by the overexpression of the CTA genes. In contrast, the methylome remained globally stable, although local alterations were detected. Taken together, these findings suggest that the early cellular response to CTA reactivation is predominantly transcriptomic, and that although a germline-like transcriptional program may be initiated, epigenetic reorganization likely operates on a longer temporal scale or manifests in a more subtle manner. These results advance our understanding of how germline gene reactivation may drive cell identity transitions during the earliest stages of zebrafish development.

POSTER · Session 1 · Early Embryonic Development · Poster number 103

Supporting-like cells constitute an alternative steroidogenic lineage conserved in amniotes

Iván Barberá-Aura, Wai-Yee Chung, Emilie Dujardin, Alicia Hurtado, Mathieu Galmiche, Chloé Mayère, Maëva Guy, Béatrice Mandon-Pépin, Vedran Franke, Yan Jaszczyszyn, Rafael D. Acemel, Serge Rudaz, Jennifer Mckey, Serge Nef, Eric Pailhoux, Darío G. Lupiáñez (1)

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In mammals, the production of sex hormones is widely considered to depend on the interstitial lineage of the gonad, which differentiates into Leydig cells in males or theca cells in females. However, certain mammalian species evidence gonadal steroidogenic activity prior to the specialization of these interstitial lineages, suggesting that alternative cell types may assume this function. Here we reveal a previously unrecognized role for supporting-like cells (SLCs), which can act as a major steroidogenic lineage during mammalian embryonic development. Through comparative single-cell transcriptomics, steroidomics and in toto organ imaging we find that in rabbits, SLCs not only contribute to the formation of gonadal rete structures, as described for other mammals, but also differentiate into a steroid-producing population. The steroidogenic program of SLCs is initially activated in both sexes but selectively maintained in ovaries, whereas in testes it is progressively replaced by that of interstitially derived Leydig cells. Evolutionary comparisons indicate that SLCs may represent an ancestral lineage that is homologous to the steroidogenic cells of non-mammalian species, which also derive from supporting precursors and share expression of cell fate regulators such as PAX2/8 and TBX1. Altogether, our findings redefine current models of gonadal lineages, revealing an unexpected plasticity in sex differentiation and exemplifying how distinct cell types can converge on analogous functions during evolution.

POSTER · Session 1 · Early Embryonic Development · Poster number 102

Exploring the Role of DNA Damage Response Pathways in Early Embryonic Development

Ana María Sevilla (1), Guillermo Rodríguez (1), Ana Alcaina (1), Iris Guerrero (1), Thomas Spruce (1), Bárbara Pernaute (1)

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Zygotic Genome Activation (ZGA) constitutes the first major developmental transition during mammalian preimplantation development and occurs at the 2-cell stage in mice. During this process, developmental control shifts from maternally inherited transcripts and proteins to a newly established embryonic transcriptional program. The extensive molecular reprogramming that takes place during ZGA likely causes a large amount of genomic stress making regulation of the DNA Damage Response (DDR) particularly relevant at this time. The DDR comprises a network of signalling and repair pathways that detect and resolve DNA lesions, thereby preserving genome stability. Two key pathways are those controlled by the kinases ATM (that senses DNA double strand breaks) and ATR (that senses replication stress). Interestingly, our recent studies have identified an unexpected attenuation of DDR activity through the ATM pathway during the first embryonic cell divisions1, which raises the question: how is genome integrity maintained during the first stages of life?

To address this we are performing a systematic characterization of the activity of key DDR pathways under basal conditions and following exposure to different damaging agents throughout preimplantation development, with a particular focus on the ZGA period. Our results show that, contrary to the ATM pathway, the ATR pathway not only appears to be fully functional at early stages, but may may also be implicated in additional cellular processes during early embryogenesis beyond its canonical DDR role.

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POSTER · Session 1 · Early Embryonic Development · Poster number 101

Investigating the basis of ATM-dependent DNA damage response attenuation around mouse zygotic genome activation

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Early mammalian embryos must preserve genome integrity during major developmental transitions, including zygotic genome activation (ZGA) and totipotency acquisition and loss. Previous work has shown that the response to DNA double-strand breaks (DSBs) is regulated in a stage-specific manner, with cleavage-stage embryos around ZGA showing reduced ATM-p53 pathway activation compared with morula-stage embryos1. Our observations indicate that total ATM protein levels are lower in early versus late stages, suggesting that ATM abundance may contribute to stage-specific differences in DNA damage response activation. However, it remains unclear how these differences relate to endogenous levels of DNA damage and whether there is a functional benefit to this ATM pathway dampening2.

Here, we are establishing a framework to quantify DNA damage and modulate ATM levels in mouse embryonic stem cells (mESCs) and preimplantation embryos. Comet assay conditions are being adapted to quantify DNA damage burden in mESCs and embryos3, with a particular focus on DSB. Current data support their use in both systems, while ongoing optimisation aims to improve robustness in early embryonic stages. In parallel, we are investigating the functional consequences that restoring ATM levels at early stages has for developmental progression. By combining damage quantification with controlled modulation of ATM levels, this approach will help determine whether stage-specific DNA damage response phenotypes in early embryos are associated with differences in DNA damage burden or with differential ATM pathway activation. Furthermore, it will allow us to gain insight into the impact that ATM-mediated DNA damage response modulation has on early embryo development.

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POSTER · Session 1 · Early Embryonic Development · Poster number 95

Mechanical forces drive gene regulation during epiboly cell movements

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During vertebrate embryogenesis, mechanical and geometrical inputs are integrated with gene regulatory mechanisms to guide cellular differentiation and organ formation. However, how these physical cues influence the epigenetic landscape of developing tissues is poorly understood. We aim to uncover this interplay in the Enveloping Layer (EVL) of zebrafish embryos during epiboly, the first morphogenetic movement dependent on the capacity of EVL cells to undergo drastic morphological flattening and regulate mechanical tension driven by the actomyosin ring near the yolk. To investigate this relationship, we employed mechanical tension and cell geometry modulation strategies: (i) changing yolk volume (remove yolk vs. increase yolk volume by injecting oil droplets), (ii) altering actin-cytoskeletal intracellular tension using drug treatments (Rockout vs. Calyculin A) and (iii) applying geometrical constrictions with different capillary stiffness to the embryo. Following these perturbations, we are integrating single-cell multiomics (scRNA/ATAC-seq) and Whole Genome Bisulfite Sequencing (WGBS) to correlate mechanical tension with chromatin accessibility and DNA methylation patterns. Our results demonstrate that mechanical modulation alters blastoderm spreading yet drives molecular progression even when morphological movements are phenotypically paralyzed. Transcriptomic analysis reveals robust genetic responses, specifically activating stress and cytoskeletal pathways while remodeling adhesion networks in tension-modified embryos. This coordinated development under physical constraints highlights compensatory mechanisms that ensure developmental robustness. In conclusion, our work demonstrates that mechanical forces are essential regulators of gene expression in zebrafish gastrulation and elucidates how cells interpret and “remember” physical inputs, providing insights into fundamental principles governing cell fate decisions and tissue architecture in developing tissues.

POSTER · Session 1 · Early Embryonic Development · Poster number 94

The role of WAPL during the maternal-to-zygotic transition in zebrafish

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Upon oocyte fertilization, the maternal-to-zygotic transition (MZT) takes place leading to i) the first transcription during embryogenesis or the zygotic genome activation (ZGA) and ii) a finely controlled maternal transcripts degradation. Along early development the 3D genome is reorganized, leading to the establishment of enhancer–promoter interactions and topologically associating domains (TADs). TAD formation depends on the balance between cohesin loading by the NIPBL/MAU2 complex and its unloading by WAPL. In zebrafish, TADs emerge after ZGA, yet whether WAPL contributes to genome architecture establishment during this process remains unknown.

Using the CRISPR–RfxCas13d system, we knocked down (KD) wapla and waplb maternally-provided mRNAs, the two WAPL paralogs present in zebrafish, and analyzed the effects of their loss of function during early embryogenesis. We found that embryos specifically lacking wapla mRNA exhibit pronounced developmental defects after ZGA. Furthermore, RNA-seq analysis at 4 hpf revealed significant downregulation of early zygotically-expressed genes in Wapla-deficient embryos, while maternal transcript clearance pathways were also disrupted, suggesting a major role for Wapla during the MZT. Importantly, these effects are highly specific, as demonstrated by cross-rescue experiments.

Together, our results identify Wapla as a key regulator of the MZT in zebrafish and suggest that proper cohesin dynamics are required for the activation of the early zygotic program. We are currently investigating the impact of Wapla KD on genome accessibility and 3D genome organization and evaluating cohesin occupancy to determine whether the observed transcriptional defects are associated with alterations in chromatin architecture and cohesin positioning during ZGA.

POSTER · Session 1 · Early Embryonic Development · Poster number 93

Tissue mechanics as an instructor of cell fate during zebrafish gastrulation

Nicholas de Jager (1)

(1) CABD

During vertebrate embryogenesis, tissue-scale mechanical forces emerge from coordinated cell flows. Consistent with emerging evidence that these forces influence transcriptional programming, we hypothesise that they, along with genetic and epigenetic signals, are the earliest instructors of cell fate decisions. To investigate how cells sense and transduce these forces into transcriptional and identity changes we aim to engineer known mechanosensing cellular components into reporters to elucidate the mechanical state of the cell. Using early-stage zebrafish development as a model, which offers a well-defined morphogenetic process that is amenable to in vivo imaging, we seek to investigate mechanical tension in the spatially heterogenous regions within the early embryo to reveal potential hidden mechanical boundaries guiding cells towards distinct transcriptional fates. We are developing a FRET-based tension sensor built on the nesprin-2G TSMod architecture (mTFP1–linker–mVenus), which reports nuclear envelope force transmission in real time, and a Piezo channel activity reporter to monitor mechanosensitive ion channel activation in the embryo. Together, these approaches aim to establish a framework linking mechanical patterning in the developing embryo to transcriptional and lineage-specific responses, with broader implications for understanding how physical forces contribute to the regulation of early development.

POSTER · Session 1 · Early Embryonic Development · Poster number 91

CRISPR-RfxCas13d optimizations and maternal screenings as a tool to discover novel regulators of

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The maternal to zygotic transition (MZT) is a universal genetic reprogramming that shifts developmental control from the factors deposited in the oocyte to the embryo’s genome through the zygotic genome activation (ZGA) coupled with the clearance of these maternally provided RNAs and proteins. A small number of regulators of the MZT have been discovered and described, however, there is still a lot of maternal transcripts whose role during MZT is unknown. To tackle this, we have optimized CRISPR-RfxCas13d to efficiently eliminate RNAs during embryogenesis in vertebrates. By using this technology in zebrafish embryos, we have been able to perform several screenings focusing on post-transcriptional and post-translational factors that have been much less studied in the MZT context. As a proof of principle, we recently identified Bckdk, a kinase that regulates histone acetylation and ZGA, as a novel post-translational regulator of the MZT. Beyond this, we have also uncovered other potential post-transcriptional MZT regulators, Atxn2l and Ddx3xa. Atxn2l (Ataxin 2-like), is an RNA binding protein that is predicted to be involved in the formation of stress granules and P-bodies, and Ddx3xa (DEAD-box helicase 3 X-linked a) is an RNA helicase. Depletion of their maternal mRNA generated epiboly defects and a deregulation of ZGA and the clearance of maternal RNAs. We are now performing genomics, transcriptomics and molecular biology approaches to analyse their role in the post-transcriptional regulation during early embryogenesis. Together, our findings demonstrate that CRISPR-Cas RNA-targeting is a systematic and powerful approach to discover novel regulators of early development.

POSTER · Session 1 · Early Embryonic Development · Poster number 89

Examining the role of Nanog phosphorylation during the maternal- to-zygotic transition.

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The maternal-to-zygotic transition (MZT) is a pivotal event in early embryonic development, characterized by the zygotic genome activation (ZGA) and the concomitant clearance of maternally supplied mRNAs and proteins. In zebrafish and other vertebrate models, several maternal transcription factors and chromatin modifiers have been identified as key regulators of ZGA. However, the contribution of post-translational modifications to this process remains largely unexplored. Recently, through a CRISPR-based RNA-targeting screen, we identified Bckdk as a previously unrecognized kinase that regulates MZT in teleosts. Subsequent phosphoproteomic analyses revealed that several critical MZT regulators, including Nanog, a pioneer factor during MZT, and Brd4, an essential histone acetylation reader, undergo phosphorylation at the onset of ZGA in zebrafish. Here, we investigated whether Nanog, phosphorylation contributes to MZT regulation. We found that overexpression of either wild-type Nanog or a non-phosphorylatable Nanog variant induced epiboly defect, whereas overexpression of a phosphomimetic form did not. Moreover, neither the phosphomimetic nor the non-phosphorylatable variant was able to rescue the epiboly defects associated with nanog loss of function. In contrast, co-injection of both variants at a balanced stoichiometric ratio restored embryonic development to levels comparable to those achieved by wild-type Nanog rescue. These findings suggest that precise regulation of Nanog phosphorylation is required for its developmental function during MZT. Ongoing studies are examining the effects of Nanog phosphorylation on protein stability, subcellular localization, and embryonic gene expression. Collectively, our results identify Nanog phosphorylation as a potential post-translational mechanism controlling MZT in zebrafish.

POSTER · Session 1 · Early Embryonic Development · Poster number 80

Investigating mechanisms behind zygotic genome activation and regulatory networks coherence by interspecies hybridization within the Oryzias

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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.

POSTER · Session 1 · Early Embryonic Development · Poster number 79

Molecular dynamics of the cytoskeleton in phagocytic protrusions of the live embryo

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The early embryo develops exposed to both internal and environmental perturbations. Phagocytosis is a fundamental defense mechanism that enables the elimination of harmful particles and damaged cells and it is mediated by phagocytic cups, dynamic protrusions that recognize, bind, and engulf targets through a zipper-like mechanism. We previously discovered a phagocytic epithelium in early embryos, which allows us to explore how these events occur in a natural context. Using an early zebrafish embryo infection model together with super-resolution techniques, we have captured actin dynamics at phagocytic protrusions, revealing their ring-like organization and improving our understanding of their nanoarchitecture. Here, we present insights into the structure of phagocytic cups and the molecular mechanisms involved.

POSTER · Session 1 · Early Embryonic Development · Poster number 78

Shaping embryo-environment interactions during preimplantation development: bacterial ingestion by human and mouse embryos

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During preimplantation development, the embryo is exposed to a dynamic microbial environment while undergoing critical steps required for implantation. We previously demonstrated that early embryos are capable of ingesting and degrading bacteria, revealing an unexpected immunological role in the early embryo.

Here, we investigate the physiological relevance and mechanisms underlying this process. Using mouse and human preimplantation embryos, we study how embryos interact with bacteria in their surrounding environment. Through live time-lapse imaging, we have directly captured bacterial uptake by early embryos showing this process is dependent on actin polymerization, indicating an active, cytoskeleton-driven mechanism of ingestion.

We are also exploring the functional consequences of these interactions using an in vitro implantation model based on endometrial cells. In this system, we aim to reproduce the encounter between preimplantation embryos and bacteria at the endometrial surface and investigate how bacterial exposure may influence embryo implantation and embryo-endometrium interactions.

Together, our results suggest an evolutionarily conserved role during the preimplantation period, potentially contributing to embryo fitness and shaping embryo-environment interactions in the presence of bacteria.

POSTER · Session 1 · Early Embryonic Development · Poster number 57

Applying smFISH to study transcriptional activation by enhancer RNAs during zebrafish development

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Enhancer RNAs (eRNAs) are non-polyadenylated non-coding transcripts that are produced from active enhancers by RNA polymerase II. Although knockdown studies suggest that eRNAs promote transcription of their cognate target genes, how they regulate nascent transcription remains mechanistically unclear. In previous work, our laboratory used csRNA-seq to identify transcription start sites (TSSs) during zebrafish embryonic development transcriptome-wide. This revealed, among other things, bidirectional transcription of the lateral stripe element (LSE), an enhancer of the proneural transcription factor neurogenin-1 (ngn1), at the bud stage. We next selectively depleted LSE eRNAs in both transcriptional directions (LSE+ and LSE−) using CRISPR/RfxCas13d and validated knockdown efficiency by qPCR. The consequences of these eRNA knockdowns for ngn1 transcription were then investigated using single-molecule fluorescence in situ hybridization (smFISH). Automated image analysis was used to quantify nuclear and cytoplasmic ngn1 transcript densities at single-cell level. Our data demonstrate that a co-depletion of both LSE enhancer transcripts indeed reduced nuclear ngn1 transcript density. This finding provides strong evidence that LSE eRNAs indeed contribute to the activation of ngn1 transcription.

POSTER · Session 2 · Stem Cells · Poster number 13

Metabolic reprogramming during early planarian regeneration: a single-cell transcriptomic perspective

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What metabolic programmes does a planarian activate in the first hours after amputation? Despite extensive knowledge of the signalling pathways triggered by injury, the metabolic state of the tissue at the moment of wounding — and how it shifts in response — remains poorly understood. In stem cell-based regeneration, metabolic reprogramming may determine which cell types respond to injury and how, acting as an early instructive input rather than a downstream consequence of tissue growth [1,2]. Planarians (Schmidtea mediterranea) offer a tractable system to address this: regeneration depends on adult stem cells (neoblasts) [3], animals tolerate a wide range of nutritional states [4], and the early transcriptional response to amputation can be captured at single-cell resolution [5].

Using fGSEA-based pathway enrichment on single-cell transcriptomic data spanning five post-amputation time points (1.5–24 hpa), we characterise the dynamics of broad metabolic programmes after injury. Our analysis reveals that glycolytic programmes are suppressed across several cell types during the early regenerative response, while beta-oxidation shows context-dependent activation at specific time points. These pathway-specific and temporally dynamic changes are not consistent with the glycolysis-biased metabolism typically associated with stem cell activation and proliferation [6,7], pointing to metabolic dynamics during regeneration initiation that warrant further investigation. Ongoing analyses including metabolic scoring, trajectory analysis and integration with functional and lipidomics data will determine how these transcriptional metabolic dynamics connect to cell fate decisions and regenerative signalling.

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POSTER · Session 2 · Stem Cells · Poster number 88

Pharmacological Modulation of 3D Genome Structure in Embryos and mESCs

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During early embryonic development, extensive chromatin reorganization accompanies the transition from totipotency to pluripotency. Although topologically associating domains and higher-order genome organization are progressively established during preimplantation development, previous work from our laboratory demonstrated that early depletion of CTCF does not severely impair transcriptional programs. These findings suggest that chromatin architecture and transcription may be partially uncoupled during early embryogenesis1.

To further explore this relationship, we are studying the effects of Curaxin, a small molecule known to disrupt chromatin organization2. Using mouse embryonic stem cells and preimplantation embryos as complementary models, we aim to understand how chromatin perturbation affects transcriptional programs associated with cellular potency.

Bulk RNA-seq analysis revealed that Curaxin treatment partially phenocopies CTCF depletion, promoting the upregulation of totipotency-associated genes while reducing pluripotency-related programs. In embryonic stem cells, Curaxin treatment increased ZSCAN4 expression to levels comparable to those observed following CTCF depletion, suggesting induction of a totipotent-like state3,4. In parallel, Curaxin-treated preimplantation embryos showed increased expression of Zscan4 and MERVL in a stage-dependent manner, together with impaired proliferation and developmental progression.

Interestingly, Curaxin failed to induce Zscan4 expression at later developmental stages in which totipotency-associated programs are already fully silenced, indicating that chromatin disruption amplifies permissive transcriptional states rather than reactivating inactive ones de novo.

Overall, our results support a close relationship between chromatin architecture, developmental competence, and proliferation during early embryogenesis. Ongoing experiments combining transcriptomic and chromatin conformation analysis will help dissect the CTCF-dependent and independent effects of Curaxin on genome regulation and cellular potency.

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POSTER · Session 2 · Stem Cells · Poster number 52

Extracellular matrix homeostasis regulates cell cycle progression and genome integrity in Drosophila germline stem cells

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Adult stem cell behavior is tightly regulated by specialized microenvironments, or niches, in which extracellular matrix (ECM) composition and mechanical properties play essential roles in maintaining tissue homeostasis. ECM components within the niche are continuously deposited from several sources and remodeled by matrix metalloproteinases. The mechanisms by which ECM remodeling influences stem cell function remain incompletely understood. Here we investigated the contribution of the ECM regulator tissue inhibitor of metalloproteinases (Timp) to cell cycle progression and genome integrity in the Drosophila ovarian germline stem cells (GSCs). Live imaging combined with quantitative cell cycle analysis revealed that loss of timp did not significantly alter the duration of mitosis but instead disrupted earlier stages of the cell cycle. Specifically, timp mutant GSCs exhibited reduced premitotic centrosome dynamics and a marked extension of the G1 and S phases. To identify the underlying cause of these defects, we analyzed markers of genomic stress and found increased DNA damage in mutant GSCs. These findings suggest the activation of DNA damage checkpoint pathways upon the loss-of-function of timp, supporting a model in which disruption of ECM homeostasis compromises genome integrity, thereby slowing cell-cycle progression. Our results establish a functional link between ECM remodeling, DNA damage responses, and stem cell proliferation, demonstrating that proper ECM homeostasis is required for normal stem cell function. Our results thus highlight the importance of the physical properties of the stem cell niche in preserving genome integrity and maintaining adult stem cell homeostasis.

POSTER · Session 2 · Stem Cells · Poster number 47

Fly males experience a physiological pre-mating response caused by sexual pheromones and Octopamine signaling

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Reproduction is paramount to animals. For it to be successful, the coordination of social behaviour, physiology and gamete production is necessary. How is the socio-sexual environment perceived and how does it affect physiology and gametogenesis? How animals cope with the high energy costs associated with reproduction? Females, ranging from insects to mammals, have provided multiple insights about this coordination, with their postmating responses as prime examples. However, the existence of similar mechanisms in males remains largely unknown. We recently described that the fly testis experiences an activation phenomenon that couples spermatogenesis with the availability of potential mating partners - males detect the presence of females by sexual pheromones, what triggers inter-organ signals boosting the proliferation of the stem cells residing at the testis.

Our recent data suggests that this phenomenon is part of a larger physiological and metabolic response affecting multiple organs and signalling axes. In presence of females, the testis displays reduced levels of available citrate and ATP, a signature of increased energy demands. Simultaneously, reserves of lipids and carbohydrates are reduced in the adipose tissue and get mobilized to the fly blood, making them available to other tissues. Interestingly, these changes are downstream of the same perception mechanism that activates the stem cells of the testis, based on pheromonal communication and Octopamine release from the brain. In sum, our findings point to the existence of a complex pre-mating response in males, akin to the post-mating response of females, aimed to prepare males for reproduction.

POSTER · Sessions 3–4 · Morphogenesis and Organogenesis · Poster number 122

Gene regulatory network analyses reveal a redundant function for Meis genes in the specification of sexual identity

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Mammalian sex determination is controlled by a balanced gene regulatory network (GRN) of female- and male-promoting factors. The expression of the SRY gene tilts this balance toward testicular fate, or toward ovarian fate in its absence. Despite significant advances in the field, sex-determining GRNs remain largely uncharacterized, limiting the discovery of new regulators. Here, we employ an expansion of METALoci, an approach that integrates 3D chromatin conformation data and epigenetic information to assign enhancers to target genes. By combining this method with single-cell RNA sequencing (scRNA-seq) data, we reconstructed temporal and sex-specific GRNs and identified Meis genes as relevant factors for sex determination. Deletion of Meis1 in XY mouse embryos results in alterations in sex specification, as indicated by SOX9 downregulation and FOXL2 upregulation in supporting cells at the gonadal poles. Interestingly, the combined deletion of Meis1 and Meis2 alleles intensifies this effect, with an abundant presence of FOXL2+ cells throughout the entire gonad. Defects in sex specification are also observed in XX embryos, with the appearance of SOX9+ cells in mutant gonads. Overall, these findings demonstrate that Meis genes act redundantly and are required for the proper specification of sexual identity during gonadal development. Our approach underscores the power of integrative genomics for identifying novel candidate genes involved in biological processes.

POSTER · Sessions 3–4 · Morphogenesis and Organogenesis · Poster number 114

Optic cup folding driven by geometry and tension of Retinal Pigmented Epithelium (RPE) cells

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While optic cup folding is known to involve specific geometric changes and migration of RPE cells, the precise mechanical and gene regulatory mechanisms orchestrating the adoption of their highly rigid geometry, and how these contribute to successful folding, remain incompletely understood. To address this gap, we investigated how the increase in mechanical tension and maintenance of an elongated geometry depend critically on the specific activation of the Wnt/β-catenin and YAP pathways in RPE cells. We have demonstrated that optic cup folding failure caused by the interference of these pathways is explained by a reduction in RPE cellular tension. We also identified transcriptional programs controlled by these pathways that regulate the mechanical properties of the actin cytoskeleton, cell rigidity, cell-to-cell and cell-to-ECM adhesions, and force-sensing ion channels. Finally, we hypothesized that the LINC complex, which transmits tension between the cell and nuclear membranes, is responsible for the nuclear entry of BCatenin and YAP transcription factors in a geometry-dependent manner. We combined quantitative imaging, functional analysis, mechanical perturbation assays, and transcriptomic analysis to generate a comprehensive view of how the coordination of mechanosensitive gene expression and changes in cellular and nuclear geometries are required for the correct formation of the eye. Our study identifies and integrates nuclear effectors (Yap and BCatenin), mechanotransduction key complexes (actin cytoskeleton and cell adhesion regulators), mechanosensitive ion channels (piezo1), and tension transducers (LINC complex) as essential components of this complex morphogenetic process.

POSTER · Sessions 3–4 · Morphogenesis and Organogenesis · Poster number 104

Cis-regulation during in vivo and in vitro organ development

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The precise regulation of gene expression during development depends on the coordinated activity of cis-regulatory elements and epigenetic mechanisms. While organoid systems provide powerful in vitro models to study organogenesis and advance regenerative medicine, they frequently fail to fully recapitulate in vivo developmental programmes. Currently, organoid fidelity is predominantly evaluated by morphological resemblance, offering a limited perspective on developmental accuracy. A critical yet underexplored aspect is the epigenetic landscape, which dictates cell identity and is essential for faithfully reproducing in vivo organogenesis.

To address this gap, this project uses zebrafish as a non-mammalian vertebrate model with regenerative capacity and conserved developmental mechanisms. Having successfully established and optimised a zebrafish retinal organoid protocol, our current work is focused on the systematically comparison between the epigenetic and regulatory features of zebrafish eye development in vivo with those of organoid-derived tissue in vitro. By generating comprehensive genomic datasets using single-cell multiome profiling, histone modification mapping, and base-resolution DNA methylome sequencing at matched developmental stages, we aim to map chromatin states and regulatory elements across both systems. Ultimately, this multi-omic framework will determine how faithfully in vitro organoids reproduce in vivo regulatory landscapes. By establishing robust benchmarks for assessing developmental fidelity, this work will advance the use of zebrafish retinal organoids as a reliable model system, contributing to the improvement and translational relevance of organoid technologies in regenerative medicine.

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POSTER · Sessions 3–4 · Morphogenesis and Organogenesis · Poster number 7

Matrix Stiffness as a Mechanobiological Regulator of Cardiomyocyte Response During Zebrafish Heart Regeneration

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The zebrafish (Danio rerio) heart serves as a valuable model for investigating the cellular and molecular mechanisms underlying cardiac development and regeneration. In contrast to mammals, zebrafish possess a robust capacity to fully regenerate cardiac tissue following injury, making them an optimal system for studying the regulatory processes involved. A key aspect of regeneration is the dynamic remodelling of the extracellular matrix (ECM), which progresses through distinct mechanical states: an initial softening phase associated with inflammatory infiltration, a stiffer fibrotic intermediate resulting from collagen and fibrin deposition, and a gradual restoration of baseline compliance as tissue repair advances. These alterations in ECM stiffness function as instructive mechanobiological signals that directly modulate cardiomyocyte behaviour, proliferation, and fate.

Building on this background knowledge, my research investigates how cardiomyocytes sense and respond to mechanical stimuli during regeneration by employing hydrogels with tunable stiffness that mimic the mechanical progression of the injured myocardium. Phenotypic and multi-omics analyses are integrated to map the mechanosensitive gene regulatory networks activated under varying mechanical conditions.

In parallel, this research explores how tissue-level mechanical forces, including hemodynamic flow and myocardial tension, shape early embryonic heart looping, a critical morphogenetic event that establishes cardiac asymmetry and chamber organization. Taken together, this work seeks to define the mechanobiological logic that connects physical forces to cardiac form and function, with broader implications for regenerative medicine

POSTER · Sessions 3–4 · Morphogenesis and Organogenesis · Poster number 87

Genome-wide dynamics of regulatory R-loops during zebrafish embryonic development

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R-loops, three-stranded nucleic acid structures consisting of an RNA:DNA hybrid and a displaced single-stranded DNA, have emerged as key epigenetic regulators involved in transcriptional control and chromatin organization. While historically viewed as mere byproducts of transcription linked to genomic instability, their in vivo regulatory roles during vertebrate embryogenesis remain largely uncharacterized. In this study, we use zebrafish as a model to perform a comprehensive genome-wide characterization of R-loops during embryonic development. By analyzing the dynamics and functional relevance of R-loops at different developmental stages, we aim to uncover their role in establishing regulatory landscapes necessary for proper gene activation and genome organization. Our findings will allow us to understand the role of R-loops in chromatin and gene regulation and may provide insights into the mechanisms underlying developmental disorders and genome instability.

POSTER · Sessions 3–4 · Morphogenesis and Organogenesis · Poster number 84

Dynamics and molecular regulation of three-dimensional packing of epithelia.

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Understanding tissue and organ organization is key to unravel the genesis of pathology. Also, the study of epithelial cells three-dimensional (3D) packing will help to understand the mechanisms underlying organ development. We have previously shown that cells in bent epithelia can undergo intercalations along the apico-basal axis. Consequently, cells adopt a particular shape named “scutoid”, phenomenon that contributes to minimize the tissue energy level. The study of the three-dimensional architecture of epithelia relays on the accurate segmentation of microcopy images, a work that can be done manually but is time consuming and inefficient. In our lab we have developed Cartocell, an AI-based method for rapid and automated image segmentation and detection of morphological patterns. In this project, we adapted the use of Cartocell to understand the dynamics and molecular mechanisms regulating epithelial 3D packing in Drosophila. We have applied this method to several fly tissues, including the egg chambers, salivary glands and the epithelium of the early Drosophila embryo at the stage of cellularization. Thanks to this method, we have confirmed the presence of scutoids in all these model epithelia. Now, we are studying the dynamics and molecular mechanisms underlying the formation and resolution of scutoids during development, by the analysis of the differential localization of candidate proteins at the apico-basal transitions, as well as carrying out genetic analysis of candidate genes.

POSTER · Sessions 3–4 · Morphogenesis and Organogenesis · Poster number 71

Investigating the dynamic cellular mechanoresponse in the early teleost embryo through multi-omic integration

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During embryonic morphogenesis, cells must rapidly adapt to environmental mechanical properties. Although these mechanoresponses are essential for development, their underlying molecular mechanisms remain elusive. Mechanotransduction operates from immediate cytoskeletal changes to downstream transcriptional and chromatin responses; however, how these distinct regulatory layers are dynamically coordinated in vivo to initiate specific genomic programs remains unknown.

Our goal is to investigate these mechanisms in vivo, identifying mechanosensitive elements within the chromatin and transcriptome. We utilize gastrulating medaka embryos as a morphogenetic model, where axis formation critically depends on mechanical forces in the context of gastrulation (Sousa-Ortega et al., Nat Comm 2023). Specifically, a customized mechanical tester is used to apply precise embryonic compression programs.

To capture response kinetics, we performed RNA-seq at 15 and 40 minutes following mechanical stimulation, complemented by ATAC-seq chromatin profiling. By integrating these transcriptomic and epigenetic datasets across time, we have reconstructed mechanosensitive gene regulatory networks (GRNs). This approach have allowed us to track mechanoresponse progression and identify several candidate mechanosensitive genes and specific Differentially Open Chromatin Regions (DOCRs) as potential regulatory hubs.

These candidate genes and regulatory regions have served as a platform for downstream functional validations to decipher their precise roles in the mechanotransduction cascade. Ultimately, characterizing these targets will provide critical insights into the temporal and structural regulation of the mechanoresponse during early teleost development.

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POSTER · Sessions 3–4 · Morphogenesis and Organogenesis · Poster number 68

Molecular dissection of an ectopic TAD boundary at the Epha4- Pax3 locus

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Mammalian genomes are organized into Topologically Associated Domains (TADs), large genomic regions that display high interaction frequencies and facilitate enhancer-promoter communication and gene activation. TADs are delimited by boundary regions that usually contain multiple binding sites for the insulator protein CTCF. We previously demonstrated that a boundary region at the Epha4-Pax3 locus is sufficient to segregate the regulatory activity of two adjacent TADs and to prevent ectopic Pax3 limb expression by Epha4 enhancers. Unexpectedly, the deletion of this boundary in its native context does not cause a fusion of these adjacent TADs and Pax3 activation. Instead, an ectopic boundary emerges at the Epha4 transcription start site (TSS), maintaining TAD segregation. This ectopic boundary has an atypical configuration, as it contains a single CTCF binding site (CBS), leading us to hypothesize that the Epha4 promoter and this CBS cooperate to confer regulatory insulation. In this project, we are performing a molecular dissection of this ectopic boundary through serial deletions of the promoter and/or the CBS. Our results suggest that transcriptional activity at the Epha4 TSS plays a major role in functional insulation, highlighting how promoters can play structural roles in 3D genome organization.

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POSTER · Sessions 3–4 · Morphogenesis and Organogenesis · Poster number 66

Study of gene network divergence during organ evolution

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Decades of investigation in development have described the complex genetic networks driving the formation of organs with specific functions, however it is still unknown how these networks evolved.

Drosophila is a segmented animal with metamerically repeated organs in adjacent segments. During evolution, these organs diverged from a common ancestor giving rise to different structures with diverse functions.

To infer how an ancestral regulatory gene network diverges, we compare the genetic inputs governing the formation of the corpora allata, the prothoracic glands, the tracheal system and the cephalic endoskeleton.

Despite their enormous morphological and functional differences, these organs evolved from a common metamerically repeated organ. Comparison between the genetic networks forming these four divergent organs can shed light on how changes in a primitive genetic network gave rise to the evolution of different organs. This will also serve to discover new elements involved on the development of the endocrine glands controlling moulting and metamorphosis. We have evidence that the snail (sna) gene is a major player specifying the formation of the endocrine glands. I will present how by analysing the regulation of a gland sna cis-regulatory element we can begin to understand how the endocrine and cephalic endoskeletal cells diverge.

POSTER · Sessions 3–4 · Morphogenesis and Organogenesis · Poster number 12

Limb dorsalization: Lmx1b mode of actions during limb development

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The establishment of dorsoventral asymmetry during vertebrate limb development, leading to the formation of distinct dorsal and ventral compartments, is essential for proper locomotion and manipulation. The LIM homeobox transcription factor Lmx1b is expressed specifically in the dorsal limb bud mesenchyme and is both necessary and sufficient for specifying dorsal limb identity. Loss of Lmx1b function results in ventralization of dorsal structures, as demonstrated in mouse models and in Nail-Patella Syndrome in humans.

Despite its central role and previous genome-wide transcriptomic and ChIP-seq studies, the downstream gene regulatory network and direct targets of Lmx1b remain incompletely characterized.

To dissect the Lmx1b-mediated gene regulatory network in a systematic temporal manner, it is necessary to isolate Lmx1b-expressing cells. To achieve this, I generated the rLARM2-GFP mouse line, which drives GFP expression under the control of LARM2, a limb-specific Lmx1b enhancer. Characterization of GFP expression demonstrated faithful recapitulation of the dorsally restricted endogenous LARM2 activity, including its anterior bias. Additionally, I have established the Lmx1b-2A-Cre mouse line in the laboratory which, when combined with the ROSAmTmG reporter allele labels cells that have ever expressed Lmx1b. Using these genetic tools, we are starting to isolate dorsal limb mesenchymal cells and to characterize the transcriptional programs downstream of Lmx1b.

In parallel, I have obtained the only ChIP-grade validated Lmx1b antibody and started ChIP-seq experiments across multiple developmental stages.

Integration of these datasets will enable the identification of direct Lmx1b target genes and provide new insights into the gene regulatory mechanisms underlying limb dorsalization.

POSTER · Sessions 3–4 · Morphogenesis and Organogenesis · Poster number 63

RNA-mediated gene regulation and chromatin organization during vertebrate embryonic development

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The precise control of gene regulation during embryonic development relies on the coordinated interplay between transcription, cis-regulatory elements, and chromatin organization. “R loops”, three-stranded nucleic acid structures formed by an RNA:DNA hybrid and the displaced single-stranded DNA, are increasingly recognized as potential regulators of transcription and genome architecture. However, their roles during vertebrate embryogenesis remain poorly understood. In this project, we use zebrafish as an in vivo model to characterize the genome-wide dynamics of R- loop formation across embryonic development. By identifying when and where R-loops form, and how they relate to gene activation and chromatin regulatory landscapes, we aim to determine whether these structures act as functional components of developmental gene regulation. This work will provide insight into new RNA-mediated mechanisms linking transcriptional control and chromatin organization during vertebrate embryonic development.

POSTER · Sessions 3–4 · Morphogenesis and Organogenesis · Poster number 53

Role of the myogenic regulatory factor Mrf4 in the coordinated development of the musculoskeletal system

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Muscles, bones and tendons must develop in a coordinated manner to form a functional musculoskeletal system. Developmental studies require an integrated perspective, although classical approaches often concentrate on each component separately. In myogenesis, research focuses on the function of the myogenic regulatory factors Myf5, Mrf4, MyoD and MyoG. Remarkably, Mrf4 function remains elusive because the available knock-outs affect the expression of the neighbouring Myf5. We generated new alleles using CRISPR/Cas9, and found that Mrf4 absence not only affects muscles, but also other tissues, especially bones. This leads to the possibility that Mrf4 is involved in the crosstalk between muscles, bones and tendons during development. Using light-sheet microscopy, we conduct 3D reconstructions of fetal and neonatal limbs to explore Mrf4 function in musculoskeletal development. Meanwhile, Mrf4 deficiency causes sex-specific phenotypes, including hypertrophy in females. Although male mutants are not hypertrophic, they present increased strength maintained into old age. Several sarcomeric proteins are deregulated in the mutants, which may mechanically explain this phenotype. To explore this possibility, we are optimising a method to study sarcomeric features using dSTORM-based super-resolution microscopy. Finally, it was shown that Mrf4 silencing in rats protects against denervation-induced atrophy, but Mrf4 KOs only confer mild protection. Mutant muscles, however, show a distinct metabolic and transcriptomic response. We are starting to decipher the precise molecular events triggered by denervation and how Mrf4 impinges on the process, considering potential sex-specific events. Overall, we aim to comprehend Mrf4 function with special focus on organ intercommunication in the musculoskeletal system.

POSTER · Sessions 3–4 · Morphogenesis and Organogenesis · Poster number 51

Integrating Morphogen Signaling and Tissue Mechanics during otic placode morphogenesis in zebrafish

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A hallmark of embryonic development is the formation of organs with reproducible shape, size, and function, yet how mechanical forces and biochemical cues coordinate to sculpt three-dimensional functional tissue remains unclear. Using the zebrafish otic placode as an in vivo model, we interrogate how mechanochemical dynamics guide sensory organ self-assembly. The otic placode transitions from a loosely organized field of precursor cells into a condensed, polarized epithelial sphere. While Fibroblast Growth Factor (Fgf) signaling specifies this precursor domain, its role in directly regulating physical cell sorting and epithelialization independent of cell fate remains uncharacterized. To address this gap, we combine quantitative live imaging, in vivo biophysical measurements, and precise Fgf perturbations to map tissue-wide forces during otic self-assembly. We identify actomyosin contractility and cell-cell adhesion dynamics as key molecular effectors driving these physical transformations. Finally, we integrate a 3D computational vertex model of placode self-assembly with in vivo rescue experiments to decouple specific biophysical parameters. By systematically altering individual mechanical properties in silico, we determine their precise contributions to cell sorting and tissue condensation. Together, this work links morphogen signaling to tissue mechanics, revealing core biophysical principles governing early inner ear morphogenesis.

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Mitochondrial redox state sets the pace of development under thermal stress

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How organisms determine the pace of their development remains one of the fundamental unanswered questions in developmental biology. Developmental rate coordinates growth, differentiation and maturation throughout the life cycle, yet the metabolic mechanisms governing developmental timing remain poorly understood. Although temperature profoundly influences developmental speed across ectotherms, how environmental cues translates into changes in developmental pace is still unclear. Here, we combine a high-throughput bioluminescence-based assay, first established in Caenorhabditis elegans and subsequently adapted to Drosophila melanogaster, with genetic manipulation of mitochondrial redox pathways to investigate the metabolic control of postembryonic development. This comparative framework enables minute-scale quantification of developmental transitions across animal models while providing stage-specific resolution in large cohorts. Using this platform, we show that modulating mitochondrial electron flow through Alternative Oxidase or increasing the NAD+/NADH ratio with a bacterial NADH oxidase (LbNox) significantly accelerates development under thermal stress. This effect is largely confined to intermolt periods, revealing that distinct developmental stages differ in their sensitivity to mitochondrial metabolic state. Together, these findings identify mitochondrial redox balance as a key regulator of developmental rate. Beyond uncovering a role for mitochondrial metabolism in developmental timing, this work establishes a robust quantitative framework for measuring developmental dynamics with unprecedented temporal precision. Our results support the existence of conserved metabolic mechanisms that coordinate developmental progression in response to environmental conditions and provide a foundation for comparative studies across animal models.

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POSTER · Sessions 3–4 · Morphogenesis and Organogenesis · Poster number 48

USP8 is required for cardiomyocyte and mitochondrial maturation through control of the embryonic-to-foetal metabolic switch

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During cardiovascular development, an embryonic-to-foetal metabolic switch from anaerobic to aerobic metabolism supports the increasing energy needs of the growing heart, in parallel with the maturation of mitochondria and cardiomyocytes. This process is transcriptionally regulated, but post-translational (PT) regulation remains elusive. Deubiquitylases (DUBs) remove the Ubiquitin PT modification from substrates, regulating their turnover, trafficking, and activity. Ubiquitin-Specific Protease 8 (USP8) is a DUB that modulates receptor tyrosine kinases (RTKs) signaling in vitro, essential for cardiovascular development. However, the in vivo role of USP8 in cardiovascular development remains unknown. We show that conditional inactivation of Usp8 in the vascular endothelium and endocardium in mice results in lethality by embryonic day (E)10.5, with defective ventricular and valve development. Molecularly, Usp8 mutants display altered activity and subcellular localization of key RTKs, including phospho-ERBB2 and VEGFR2. These changes are associated with a transcriptomic profile indicating hypoxia and catabolism. Conversely, early myocardial inactivation of Usp8 leads to embryonic death at E12.5. These mutants exhibit a proteomic signature of mitochondrial dysfunction, including reduced oxidative phosphorylation and increased lactic fermentation, along with ultrastructurally and functionally immature mitochondria. Ubiquitinome analysis suggests proteins related to cardiac muscle contraction (ACTC1, TPM1, MYL2), calcium handling (ATP2A2), and lipid metabolism (EHHAH) as USP8 targets, which could explain the metabolic phenotype. Both Usp8 inactivation models show cell type-specific accumulation of ubiquitylated proteins within enlarged intracellular vesicles, and global upregulation of the metabolic stress sensor HIF1α. Together, our findings identify USP8 as an essential regulator of cardiac development and the embryonic metabolic switch.

POSTER · Session 5 · Neurodevelopment · Poster number 112

Differential development of the optic tectum of amniotes as a source of evolutionary divergence

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The tectal mesencephalon -superior colliculus for mammals or optic tectum for non-mammals- plays a key role in the visual pathway of the brain. This structure is thought to be very conserved between vertebrates due to its layered composition, retinotopical arrangement and similar connections. Nonetheless, the differences between the relative size of the structure, lamina number, and the relevance in the visual pathway show that there are clear differences between superior colliculus and optic tectum. There is little to no information about the processes leading to these evolutionary differences. In this study, we aim to find the conserved and differentiated characteristics of the development of the tectal mesencephalon in chicken (Gallus gallus), gecko (Paroedura picta), and mouse (Mus musculus). To achieve this goal, EdU birthdating was used to highlight the creation of the optic tectum layers and cells. Moreover, retrograde tracing techniques were employed to locate the major axonal pathways projecting outside the optic tectum; and transcriptomics data analysis to study the molecular features of the tectal mesencephalon of different amniote species. Preliminary integration of these data suggests that the neurogenic waves of the chicken, gecko and mouse could be very similar. Nevertheless, not all connections seem to be present in all species. Above all, some conserved connections may be formed in different moments of the development of the tectal mesencephalon. These results show that the optic tectum and the superior colliculus may not be as conserved as previously thought, given some differences in their developmental trajectories explaining the adult divergences.

POSTER · Session 5 · Neurodevelopment · Poster number 110

Generation of Patterned Human Spinal Cord Organoids to Study Neural Tube Defects

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The human central nervous system (CNS) develops through tightly regulated morphogen signaling pathways that establish dorsoventral (DV) patterning and generate distinct neural progenitor domains. Disruption of these developmental processes can contribute to neural tube defects (NTDs), including spina bifida. Human spinal cord organoids derived from pluripotent stem cells provide a valuable platform to model these early developmental events in vitro. Here, we generate ventralized human spinal cord organoids through treatment with retinoic acid (RA) and the Sonic Hedgehog (SHH) pathway agonist SAG to recapitulate ventral spinal cord development. Under these conditions, organoids establish ventral identities, including floor plate populations and progenitor domains such as the motor neuron progenitor (pMN) domain. This system enables the study of spatial organization and DV patterning in a human context. Using this model, we aim to investigate mechanisms underlying NTDs, with a particular focus on the planar cell polarity (PCP) pathway component FUZ. Mutations in FUZ have been associated with spina bifida and motor dysfunction, although the developmental mechanisms involved remain poorly understood. By examining the effects of altered FUZ function in human spinal cord organoids, we seek to understand how PCP pathway dysregulation impacts ventral spinal cord patterning and neural development. Altogether, this work establishes human spinal cord organoids as a relevant model to study human spinal cord development and the cellular mechanisms contributing to neural tube defects.

POSTER · Session 5 · Neurodevelopment · Poster number 109

Neural tube defects and the PCP pathway: deciphering human spinal cord morphogenesis with neural tube organoids

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The human central nervous system is formed through a highly complex morphogenetic process known as neurulation. Disruptions in this process can cause neural tube defects (NTDs), such as spina bifida, a congenital malformation of the posterior spinal cord that arises early in embryonic development. Genetic variations in the planar cell polarity (PCP) pathway, a signaling cascade coordinating cell orientation across the tissue plane, have been strongly linked to NTDs. The precise cellular mechanics underlying these defects have been historically difficult to study in vivo.

Here, we generated mutant human embryonic stem cells lines carrying patient derived-PCP mutations to investigate how PCP disruptions alter spinal cord morphogenesis and leverage our established human spinal cord organoid model to recapitulate early neural tube development in vitro.

Using our organoid model, we aim to visualize how specific PCP mutations impact key morphogenetic events in early neural development.

POSTER · Session 5 · Neurodevelopment · Poster number 107

Cell-type-specific profiling of transcription factor binding in C. elegans.

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Transcription factors (TFs) are the main orchestrators of gene expression. An universal feature of TFs is their pleiotropy, they are usually expressed in several cell types and tissues and their specific actions are determined by the context, mainly the combined action with other TFs. Caenorhabditis elegans nervous system is composed by 118 neuron types, which are specified by combinations of TFs termed terminal selector (TS) collectives. We do not know the cell-type specific binding profiles for those TFs. In addition, a few TFs are recurrently used as TS for a broad number of different neuron types. Due to their pleiotropic actions, conventional approaches, such as whole-worm chromatin immuno precipitation followed by sequencing (ChIP-seq) fail to provide cell-type specific information.

We aim to overcome these difficulties by using a combination of genetic tools to obtain C. elegans strains in which a given TF will be endogenously and specifically tagged in a given neuron type and then its chromatin binding profile will be determined by CUT&RUN. We are using CRISPR to knock in a cassette that when combined with a strain expressing a flipase recombinase in a neuron-type specific manner, the endogenous TFs switches to TF::eGFP::3xFLAG tagging. Recombined strains will be later used to profile TF binding using anti-GFP nanobodies and CUT&RUN. We are currently establishing this methodology to profile different TFs that act as TS of specific neuron types and gain insights in their mode of action.

POSTER · Session 5 · Neurodevelopment · Poster number 106

Unveiling the mechanisms of non-CpG methylation reading and writing in neural development

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Non-CpG methylation (mCH) is present at low basal levels across most tissues but selectively accumulates in cells with low mitotic rates, such as neurons and oocytes. In mammals, the dysregulation of mCH has been implicated in cancer, cardiovascular diseases, and neurodevelopmental conditions, most notably Rett syndrome (RTT). RTT is of particular interest due to the evolutionarily conserved relationship between mCH and its primary reader protein, MECP2; mutations in the MECP2 gene are the leading cause of this syndrome in humans. While MECP2 has been extensively characterized in recent years, the precise causal mechanisms by which mCH alterations contribute to this process remain largely elusive. To address this knowledge gap, we employ zebrafish (Danio rerio) as an in vivo model. Zebrafish possess two paralogs of the DNA methyltransferase 3A (dnmt3a) gene, dnmt3aa and dnmt3ab, whose targeted depletion leads to a brain-specific reduction in mCH levels. By utilizing specific knockout (KO) lines for Dnmt3aa/ab depletion alongside Mecp2 KO lines, we employ a multi-omics and functional approach, including RNA-seq, Whole-Genome Bisulfite Sequencing (WGBS), and behavioural assays, to characterize the in vivo role of mCH in the central nervous system. Ultimately, this study aims to elucidate the molecular implications of non-CpG methylation in the origin of Rett syndrome.

POSTER · Session 5 · Neurodevelopment · Poster number 82

The Role of Vangl2 in Cellular Organization and Polarity During the Onset of Neurulation in Mouse

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The planar cell polarity (PCP) pathway plays a fundamental role during neurulation by coordinating the cellular behaviours required for neural tube closure and embryonic tissue organization. Neural plate shaping depends on convergent extension, a mechanism involving the rearrangement of polarized cells within the plane of the tissue. The embryonic node also acts as a key organizer during early development, contributing to axial patterning and generating structures such as the notochord, which provides inductive signals that influence neural tube formation. These coordinated processes are largely regulated by the non-canonical Wnt/PCP signalling pathway. Mutations in PCP components are associated with severe neural tube defects, including craniorachischisis, although the underlying cellular mechanisms remain incompletely understood. Vangl2 mutant mouse embryos fail to initiate neural tube closure and remain open along the body axis. They exhibit abnormal midline morphology, including a flattened neural plate and expansion of the floor plate and notochord, associated with increased Shh signalling. Similar defects can be reproduced experimentally by disrupting PCP signalling with the ROCK inhibitor Y27632 or by altering cytoskeletal organization using cytochalasin D or blebbistatin. These treatments affect neural tube closure and alter node and notochord morphology, indicating a crucial role for PCP-dependent tissue organization during neurulation. In this study, we analyse Vangl2 mutant and pharmacologically treated embryos to investigate how these perturbations affect neuroectodermal cell behaviour and tissue dynamics. Our aim is to characterize the morphogenetic alterations that precede midline formation and neural tube closure, providing insight into the cellular basis of PCP-associated neural tube defects.

POSTER · Session 5 · Neurodevelopment · Poster number 81

Establishing Cellular Models to Study VANGL2 Regulatory Dysregulation Associated with Neural Tube Defects

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Neural tube defects (NTDs) are among the most common congenital malformations and have been strongly associated with disruption of the planar cell polarity (PCP) pathway. Variants in VANGL2, a core PCP gene, are known to cause severe neurulation defects in mouse models, ranging from craniorachischisis in homozygous mutants to milder forms of spina bifida in heterozygotes. However, the contribution of non-coding regulatory variation to VANGL2 dysregulation and NTD susceptibility remains poorly understood. In this study, we investigated regulatory regions of VANGL2 gene to assess their potential involvement in NTD pathogenesis. Analysis of whole-genome sequencing datasets from patients with myelomeningocele identified six potentially relevant variants within the promoter and proximal enhancer region of VANGL2. Several of these variants were recurrent among affected individuals, including a rare promoter variant that appeared at a higher frequency than expected, suggesting a possible role in gene regulation and disease susceptibility. To explore the biological consequences of altered VANGL2 expression, cellular models are being used to evaluate the effects of VANGL2 downregulation on cell morphology and PCP-associated phenotypes. Reduced VANGL2 expression could result in alterations in cellular organization and cytoskeletal architecture, supporting a role for precise VANGL2 regulation in maintaining normal cellular behaviour. Together, these findings would highlight the importance of regulatory mechanisms controlling VANGL2 expression and suggest that non-coding variants may contribute to the genetic architecture and phenotypic variability of NTDs. This work will provide new insights into how disruption of PCP pathway regulation may influence early neural tube development and disease.

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High throughput spatiotemporal analysis of vertebrate hindbrain ontogeny

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The development of the Central Nervous System (CNS) demands a tight coordination of cell proliferation and neuronal or glial cell fate acquisition and differentiation. This allows the formation of a brain of the right size and shape, with the correct cell types and proportions. Said process is precisely regulated by Gene Regulatory Networks that govern the patterning and emergence of neuronal diversity within the CNS.

The zebrafish hindbrain is our model to understand the origin and cell diversity of the CNS. We have assessed two distinct lineages of the posterior brain; the neurogenin1 and ptf1a lineages, responsible for the generation of mostly glutamatergic and GABAergic neuronal fates, respectively, thus being key for neuronal diversity. Both lineages diverge in their differentiation dynamics and neuron clonal output, with key antero-posterior differences. Furthermore, at a given timepoint, they differ in their Notch signaling response, in a way that suggests the time during which they have been expressed is important. This results as well in Notch differentially regulating glutamatergic and GABAergic final neuronal fate acquisition.

Finally, we have looked into the mechanisms that CNS progenitors use for cell fate acquisition to become neurons. We have confirmed an intriguing distribution of ubiquitinated proteins within the hindbrain, with these proteins accumulating in the neuronal domain. Moreover, we have observed that a higher accumulation of these leads to an increase in neuronal differentiation. This could be the result of a repurposing of cellular routines, like the recycling of damaged proteins, to induce a neuronal fate.

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Temporal dissection of neural progenitor competence in the zebrafish hindbrain

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The precise generation of neuronal diversity, including the correct numbers and proportions, is fundamental for vertebrate brain development. While the progression from neural progenitor cells (NPC) to mature neuron is well understood, the extent to which their competence — the potential to generate a specific neuronal subtype — is plastic over time and across different NPC populations remains an open question.

To investigate the temporal dynamics of NPC competence as a key mechanism regulating neuronal subtype specification, we use the zebrafish embryonic brainstem as a model system. Leveraging multiple transgenic lines, we have already monitored the contribution of different NPC lineages to glutamatergic and GABAergic circuits across different temporal windows. To study mechanisms by which the competence of different NPCs evolves, we focused on the temporal TF onecut1. We found that it is expressed in differenciating NPCs and early-born neurons, reminiscent of other vertebrates and invertebrates, suggesting that it participates in a temporally specified neuronal maturation program. We have found that onecut1 is expressed across multiple NPC lineages, that it is required for early neurogenesis, and we are currently investigating it's role in neuronal fate choice.

Moving forward, we plan to challenge NPC competence directly through heterochronical expression of onecut1 and other temporal TF. Using our method TEMPO, we will control the population and the time of re-specification. This approach promises to deliver key insights into the heterogeneity of NPC competence and the mechanisms controlling neuronal diversity, which could inform the development of cell therapy strategies for neurodevelopmental and neurodegenerative diseases.

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No evidence for a conserved temporal neurogenic program in the embryonic sauropsid brain

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Neurogenesis in the vertebrate brain has traditionally been viewed as the output of spatially restricted genetic programs imposed by conserved morphogenetic gradients. Work in mammals has since revealed an additional, global temporal transcriptional code that orders neuronal production over time, partitioning progenitors and neurons into early, intermediate, and late identities. Whether this temporal program predates mammals or is restricted to that lineage remains an open question. To address it, we asked whether the mammalian temporal neurogenic code operates in sauropsids. Using In Situ Sequencing (ISS) in neurogenic territories of the embryonic chick telencephalon, we mapped both spatially and quantitatively, the expression of genes linked to early (e.g., Onecut, Hmga1/2), intermediate (e.g., Pou2f2, Zfhx3), and late (e.g., Nfia/b, Tcf4) neurogenic states across developmental stages covering early, mid, and late neurogenic windows. The chick transcriptional landscape did not reproduce the temporal sequence reported in mammals: we found neither an ordered, stage-wise activation of these markers nor a clear separation between early-, intermediate-, and late-type molecular signatures. We therefore find no support for conservation of the global mammalian temporal program in the sauropsid telencephalon, and propose instead that this code may represent a mammalian lineage-specific innovation rather than a shared feature of amniote neurogenesis. More broadly, the study illustrates how spatial transcriptomics can resolve long-standing questions in evolutionary developmental neurobiology.

POSTER · Session 5 · Neurodevelopment · Poster number 73

Decoding the developmental origins of thalamic diversity in amniotes

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The thalamus is a central hub for sensory and higher-order information processing across vertebrates, yet the developmental mechanisms that generated its remarkable cellular diversity during evolution remain poorly understood. Here, we investigate the evolutionary origins of thalamic organization by combining single-cell transcriptomics, developmental analyses, and connectivity studies across amniotes, with a particular focus on birds and reptiles and comparative integration with mammals.

To characterize the molecular programs underlying thalamic development, we generated large-scale single-nucleus RNA sequencing datasets from embryonic thalamus in chicken and gecko. These datasets reveal a highly conserved transcriptional landscape across major neuronal populations and provide unprecedented resolution to reconstruct developmental trajectories and identify lineage-specific cell types. Comparative analyses further uncover evolutionary innovations, including neuronal populations associated with the mammalian anterior thalamic complex, highlighting molecular adaptations that may underlie species-specific functions.

To investigate how cellular diversity emerges, we combined transcriptomic data with EdU-based birthdating approaches. In both species, we identified conserved neurogenic gradients, with lateral and ventral nuclei generated earlier than medial regions and the dorsomedial nucleus consistently arising last. These findings support the existence of developmental chronotopy, a common organizational principle linking neuronal birthdate with spatial arrangement, while also revealing previously unrecognized intra-nuclear gradients that may contribute to functional specialization.

Finally, retrograde tracing experiments suggest that developmental timing may influence connectivity patterns, with early-and late-born neurons contributing to distinct circuit architectures. Together, these results provide an integrative framework to understand how conserved neurogenic programs and evolutionary innovations shape thalamic diversity across amniotes.

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Modulating Callosal Projection Neuron Plasticity during Cortical Development by In Vivo Reprogramming

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The development of interhemispheric connectivity critically depends on the proper specification and maintenance of callosal projection neurons (CPNs), a specialized population of intra-cortical excitatory neurons, which links distant cortical areas, have expanded and diversified throughout mammalian evolution, paralleling the rise of complex associative circuits and higher-order cortical functions such as multimodal processing, decision-making, and social cognition. While many cortical neurons initially extend callosal projections, a substantial fraction undergoes developmental refinement, adopting local circuit identities. Whether CPN fate is irreversibly predetermined or actively maintained during maturation remains unclear.

In this project, we investigate the molecular and developmental plasticity of CPNs and test the potential of in vivo cellular reprogramming to maintain or re-engage a callosal identity. Using in utero electroporation combined with Tet-ON inducible system to transiently express the Yamanaka factors (OSKM) in postmitotic cortical neurons, enabling controlled partial reprogramming. This approeach led to a significant increase in the proportion of CPNs (up to 69% compared to ~50% in controls).

These findings support the existence of an early “callosal program” that can be modulated after neuronal differentiation. Ongoing work aims to elucidate the epigenetic mechanisms underlying this plasticity and to test whether reprogramming can promote circuit restoration following injury. This study provides new insights into neuronal fate stability and highlights reprogramming as a promising strategy for cortical repair.

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POSTER · Session 5 · Neurodevelopment · Poster number 64

Single-Cell transcriptomic Profiling of Circulating Blood Cells in Newborn mice Following Hypoxic Ischemic Encephalopathy

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Neonatal hypoxic-ischemic encephalopathy (HIE) is a severe neurological condition associated with high mortality and long-term disability. Cerebral injury triggers a strong inflammatory response involving peripheral leukocytes and brain-resident cells, which contribute to tissue damage and repair. However, cellular dynamics and diversity remain under investigation to identify temporal mechanisms, biomarkers, and potential therapeutic strategies. Single-cell RNA sequencing (scRNA-seq) is a powerful tool for studying cellular heterogeneity in development and disease. Here, we used scRNA-seq to investigate cellular heterogeneity in circulating blood cells during neonatal HIE.

P7 mouse pups were used to model human neonatal HIE brain injury. Mice underwent unilateral common carotid artery ligation followed by systemic hypoxia to induce HI brain injury. Sham controls underwent the same surgical procedure without artery ligation or hypoxic exposure. At 48 h post-surgery, brains were collected for flow cytometry and immunofluorescence (IF) analysis, while circulating blood cells were analyzed by scRNA-seq and multiparametric flow cytometry.

Differential gene expression-based clustering identified known and novel markers for major PBMC populations and delineated 27 cell subsets in neonatal blood. Injury altered immune cell composition, including depletion of intermediate and non-classical monocytes with reparative transcriptional profiles. We are investigating whether this depletion correlates with brain injury severity and reflects their migration from circulation into injured brain tissue.

We propose that the frequency of monocytes with a reparative profile in peripheral blood could be used as a novel biomarker to assess the progression of HI brain damage.

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Characterization of immune and vascular responses to neonatal hypoxic-ischemic injury in a murine model

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Neonatal hypoxic-ischemic encephalopathy (HIE) is a major cause of mortality and long-term neurological disability. Following injury, a robust inflammatory response involving peripheral leukocytes and brain-resident immune cells contributes to disease progression and tissue repair. Recent single-cell transcriptomic analyses from our group identified a reduction of a circulating monocyte subset with a reparative transcriptional profile after hypoxic-ischemic (HI) injury. Here, we investigated whether these changes are associated with immune cell recruitment into the injured brain and characterized inflammatory and vascular responses within the damaged tissue.

HI injury was induced in postnatal day 7 mice using the Rice–Vannucci model. Forty-eight hours after injury, leukocyte populations in blood and brain from HI and sham animals were analyzed by multiparametric flow cytometry using a 9-color panel. Brain sections were further characterized by immunofluorescence to assess microglial activation, leukocyte distribution, and vascular organization.

Preliminary analyses reveal injury-associated alterations in leukocyte composition. Ongoing studies are assessing whether specific monocytic populations reduced in circulation accumulate within the injured brain following HI. Initial findings suggest differential immune cell recruitment between ipsilateral and contralateral hemispheres. In parallel, immunofluorescence analyses indicate increased microglial activation and vascular remodelling in the injured hemisphere, including changes in vascular density and branching patterns. These responses correlate with injury severity, with more extensively damaged brains displaying more pronounced inflammatory and vascular alterations.

Together, these approaches provide a comprehensive view of immune cell dynamics and tissue responses following neonatal HI injury and may facilitate the identification of biomarkers and therapeutic targets for neonatal HIE.

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POSTER · Session 5 · Neurodevelopment · Poster number 49

Conserved Gene Regulatory Networks driving hierarchical cellular diversification

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During development, a single genome generates diverse cell types through context-dependent gene regulation. Single-cell studies have revealed that this diversification is hierarchically organized, with broad cell classes progressively subdividing into finer types and subtypes. However, the mechanisms that establish and maintain these cellular hierarchies remain poorly understood.

Using the highly resolved nervous system of Caenorhabditis elegans, we identify an evolutionarily conserved role for the T-box transcription factor TBX-2 as a selector of neuronal subtype identity. TBX-2 expression is restricted to specific neuronal subtypes, and gain- and loss-of-function analyses demonstrate that it is both necessary and sufficient to establish and maintain subtype identity across multiple neuronal contexts. Mechanistically, TBX-2 acts primarily through direct repression of alternative transcriptional programs, preventing switches to closely related subtype fates.

Evidence from vertebrate systems suggests that this function is conserved. In the mammalian cochlea, Tbx2 is required to maintain inner hair cell identity by repressing outer hair cell fate. Re-analysis of published datasets further reveals that Tbx2 is expressed in neural crest cells and becomes enriched in mechanoreceptive neurons of the dorsal root ganglia (DRG), a sensory system with remarkable subtype diversity.

Building on our findings in C. elegans, we are currently working on the establishment of an in vitro sensory neuron differentiation system that recapitulates multiple DRG subtypes. This platform will enable perturbation of Tbx2 expression and high-resolution dissection of the transcriptional and chromatin mechanisms by which Tbx2 controls cell subtype specification.

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POSTER · Session 6 · Evo-Devo · Poster number 121

Flexibility of cell fates and functions across sex determination systems revealed by comparative single-cell analyses

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Sex determination in vertebrates can be initiated by a wide range of genetic or environmental triggers. Yet, the degree to which gonadal cell types and genetic programs are conserved remains unresolved. Here we employed single-cell transcriptomics to characterize the temperature-dependent sex determination (TSD) program in gonads from the turtle species Trachemys scripta. Comparative analyses against species with genetic sex determination, like mouse (XY) and chicken (ZW), revealed a marked divergence in cell type repertoires and functions during vertebrate evolution. Unlike mammals, fetal Leydig cells are absent from the early gonads of T. scripta, where the supporting lineage expresses genes required for androgen synthesis. Evolutionary reconstructions show that this lineage derives from a Pax2-positive mesenchymal population, suggesting an ancestral condition in Archelosauria that differs from the primarily coelomic epithelium origin in the mammalian clade. Transcriptional dynamics and co-expression analyses revealed the recruitment of lineage-specific transcription factors, including Twist1 or Runx1, into the genetic programs of vertebrate clades. Our findings reveal extensive plasticity of the cellular and genetic mechanisms of vertebrate sex determination and suggest that this flexibility is a key feature of gonadal evolution.

POSTER · Session 6 · Evo-Devo · Poster number 118

Multi-omics characterization of mole’s limb development in Talpa occidentalis

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How limb diversity arises from changes in development remains an open question in the evo-devo field. Since tetrapods first appeared, limbs have been modified in many ways, from changes in digit patterning to large-scale changes in skeletal structure. A striking example of this is the mole’s limb, which has been completely remodeled into a highly optimized appendage for underground environments. This includes alterations in proximal skeletal elements such as flattened enlarged humerus, as well as distal ones, including digit shortening (brachydactyly), an increased number of sesamoid bones and the emergence of a novel enlarged sesamoid bone that forms an additional “thumb”.

To characterize the molecular mechanisms driving this specialized phenotype, we generated single cell RNA-seq and ATAC-seq (scRNA-seq, scATAC-seq) dataset from a stage equivalent to mouse E13.5, when the autopod is already specified. We performed an inter-species integration, with scRNA-seq data from mouse’s limbs, to ensure that cell type annotations were comparable between both species. Thereafter we focused on analyzing skeletogenic populations, this analysis revealed a unique cell cluster in the mole dataset with a combination of markers related to cartilage formation. We further investigated this population performing in situ Hibridization Chain Reaction, which revealed that the spatial localization and expression patterns of these genes coincide with regions of cartilage formation.

Further analysis of scATAC-seq datasets will identify candidate regulatory regions responsible for this differential gene expression between species. We will then train deep learning models using CREsted to pinpoint the motifs potentially driving regulatory activity within these regions.

POSTER · Session 6 · Evo-Devo · Poster number 117

Evolution of gene regulatory landscapes underlying phenotypic adaptations in moles

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Cis-regulatory elements (CREs) are genomic elements that regulate the precise gene expression pattern critical for specifying cellular, tissue and organ identities during development. Most developmental genes are regulated by multiple and often redundant CREs that are brought into physical proximity through the three-dimensional organization of the genome. How CREs and their 3D spatial architecture collectively generate precise transcriptional controls is still poorly understood and remains a major bottleneck in decoding gene regulatory landscapes. To address this challenge, we leverage naturally evolved regulatory landscapes in the Iberian mole. Moles have many unique features in their adaptation for a subterranean lifestyle, such as modified forelimbs, elongated snouts and degenerated eyes. Comparative genomic analyses revealed that the TFAP2A regulatory landscape contains the highest number (38) of mole-accelerated noncoding sequences that are otherwise highly conserved across mammals. In addition, the TFAP2A locus has reduced from 2Mb in mouse to 1Mb in mole. Therefore, the mole TFAP2A locus presents as an exceptional model for investigating how sequence and architectural changes in the gene regulatory landscape can allow for evolutionary innovation while maintaining developmental robustness. In this ongoing project, we will generate a series of transgenic mice carrying progressive replacements of the Tfap2a regulatory landscape with its mole counterpart using novel synthetic biology approaches. Since TFAP2A is an essential and pleotropic gene, it presents as a model testbed for uncovering insights into evolution of regulatory landscapes.

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POSTER · Session 6 · Evo-Devo · Poster number 115

When timing matters: cell cycle length in amniote neurogenesis

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The diversity of brain size and morphology among amniotes raises important questions about the developmental mechanisms underlying this variability. Among these, cell cycle dynamics in neural progenitor cells are key regulators linking neurodevelopmental processes to evolutionary outcomes. In particular, cell cycle duration influences neuronal output, contributing to interspecific differences in brain size.

Comparative studies suggest that cell cycle length differs among amniote clades, with reptiles generally showing longer cycles than birds and mammals, which display faster proliferative dynamics. In addition to interspecific differences, cell cycle duration varies across development, with early stages showing shorter cycles and later stages a progressive lengthening that facilitates differentiation. However, available data remain limited to a few model species, and brain regions.

To address these gaps, we analysed cell cycle dynamics in three representative amniotes at early, intermediate, and late embryonic stages: mouse (Mus musculus), chicken (Gallus gallus), and gecko (Paroedura picta). Using dual thymidine analogue labelling (EdU/BrdU) combined with Sox2 immunostaining to identify neural progenitors, we quantified proliferative dynamics by measuring the proportion of labelled cells.

This approach enabled estimation of cell cycle length across developmental stages and eight brain regions spanning the prosencephalon to the rhombencephalon, including both alar and basal domains. Our results expand current comparative datasets and provide new insights into how spatiotemporal modulation of the cell cycle contributes to neurogenesis and the evolutionary diversification of brain structure in amniotes.

POSTER · Session 6 · Evo-Devo · Poster number 113

Effects of foreign transcription factors in vertebrate gene regulatory networks

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The first appearance of new genes and proteins often implies an unexpected encounter between these new molecular entities and the cellular environment of the organism in which they originated. However, since only successful encounters are evolutionarily retained, general principles governing the earliest stages of molecular innovation remain difficult to infer. To assess how first-time interactions take place, we artificially generated unexpected encounters by putting in contact molecular structures that had never interacted before. In particular, we expressed mammal ARGFX and LEUTX and plant-specific YAB1 and WRKY40 transcription factors (TFs) in the completely foreign chromatin environment of zebrafish embryos, a novel heterologous context. Through a multidimensional genomic approach, involving gene expression, chromatin accessibility and protein-chromatin binding, we show that these TFs possess an intrinsic capacity to engage with and interpret a foreign chromatin landscape. Across the diverse TFs evaluated, we identified intrinsic functional properties, including sequence-specific binding common to all factors, pioneering activity in the case of LEUTX, and gene repression, which was particularly pronounced among plant-derived TFs. We also reconstructed both the pre-existing zebrafish gene regulatory networks (GRNs) and the novel GRNs emerging from the ectopic TF activity. By defining transcription factor–context–specific interactions and systematically comparing network topology across conditions, we quantified the extent and direction of GRN rewiring. This allowed us to determine how newly formed regulatory connections relate to ancestral host networks and to the TFs' original regulatory logic, and ultimately how such rewiring could reshape cellular identity and development.

POSTER · Session 6 · Evo-Devo · Poster number 111

UNCOVERING CELL-TYPE DIVERSITY EVOLUTION IN AMNIOTE DIENCEPHALON

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The diencephalon processes a series of functions such as sensory and motor signals. The major structures of the diencephalon (the prethalamus, thalamus and pretectum) likely reveal a variety of evolutionary trends such as diversification, conservation and innovation, making it highly interesting to study brain evolution. Understanding how its cellular diversity, connectivity, and developmental programs have evolved remains a major challenge in neuroscience. Thus, through the lens of evolutionary developmental biology (EvoDevo), this work aims to uncover the molecular profile of specific cell types in the amniote diencephalon to understand its cellular diversity. We analysed single-nuclei RNA sequencing data of the diencephalon of representative species of the major amniote clades in order to build the first diencephalic transcriptomic atlas in these species. We analyzed single nuclei RNA sequencing data from chicken (Gallus gallus) and Madagascar ground gecko (Paroedura picta) neural tissue across several key developmental stages. We also analyzed mouse (Mus musculus) data through the information available in the literature. The intra- and inter-species comparisons of the diencephalic transcriptomic cell-type profiles will explain when and how lineage-specific innovations arise through amniote brain evolution. The preliminary large-scale transcriptomic atlas of different developmental stages for both species indicates a possible conserved molecular profile across major neuronal populations, uncovering a surprising conservation of cell types on a widely divergent anatomical structure. Moreover, we seek to uncover the cell-type novelties that raised during diencephalic evolution.

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Evolution of myosin class II genes in jawed vertebrates: insights into heart evolution and adaptation

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Class II myosins are a family of motor proteins that are essential for cell contraction. In metazoans they have largely diversified into multiple subfamilies with diversified functional implications in muscles, including the heart of vertebrates. Recent studies have shown that the heart’s myosin composition is not as conserved as previously thought, suggesting a higher degree of cardiac specialization over vertebrate evolution. Here, we present a comprehensive evolutionary analysis of myosin II genes across all major jawed vertebrate groups. The last common ancestor of gnathostomes contained nine myosing class II genes, which have largely expanded over vertebrate evolution. We found that classical cardiac myosins Myh6 and Myh7 genes originated in sarcopterygians due to a lineage-specific ancestral duplication, while a proto-Myh6/7 gene largely expanded in different lineages of actinopterygians independently. We also found secondary losses and lineage-specific duplications in orthologs of the cardiac-related Myh7B and Myh15 genes in different groups. Lastly, we investigated the expression patterns of cardiac myosin II genes during embryogenesis of a cartilaginous fish, the small-spotted catshark Scyliorhinus canicula, providing insights into the putative adaptative roles of myosins in heart evolution. Taken together, our comprehensive study provides the most detailed overview of the evolution of class II myosin repertoires in jawed vertebrates, enabling the inference of vertebrate the ancestral complement and providing a solid understanding of lineage-specific evolution of the family, possibly linked to different adaptative avenues of the vertebrate heart.

POSTER · Session 6 · Evo-Devo · Poster number 97

Tracing the Evolutionary Origins of Human Motor Neuron Progenitor Expansion

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Human spinal cord development is characterized by an exceptional expansion of motor neuron (MN) production, with comparative studies indicating increased MN numbers relative to other mammals. Recent studies have identified a distinct MN progenitor population co-expressing NKX2.2 and OLIG2, termed ventral motor neuron progenitors (vpMNs), which exhibit prolonged neurogenic potential in humans. However, whether vpMNs represent a human-specific developmental feature or a conserved primate trait remains unresolved, as do the mechanisms contributing to their expansion.

Here, we investigate the evolutionary basis of pMN development by comparing human and non-human primate spinal cord organoids derived from induced pluripotent stem cells. Using comparative developmental approaches, we aim to characterize pMN emergence, diversification, and neurogenic behavior across species, providing insight into how differences in progenitor dynamics may contribute to human spinal cord development.

We further explore potential cellular mechanisms underlying species-specific differences, with a focus on the centrosome–primary cilium axis and its role in regulating developmental signaling, progenitor expansion, and cell fate decisions. Together, this work seeks to uncover the evolutionary and cellular principles shaping motor neuron development in primates.

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POSTER · Session 6 · Evo-Devo · Poster number 86

The Oikopleura “noisesome”: developmental genetic response to anthropogenic marine noise pollution.

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There is growing concern about how noise from human activities might have an impact on marine life and oceans health. While up to date most of the attention has been paid to the impact of noise in vertebrates, studies on invertebrates are scarce, limiting our knowledge on how noise affect marine trophic webs. In this context, the DeuteroNoise JPI-Oceans consortium aims to characterize the impact of noise pollution on several species of invertebrates in selected basins across Europe (Italy, Norway, Spain and Romania). Within this framework, the present study focuses on Oikopleura dioica, a fast-evolving planktonic tunicate with key ecological and evolutionary relevance. Our approach involves reproducing field-recorded noise profiles in controlled laboratory conditions and assessing their effects on O. dioica across life stages. Using transcriptomic profiling and differential gene expression analysis, we are identifying genes responsive to noise-induced stress, which we term as “noisesome”. We are particularly interested in: (a) characterizing the earliest genetic onset of response during embryonic development, (b) identifying genes affected across ontogeny, and (c) comparing noise responses between developmental stages and adulthood. By integrating molecular and phenotypic data, we aim to discover candidate biomarkers for noise stress, and to evaluate whether a conserved “noisesome” signature exists across invertebrate taxa within the DeuteroNoise consortium. Our findings contribute to understanding how genomic diversity and plasticity mediate adaptation or vulnerability to acoustic pollution, an emerging environmental pressure under climate change. This work underscores the importance of including fast-evolving, non-model organisms in evolutionary genomics to address contemporary ecological challenges.

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POSTER · Session 6 · Evo-Devo · Poster number 85

Changes in the 3D chromatin structure during ageing using the turquoise killifish

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Aging has emerged as a growing societal challenge driven by increasing life expectancy. Understanding how aging affects gene regulation is essential for uncovering the mechanisms underlying age-related diseases. Our research investigates how senescence influences gene transcription through alterations in three-dimensional (3D) chromatin architecture, which plays a crucial role in genomic compartmentalization, facilitating or hindering interactions between gene promoters and regulators, crucial for accurate gene transcription.

Using Nothobranchius furzeri, the shortest-lived vertebrate bred in captivity, we study how aging remodels chromatin structure across different organs and sexes. By integrating Hi-C chromatin conformation assays with transcriptomic and epigenomic analyses, we examine how chromatin structure, gene regulation, and transcription change during aging across multiple organs and between sexes.

Our objectives include generating multi-organ chromatin maps at different aging stages and performing comparative analyses with other vertebrates to identify chromatin features associated with lifespan and age-related diseases. Preliminary results reveal that aging impacts organs differently and that these effects are often sex-specific, highlighting the importance of a multi-organ and sex-aware approach to aging research.

Ultimately, this work seeks to determine whether age-associated changes in chromatin architecture reflect stochastic genomic disorganization or regulated physiological processes. By addressing these questions, we hope to provide new insights into the mechanisms underlying aging and age-related diseases, potentially leading to novel therapeutic strategies.

POSTER · Session 6 · Evo-Devo · Poster number 83

Long sperms, mitochondrial variants and the evolution of a novel gene

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Our group is interested in how gene networks control organogenesis at the cellular level. We have found that the gene network of Drosophila required for the formation of the ectodermal posterior spiracles has been partially co-opted into the testis mesoderm where is required for the sperm’s release (spermiation). The Drosophila sperm tails are characterised for being extremely long compared to other Diptera species. In this talk, I will present our discovery of ripley, a new gene that has recently evolved in the engrailed intergenic region. We have evidence suggesting that ripley has evolved to compensate the sterility caused by wild type mitochondrial variants present in the population affecting the release of the large sperms during spermiation.

POSTER · Session 6 · Evo-Devo · Poster number 75

Decoding age-related changes on cell-type specific GRNs in the naturally short-lived African turquoise killifish

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Aging reshapes gene expression through epigenetic dysregulation and chromatin remodeling, yet the cell-type-specific regulatory logic driving this process remains largely unknown. Here we present a multi-omic approach to decode gene regulatory network (GRN) dynamics across the lifespan using the turquoise killifish, a naturally short-lived vertebrate model. Using snMultiome (snRNA-seq + snATAC-seq), we will profile three aging-relevant tissues — brain, liver and heart — in both males and females, and reconstruct age- and cell-type-specific GRNs by identifying key transcription factors, target genes and cis-regulatory elements. This physiological aging atlas will then serve as a reference to assess how age- driven GRN reorganizations are conserved, accelerated or fundamentally altered in three age-related disease models: diet-induced steatohepatitis, cardiac arrhythmia and a Parkinson's disease-like model. Together, this work aims to identify the key regulatory modules driving aging and their role in pathological contexts, potentially revealing novel therapeutic targets.

POSTER · Session 6 · Evo-Devo · Poster number 70

Evolutionary Remodeling of the HoxD Regulatory Landscape Contributes to Forelimb Adaptations in Moles.

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Phenotypic adaptations are often driven by evolutionary changes in non-coding genomic regions, which can modify gene expression without altering protein-coding sequences. The mole provides an exceptional model to investigate this process, as its forelimbs display remarkable adaptations to a subterranean lifestyle, including brachydactyly, supernumerary sesamoid bones, and the presence of a pseudo-thumb that enhances digging performance1. Additionally, recent studies have established the importance of the HoxD gene cluster in proximodistal limb patterning during embryonic development. HoxD expression is controlled by regulatory landscapes flanking the cluster, and perturbations of these regions can result in severe limb malformations2.

Here, we investigate how evolutionary changes in regulatory landscapes may have contributed to the emergence of the specialized mole forelimb. Using transcriptomic, epigenomic, and chromatin conformation approaches, we characterized the regulatory architecture of the HoxD locus during limb development. Our analyses revealed both spatial and quantitative differences in HoxD gene expression in moles compared with other mammals. Furthermore, we identified changes in the interaction frequency between distal HoxD genes and non-coding regulatory elements located within the centromeric regulatory landscape, which is responsible for patterning the distal region of the limb.

These findings suggest that evolutionary rewiring of HoxD regulatory landscapes contributed to the emergence of novel skeletal features in the mole forelimb. Ongoing functional validation through enhancer reporter assays and interspecies in vivo replacement of large mole-derived regulatory regions in mice will test the causal relationship between these regulatory changes and the derived mole phenotype under a synthetic biology framework3.

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POSTER · Session 6 · Evo-Devo · Poster number 69

Impact of Whole-Genome Duplications (WGD) in the Regulation of Sex Determination

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A whole-genome duplication (WGD) refers to the process by which an organism acquires an extra copy of its entire genome. Such a drastic event provides raw genetic material for evolutionary innovation, as duplicated genes can acquire novel expression patterns through changes in regulation. However, WGDs cause major alterations in gene dosage, raising the question of how Gene Regulatory Networks (GRNs) accommodate these changes while remaining functional. This question is particularly relevant for sex determination, a dosage-sensitive process by which the phenotypical sex of an individual is established, and which must remain functional after a WGD to ensure species reproduction.

An ideal system to explore this question are the closely related amphibian species Xenopus laevis and Xenopus tropicalis. These species diverged relatively recently (48 million years ago), with the X. laevis lineage having undergone a WGD. This event generated two subgenomes that have since evolved asymmetrically through gene loss, retention, subfunctionalization and neofunctionalization. We hypothesize that the divergent fates of duplicated genes have reshaped the GRNs controlling sex determination in X. laevis, enabling compensation for WGD-induced gene dosage changes.

To test this hypothesis, we are generating a cell atlas (scRNA-seq, scATAC-seq, and Hi-C) of gonadal development in both the tetraploid X. laevis and diploid X. tropicalis. These atlases allow us to reconstruct and compare the GRNs controlling sex determination between species, identifying divergent gene fates and novel regulatory changes that may have enabled dosage compensation following WGD.

POSTER · Session 7 · Developmental Disorders · Poster number 116

p53-induced senescence contributes to tumorigenesis by provoking an enhanced response to reduced hormonal requirements.

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Mutations in the p53 tumour suppressor are common in cancer, often causing protein upregulation and increased resistance to cell death. Here we use a Drosophila model of sustained p53 activation in apoptotic-deficient cells, which triggers cellular senescence. These senescent cells, while arrested in the cell cycle, can influence neighbouring cells by promoting non-autonomous proliferation via the SASP, causing them to overproliferate and form tumour overgrowths. Importantly, tumour cells activate Dilp8, which, acting on the neuroendocrine centres, inhibits the synthesis of ecdysone, a hormone essential for cell proliferation and developmental transitions. This observation raises the question of how tumour cells maintain their ability to grow in a macroenvironment depleted of insulin and ecdysone, both of which are essential for tissue growth and proliferation. We demonstrate that senescent-induced tumours become extremely sensitive to the low ecdysone and insulin levels through the acquisition of a juvenile state mediated by the transcription factor Chinmo and activation of the mTOR pathway. Chinmo, a downstream effector of JAK/STAT signaling, acts as a key regulator of temporal identity, stem cell maintenance, proliferation, and prevents differentiation. Chinmo is highly expressed in the proliferating cells of the tumour and it is also essential for its overgrowth. Mechanistically, Chinmo can increase the ecdysone response independently of its ligand, as well as transcriptionally activate components of the Insulin pathway and of the cell cycle. These results demonstrate that Chinmo enhance cells' responsiveness to signals secreted by senescent cells, establishing its key role in tumorigenesis and proliferation by bypassing hormonal requirements.

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Ribosomopathies as Disorders of Polyploid Tissues

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Ribosome biogenesis is an essential and highly conserved process required for protein synthesis, growth, and development. Defects in this pathway cause ribosomopathies, a group of human developmental disorders characterized by striking tissue-specific phenotypes despite the ubiquitous requirement for ribosomes. To investigate the developmental basis of this tissue specificity, we used Drosophila melanogaster as a disease model and examined the functions of the ribosome biogenesis factors Nol9 and Las1L.

Nol9 and Las1L are evolutionarily conserved and broadly expressed during development. Tissue-specific depletion of either factor in the tracheal system resulted in impaired air filling, reduced body size, defective terminal cell growth and branching, and decreased viability. These defects emerged during larval stages, coinciding with the onset of polyploid growth, suggesting an important role for ribosome biogenesis in supporting the biosynthetic demands of enlarged cells. To explore this relationship, we analyzed Fizzy-related (Fzr), a key regulator of endoreplication. Loss of fzr phenocopied several tracheal defects observed following Nol9 or Las1L depletion, confirming that endocycle progression is required for normal tracheal development. However, fzr overexpression failed to rescue Nol9- or Las1L-dependent phenotypes, suggesting that ribosome biogenesis and polyploidization contribute to tracheal growth through distinct but complementary mechanisms.

Together, these findings identify Nol9 and Las1L as key regulators of polyploid cell development, demonstrate that ribosome biogenesis is required for the generation of polyploid cells essential for organogenesis, and suggest that defective polyploidization may represent a previously underappreciated mechanism underlying the tissue-specific pathology of ribosomopathies.

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Modeling 1q21.1 Copy Number Variations in Human Neural Organoids Reveals Dosage-Sensitive Effects on Neurodevelopment

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Human evolution is characterized by a dramatic expansion of the central nervous system (CNS), with humans generating substantially more neurons and exhibiting a prolonged neurogenic period compared with other species. These features reflect differences in neural progenitor cell (NPC) dynamics, including cell-cycle regulation, potentially influenced by human-specific genes arising from evolutionary segmental duplications. The 1q21.1 locus, which is enriched in such genes, is frequently affected by copy number variations (CNVs) associated with neurodevelopmental disorders (NDDs). Deletions at this locus are linked to microcephaly and motor impairments, while duplications are associated with macrocephaly, although the underlying cellular and molecular mechanisms remain poorly understood.

Here, we use human induced pluripotent stem cell–derived 3D neural organoids, including patient-derived lines, to investigate the impact of 1q21.1 dosage alterations on CNS development. We show that organoids carrying 1q21.1 deletions are significantly reduced in size and exhibit widespread dysregulation of neurodevelopmental programs, including defects in cell adhesion, and cytoskeletal organization. In contrast, duplication organoids display more variable size phenotypes and comparatively subtle transcriptional changes.

Together, these findings provide a cellular and molecular framework for understanding 1q21.1-associated neurodevelopmental disorders and highlight how dosage-sensitive genes in this locus contribute to human CNS development.

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Mecp2 mutant zebrafish as a model for transcriptomics-based high-throughput drug discovery in Rett syndrome

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Rett syndrome (RTT) is a rare neurodevelopmental disorder primarily caused by mutations in the X-linked gene MECP2 leading to severe cognitive, motor, and communication disabilities predominantly in females. Although ubiquitously expressed, MECP2 highly accumulates in neurons during early postnatal development. Therefore, its dysfunction triggers profound neurological deficits alongside peripheral, systemic symptoms. Currently, therapeutic options remain critically limited, with only one FDA-approved drug available for symptom attenuation. Previous drug screens rely on in vitro cultures, which fail to capture RTT's multisystemic pathophysiology, or mammalian models, which are unsuitable for high-throughput screening (HTS). Furthermore, conventional assays utilizing single reporters cannot address the widespread, downstream transcriptional dysregulation caused by MECP2 mutations. To accelerate drug discovery, we propose an automated HTS platform using mecp2 mutant zebrafish larvae to evaluate over 3,000 FDA-approved compounds for drug repositioning. Since MECP2 is functionally conserved in vertebrate brains, zebrafish offer a useful whole-organism model that mirrors some of RTT's complex pathophysiology. Instead of single reporters, we will employ low-depth RNA-seq via Pooled Library Amplification for Transcriptome Expression (PLATE-Seq) to identify compounds that revert the entire transcriptome toward a wild-type state. As a proof of concept, we confirmed that this low-depth strategy effectively distinguishes mecp2-mutant from wild-type transcriptomic profiles during early development, establishing a cost-effective, robust, and scalable framework for HTS. Ultimately, this strategy helps elucidate druggable pathways associated with MECP2 impairment, uncovering novel mechanistic insights into RTT pathogenesis while complementing mammalian and in vitro models.

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POSTER · Session 7 · Developmental Disorders · Poster number 72

In vivo optimization of CRISPR-Cas systems for disease modelling in zebrafish

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CRISPR-Cas technologies have revolutionized genome engineering and are used for disease modelling and recently even for gene therapies. However, they currently face important limitations including PAM-dependent restrictions that limit access to genomic targets, off-target activity, chromosomal translocations and potential immune responses against Cas proteins. To overcome these challenges, our laboratory is focused on expanding the CRISPR-Cas toolbox through the implementation and optimization of alternative nucleases in vivo using zebrafish as a model. FCA-anCas is an ancestral Cas nuclease (anCas) computationally reconstructed through sequence inference and previously characterized in mammalian cell culture where did not show genome editing activity. Here, we demonstrate that FCA-anCas displays a relaxed PAM requirement and nickase-like activity that may help to increase target accessibility and provide novel editing strategies upon proper experimental conditions, complementing conventional CRISPR-Cas systems. In parallel, we are optimizing eSpOT-ON, an engineered version of Parasutterella secunda Cas9 (ePsCas9). This system exhibits reduced off-target activity and lower frequencies of chromosomal translocations compared with SpyCas9 in mammalian cells. Furthermore, eSpOT-ON generates DNA breaks with cohesive ends, a property that could facilitate precise genome modifications and improve knock-in (KI) strategies. Our results show that eSpOT-ON is active in zebrafish and may outperform SpyCas9 in some targets. We are currently comparing eSpOT-ON with SpyCas9 performance triggering KI in zebrafish. Overall, our work aims to expand the repertoire of genome editing technologies available for in vivo applications, providing more precise, versatile and potentially safer CRISPR-Cas systems for biological research, disease modelling and future therapeutic applications.

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Modeling ocular anomalies with Retinal Organoids: Insight from DYRK1A syndrome

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DYRK1A syndrome is a rare, and frequently underdiagnosed disorder caused by de novo dominant mutations in the DYRK1A kinase. The syndrome is characterized by developmental delay, autism spectrum disorders, microcephaly, and a variable range of ocular abnormalities, underscoring the essential role of DYRK1A in early embryonic development. Most diagnosed cases present microcephaly,with truncated proteins with disrupted kinase domain. However, a small subset of patients with macrocephaly carries truncating mutations preserving this domain, suggesting distinct molecular mechanisms.

We aim to investigate how mutations in this pleiotropically expressed protein influence eye development and how different types of mutations can lead to contrasting phenotypes. To address this, we use retinal organoids—three-dimensional cultures that faithfully recapitulate embryonic eye development, from early eye-field specification to retinal maturation. We employed the parental H9 embryonic stem cell line and its isogenic DYRK1A-mutant derivatives generated via CRISPR-Cas9 genome editing. These variants carry either the R255Stop (g37490273>T) or Q576Stop (g37511965>T) mutations in heterozygous and homozygous backgrounds. The former is associated with microcephaly, whereas the latter correlates with patients with macrocephaly, providing an excellent system to dissect the molecular and cellular mechanisms underlying DYRK1A syndrome.

Retinal organoids derived from homozygous mutants revealed impaired retinal specification in R255Stop organoids compared with wild-type, while Q576Stop organoids displayed accelerated developmental progression. Results using heterozygous lines suggest a dose-dependent context. These findings highlight the critical role of DYRK1A in eye development and demonstrate opposing phenotypic effects of distinct DYRK1A mutations, paving the way for identifying downstream pathways.

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POSTER · Session 7 · Developmental Disorders · Poster number 58

Transcriptional Dissection of Diverse SOX2 Mutations Driving Divergent Ocular Developmental Defects in MAC Syndrome (Microphthalmia, Anophthalmia, and Coloboma)

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Congenital visual disorders, such as microphthalmia, anophthalmia, and coloboma (MAC), are thought to originate from disruptions in eye morphogenesis during embryonic development. These are rare disorders that severely impair vision. Mutations in the SOX2 gen are the most frequent causes of diagnosed MAC conditions. This gen encodes for a transcription factor (TF) required for early eye field patterning and later eye development stages in most vertebrates. We aim to understand the molecular mechanisms involved in eye malformations in a SOX2 mutated context, and the molecular basis for which different mutations in SOX2 lead to such variable pathological outcome in patients.

In our study, we use CRISPR-Cas9 based genomic editing to generate SOX2 mutated cell lines derived from human iPS cells, and differentiate, and their parental cell line, into retinal organoids, to understand the effect of these mutations in every eye developmental stage. We generated SOX2.S18Ter cell line - which disrupts the DNA-binding domain, expecting a loss of function effect -, and SOX2.G129Ter cell line, disrupting the transactivation domain of the TF. In the latter case, we expect a dominant competition of the mutated protein to their WT form, suggesting a more phenotypic impact. Our goal is to characterize the impact of these mutations on neural retina and retinal pigment epithelium development using retinal organoids as a eye developmental model and elucidate the molecular mechanisms by which SOX2 regulate eye developmental gene networks and how different mutations in these genes relate to the pathological phenotypes observed in MAC patients.

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POSTER · Session 7 · Developmental Disorders · Poster number 55

Developmental signaling pathways co-opted by glioblastoma: glia–neuron communication as a driver of tumor progression

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Glioblastoma (GB) represents a paradigmatic example of how developmental programs are hijacked during tumorigenesis. Using Drosophila melanogaster we have investigated how conserved signaling pathways governing central nervous system development are repurposed to sustain GB growth, with a particular focus on glia–neuron communication.

Our work demonstrates that GB progression relies on bidirectional interactions between glial tumor cells and neurons, rather than being a purely cell-autonomous process. Tumor cells establish functional synapse-like contacts with neurons, requiring canonical synaptic components to promote tumor expansion. These structures enable the transfer of signaling molecules and highlight the relevance of neuronal activity in shaping tumor behavior. In particular, GB cells “vampirize” neuronal signals such as WNT, triggering a JNK/MMP signaling loop that enhances both tumor infiltration and neurodegeneration.

We further show that multiple conserved pathways central to neural development, including WNT, JNK, insulin signaling, and Myc, are reactivated in GB. Insulin signaling emerges as a key mediator linking metabolic control with neurodegeneration, while circadian regulators such as cryptochrome (cry) modulate tumor growth through Myc accumulation and synchronization with environmental cues. These findings reveal the integration of developmental pathways in tumor progression.

Our results position glia–neuron communication as a central hub coordinating these pathways. This highlights that GB does not simply disrupt the neural environment but actively exploits developmental communication systems to sustain growth and invasion.

Our work suggest that glioblastoma progression reflects a pathological reactivation of developmental programs, and therefore, these interactions may open new therapeutic avenues aimed at disrupting tumor–brain crosstalk

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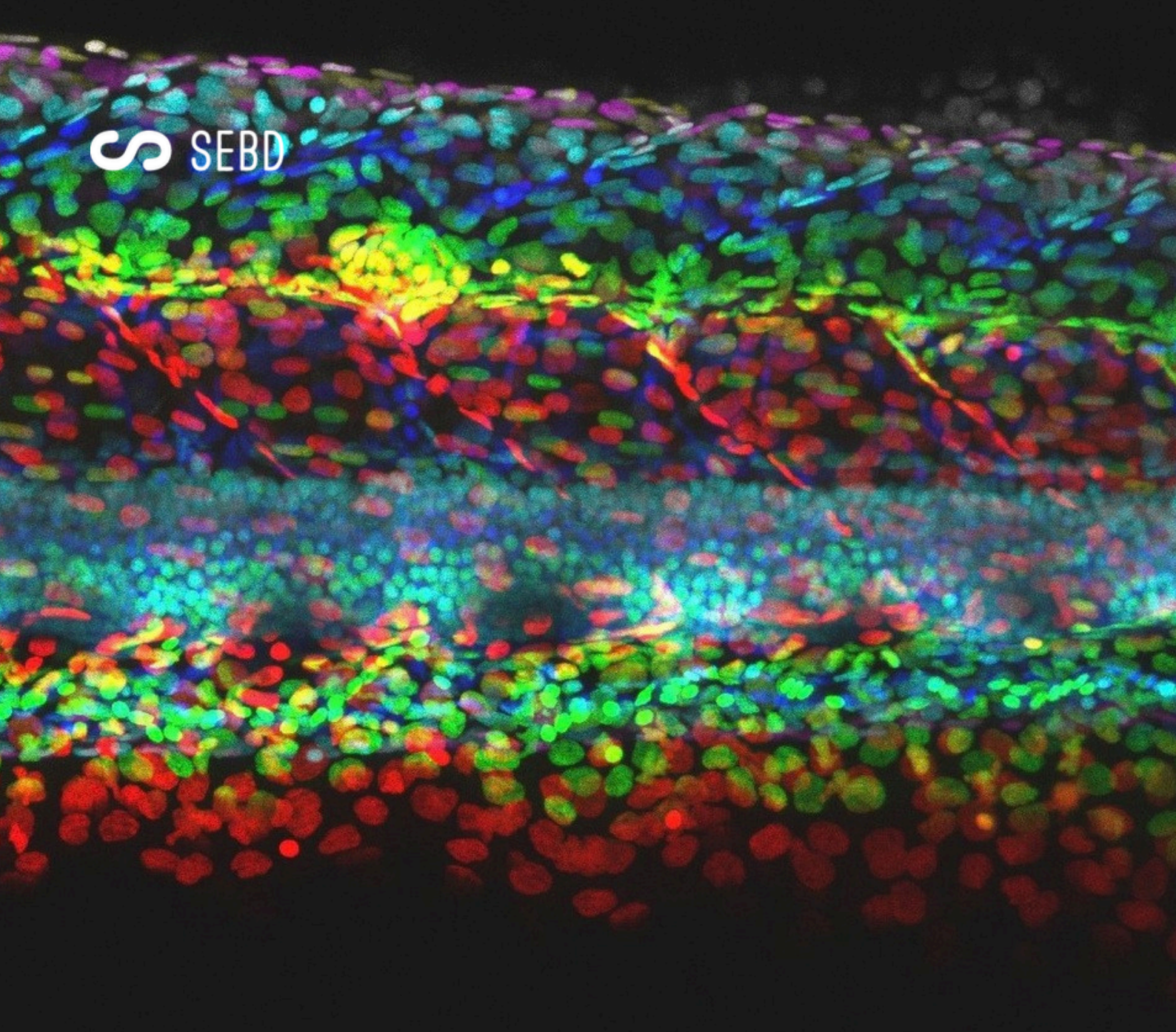
POSTER · Session 7 · Developmental Disorders · Poster number 54

Loss of tcf4 alters motor circuits and behavior in a zebrafish model of Pitt–Hopkins syndrome

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Loss-of-function mutations in TCF4 cause Pitt Hopkins syndrome (PTHS), a rare neurodevelopmental disorder characterized by intellectual disability and motor dysfunctions, including hypotonia and impaired coordination. Despite the clear genetic basis of PTHS, the pathophysiological mechanisms of the disease remain largely unknown. Notably, the role of TCF4 in motor system development and functioning is still unknown. A tcf4 knock-out zebrafish model was employed to investigate PTHS motor phenotypes. Behavioral assays revealed a reduced touch-evoked escape response in tcf4 +/- embryos, indicating poor motor output. Consistently, tcf4 +/- embryos showed reduced axonal arborization of trunk motor neurons leading to defective innervation of ventral muscle fibers. Co-staining of pre- and postsynaptic terminals demonstrated a reduction in functional neuromuscular junctions in tcf4 knock-out embryos, suggesting that compromised synaptic transmission and muscle activation may underlie muscle hypotonia. Given the motor coordination deficits observed in PTHS, we also examined cerebellar development. While Purkinje cell morphology appeared preserved, glutamatergic neurons in the corpus cerebelli were selectively affected in tcf4 +/- embryos, pointing to cerebellar circuit dysfunction. Ongoing behavioral analyses in adult zebrafish aim to assess long-term motor performance too. Together, these data provide the first functional evidence linking tcf4 to the development of motor circuits and behavior.



09 / 09

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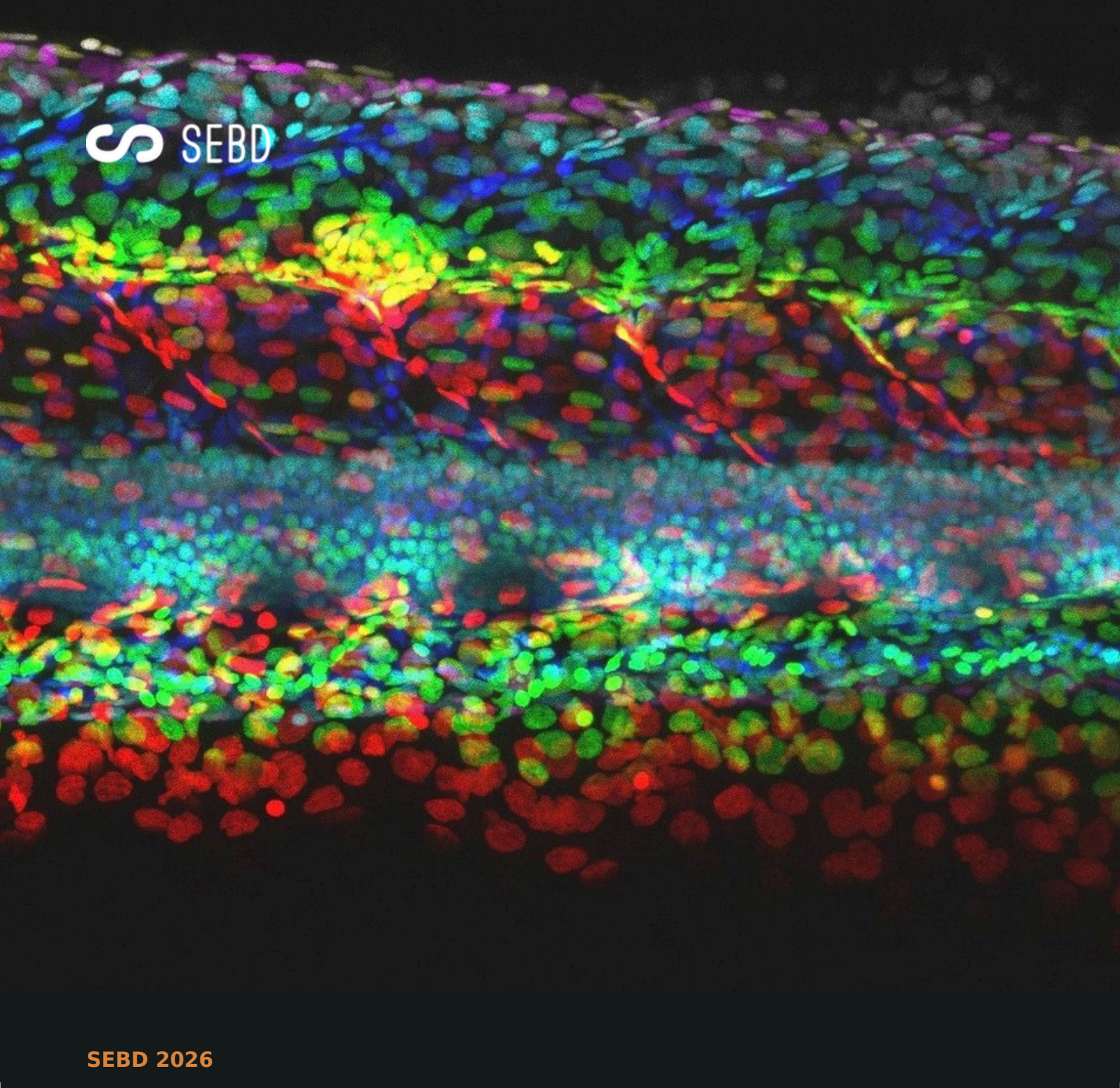
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